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Microbial transglutaminase for cold-set binding of unsalted/salted pork models and restructured dry ham

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ABSTRACT

The viability of using microbial transglutaminase (MTGase) as a cold-set binder for restructuring and manufacturing deboned dry ham (RDH) was evaluated. The influence of meat pre-treatment, preparation of the MTGase, packing system and set temperature on the binding rate and force was tested using pork models and deboned legs. The best binding parameters were obtained when meat surfaces were evenly distributed with salts (NaCl, KNO_3, $NaNO_2$) and then washed with a saline solution (W), afterwards powder (P) or liquid (L) MTGase was applied, and simultaneous salting and vacuum packing (S) set at 7 °C were performed. The RDH manufactured following these procedures (WPS and WLS) was stable during drying and could resist the handling and production process. Binding force increased ($p < 0.05$) during 8 weeks of drying. Scanning electron microscopy analysis showed an increase of cross-links during the drying period of RDH related to the increase in binding force.

1. Introduction

The meat industry is effectively using different systems of cold-set bindings (alginates, fibrinogen and thrombin, transglutaminase of different origins, etc.) to manufacture restructured products from small meat pieces and to improve the stability, texture and consistency of meat and fish gels (Cambero, López, de la Hoz, & Ordoñez, 1991; Mandigo, 1988; Vigneron, 1988). There have been numerous studies (Kuraishi et al., 1997; Paardekooper & Wijngaards, 1988; Wijngaards & Paardekooper, 1988) with satisfactory results confirming that these systems develop enough bonds to eliminate the cavities produced in the meat pieces during the deboning process. Dry-cured ham manufacture is performed using either portions or whole pork legs. During the manufacture of dry-cured hams in Spain, portions of the coxis bone are retained while in France or Italy technology usually implies the almost total elimination of this bone. Sometimes, a small coxis portion (anchetta) is not removed to avoid cavity formations and thus facilitate the drying process. A clear expansion of the market (about 73% for exports) of deboned dry-cured ham is being observed (Elizalde, 2008). To satisfy these demands of the consumers, usually the meat industry produces bone-less dry-cured hams after the salting and drying process. Traditionally manufactured hams and deboned dry-cured hams may be commercialised whole or as portions or slices. Deboned hams are the most suitable ones for cutting or slicing. This fact highlights a motivation to develop an adequate procedure for dry-cured ham manufacture using deboned fresh legs as raw material. The production of dry-cured ham essentially consists of salt diffusion into the meat and progressive dehydration. The aim of the process is to stabilize the ham by decreasing the water activity (a_w) and, at the same time, the development of the appropriate biochemical reactions that produce the typical sensory characteristics. This process may affect the action of the cold-set binders, in this case microbial transglutaminase (MTGase) systems. The transglutaminase (TG) enzyme catalyzes cross-linking between protein molecules (Yokoyama, Nio, & Kikuchi, 2004) by an acyl transfer reaction between the γ-carboxyamide group of a peptide-bound glutaminyl residue (acyl donor) and a variety of primary amines (acyl receptors), including the amino group of lysine. The TG currently utilized has a microbiological origin (MTGase), which has been tested in the manufacture of several meat and fish products: i.e. frankfurters (Jimenez-Colmenero, Ayo, & Carballo, 2005), beef protein gels (Dondero, Figueroa, Morales, & Curotto, 2006) and restructured pork products (Flores, Boyle, & Kastner, 2007). No study has been found related to the use of TG on dry-cured ham processing. Several authors (Costa, Bergamin, Silveira, & Felício, 2008) added MTGase in an aqueous solution to muscle groups separated after completion of salting to obtain bone-less restructured dry-cured hams. These authors reported that MTGase affected the protein functional properties, specially the binding capacity, thus the use of MTGase in dry-cured hams may be responsible for enhancing their springiness and cohesiveness. The acceptability of deboned dry-cured ham involves many factors, such as easy slicing and control of "deep spoilage" (*cala* in Spanish). In the dry-cured ham

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industry the "*cala*" describes a group of changes, characterised by off-odours, which make the product unacceptable for consumption, with corresponding economic losses implied. In Spanish processes, about 1–2% of manufactured hams undergo deep spoilage and have to be discarded (Paarup, Nieto, Peláez, & Reguera, 1999).

All these facts lead to the possibility of using cold-set binders to form meat pieces that can be manufactured, using modified procedures, into what is expected of Spanish dry-cured ham.

The main objective of this work has been the evaluation of the microbial transglutaminase (MTGase) for fresh deboned pork leg restructuration before or after a salting and drying process in order to ultimately produce deboned dry-cured ham. For this purpose, the effect of factors such as meat treatment, preparation of MTGase, packing procedure and binding temperature on the binding parameters of pork model complexes and restructured dry ham manufactured following a process resembling the traditional one were evaluated. Also, the microstructure of the resulting binding surfaces was analyzed by scanning electron microscopy.

2. Material and methods

2.1. Experiments with pork model complex

2.1.1. Experimental design

The variables (meat treatment, preparation of the microbial transglutaminase, packing and binding temperature) used to obtain the pork model complex are described in Table 1.

The pork model complexes were manufactured at three different times (February, May and October). In the three cases, each individual pork model complex was manufactured in triplicate; a different pork piece being used in each case.

2.1.2. Meat preparation and treatment

Pork cubes ($10 \times 10 \times 10$ cm) were obtained from fresh pork (*M. biceps femoris*) purchased at 48 h *post mortem* from a local abattoir. The visible fat and connective tissue were removed from the meat cubes. Afterwards, as indicated in Table 1, the pork cubes received one of the following procedures (U, I and W): (U) without additional treatment; (I) immersion in a saline (NaCl with about 200 ppm of KNO_3 and 100 ppm of $NaNO_2$) aqueous solution (3%, w/v) for 10 min at 4 °C after which the excess liquid was removed using a filter paper; and (W) even distribution of a mixture of salts (NaCl with about 200 ppm of KNO_3, and 100 ppm of $NaNO_2$) on the surfaces for 1 min and, after 10 min of setting time, the pork cubes were washed with an aqueous solution of the same salt mixture (NaCl with about 200 ppm of KNO_3, and 100 ppm of $NaNO_2$) after which the excess liquid was removed using a filter paper.

2.1.3. Preparation of microbial transglutaminase (MTGase)

Microbial transglutaminase (ACTIVA EB, binding effect, Ajinomoto Co., Inc., Tokyo, Japan) was obtained as a free sample from Impex Química S.A. (distributor in Spain). ACTIVA EB, used in a freeze-dried form, was composed of sodium caseinate (60%), maltodextrin (39.5%) and microbial transglutaminase (MTGase) (0.5%), as an enzyme with the ability to cross-link proteins, with an activity of 50 units g^{-1}.

Two different procedures for applying MTGase (Table 1) on binding meat surface were used (P, L): (P) powder, and (L) liquid, composed by a solution of MTGase at 0.1% in aqueous solution of NaCl 3% (w/v), with a transglutaminase activity of approximately 10 units g^{-1}.

The powder (P) or liquid (L) MTGase was carefully spreadd trying to cover one of the pork cube surfaces.

2.1.4. Preparation of the pork model complex (M/MT/M)

One pork cube (U, I or W) surface covered with the MTGase (P or L) was put into contact with a similarly processed pork cube (U, I or W) surface and the complex (meat/MTGase/meat, M/MT/M) was packed.

2.1.5. Packing

The pork model complexes (M/MT/M) were packed (Table 1) using three different procedures (A, V and S): (A) in plastic containers at atmospheric pressure; (V) vacuum-packed (20 kPa) in plastic bags, and (S) vacuum-packed (20 kPa) in plastic bags with M/MT/M covered with a dry mixture of salts (simultaneous binding and salting procedure). The mixture of salts (NaCl with 200 ppm of KNO_3, and 100 ppm of $NaNO_2$) was similar to that used for dry-cured ham manufacturing and the amount used was equivalent to 2% of the weight of the pork model complex (Santos, Hoz, Cambero, Cabeza, & Ordóñez, 2008).

2.1.6. Binding temperature

Pork model complexes were set at three (Table 1) different temperatures (0, 7 and 24 °C) during the first 24 h (binding period).

After the different binding periods, the pork model complexes (M/MT/M) were removed from the plastic containers and those corresponding to the procedure S (Table 1) were brushed to eliminate the excess of salt. Afterwards, the analyses were performed.

Table 1
Variables used for the preparation of meat model complex and restructured dry ham.

Meat treatment	Symbol
Untreated	U
Immersed in aqueous solution of a mixture of salts (NaCl with 200 ppm of KNO_3, and 100 ppm of $NaNO_2$) at 3% (w/v) for 10 min	I
Even distribution of (1 min) a mixture of salts (NaCl with 200 ppm of KNO_3, and 100 ppm of $NaNO_2$) on the surfaces, setting time 10 min, and washed with aqueous solution of the mixture of salts at 3% (w/v)	W
Preparation of the microbial transglutaminase (MTGase)	
Powder	P
Liquid, solution of MTGase at 0.1% (w/v) in aqueous solution of NaCl 3% (w/v) with a transglutaminase activity of approximately 10 units g^{-1}	L
Packing	
Atmospheric pressure in plastic containers	A
Vacuum packing at 20 kPa in plastic bags	V
Vacuum packing at 20 kPa in plastic bags with meat product covered with the mixture of salts (simultaneous binding and salting procedure)	S
Binding temperature	
0 °C	0
7 °C	7
24 °C	24