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Dry-cured ham restructured with fibrin

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ABSTRACT

The viability of a fibrinogen–thrombin system (FT) to bind fresh deboned hams for incorporation in the salting and ripening processes, to produce cured ham, was studied. The effects of the different processing variables (pH, NaCl concentration, temperature and gelation time) on FT, a meat emulsion mixed with FT, fresh pork portions and deboned hams restructured with FT were analyzed. The most stable and firmest fibrin gels were obtained after 6 h of adding the FT, with less than 2% NaCl and pH 7–8.4.

Scanning electron microscopy of the fibrin gel showed fibrillar structures with a high degree of cross-linking and a high density. Two structures were found in the binding area of restructured meat; one in the central part with similar characteristics to fibrin gels and, another in the area of contact between the meat surfaces, where a filamentous structure connected the fibrin gels with the muscle bundles.

1. Introduction

Traditionally, ripened ham has been commercialized as a whole piece whereas today the most successful products are the ready-to-eat formats, and there has been a clear expansion of the market (about 73% for exports) for deboned dry-cured ham, either whole or as portions (200–500 g) or slices (Elizalde, 2008). The manufacture of dry-cured ham from fresh deboned ham offers advantages during the production, storage and transport because it decreases the piece weight and volume in a quantity equivalent to the size of the bone. Deboned hams are also the most suitable for cutting and slicing. There are, therefore numerous reasons for developing a procedure with which to manufacture dry-cured ham using deboned fresh pork hams.

For some time the meat industry has been using a range of cold-set binding systems (alginates, blood plasma factors and enzyme complexes) to make restructured products from smaller pieces of fresh meat and to improve the stability, texture and consistency of meat and fish gels, in all cases with great success (Cambero, López, de la Hoz, & Ordóñez, 1991; Dimitrakopoulou, Ambrosiadis, Zetou, & Bloukas, 2005; Vigneron, 1988). These agents can be considered as new types of additives or coadjuvants, "binding agents", in food production and share the ability to interact with meat components. Different blood plasma preparations can be used as binding agents (Tsai, Tseng, Yang, & Chen, 2006; Herrero et al., 2007; Herrero, Cambero, Ordóñez, de la Hoz, & Carmona, 2009). Most of them owe their cohesive effect to the formation of fibrin from fibrinogen in the presence of thrombin. Fibrinogen is an elongated protein and is made up of globular domains at each end connected by α-helical coiled coils to a globular region in the middle (Standeven, Ariëns, & Grant, 2005). Thrombin converts soluble fibrinogen to insoluble fibrin polymer by cleaving fibrin peptides from the central domain, exposing knobs that can then interact with holes that are always exposed at the ends of the molecule, giving rise to a half-staggered structure called the protofibril. When the protofibrils grow long enough they aggregate laterally to form fibres, which then branch to yield the three-dimensional network of the fibrin clot or stable fibrin gel (Gentry, 2004). The fibrin complex is stable, both to heat treatment and freezing, and can interact with myofibrillar proteins and with collagen (Paardekooper & Wijngaards, 1988). Results of a range of experiments in this field suggest that these systems could be used to eliminate the cavities produced by the deboning of meat portions. The work described here is part of this same line of research. The final objective of this study is to restructure fresh deboned pork hams using fibrinogen–thrombin system as cold-set binding agent, in order to be seasoned and ripened in a similar process to that used in the production of cured hams.

2. Materials and methods

Fibrinogen (5.0 mg/mL) and thrombin (20 NIH/mL) solutions, from porcine blood plasma combined commercially as Fibrimex™, were obtained from Sonac B.V. (Loenen, Netherlands). Before use, independent fibrinogen (F) and thrombin (T) were thawed in

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plastic containers immersed in agitated water at 25 °C for 60 min. For each experiment with fibrinogen and thrombin, 10 parts of fibrinogen were mixed with 1 part of thrombin (FT, 10/1, v/v) and shaken for 30 s.

Fresh pork from deboned whole ham was purchased from a local abattoir at 48 h post-mortem. To achieve homogeneous samples, only meat from female white pigs (Landrace × Large White) fed in confinement with a commercial diet was acquired. Animals were stunned, slaughtered and exsanguinated at a local slaughterhouse at 100.3 ± 8.01 kg live-weight (about 6 months of age).

2.1. Experimental layout

The following four experiments were conducted,

2.1.1. Fibrinogen–thrombin (FT) mixtures

Different FT mixtures were prepared to study the following process conditions:

(a) pH of the FT mixtures. First the pH of the fibrinogen was adjusted to values between 5 and 8 (using 0.5 M HCl) and then thrombin was added.
(b) The concentration of NaCl in FT mixtures. A volume of 100 mL of fibrinogen was mixed with the corresponding amount of NaCl to obtain final concentrations in the FT mixture of 0%, 0.25%, 0.5%, 1%, 2%, 3%, 10%, 20% of NaCl (FT/NaCl, v/w) and then thrombin (10 mL) was added.
(c) Gelation temperature. The FT mixtures were kept at 0, 6 and 10 °C.
(d) Gelation time (0–48 h). The FT mixtures were stored at the previously indicated temperatures for up to 48 h.

Each individual FT mixture was produced in triplicate.

2.1.2. Meat emulsion supplemented with FT mixture (ME-FT model system)

Mixtures of meat emulsion were supplemented with different amounts of FT mixtures [0%, 10%, 20%, 30%, 40% and 50% of FT in the final ME-FT mixture, (w/v)]. The conditions employed for preparing the FT mixtures were those selected after the experimental procedure described in Section 2.1.1.

To make the meat emulsion (ME), the fat and connective tissue were removed from the fresh pork (*biceps femoris* muscle). Then, meat was added to a cutter (Robot Coupe R 8 V.V., Vincennes Cedex, France), where the material was cut and, after that, mixed with water or NaCl aqueous solution (100 parts of pork/11 parts of water or aqueous solution, w/w) to obtain a homogeneous mixture. The process was performed by controlling the temperature to maintain the product at 0–2 °C.

The ME-FT models were prepared in a mixer by adding the ingredients in the following order: meat emulsion (meat and water or NaCl aqueous solution), F, and T (w/v/v). Fibrinogen and thrombin (10/1, v/v) were incorporated in the amounts required to obtain a known percentage of FT in the final mixture ME-FT (w/v). Different NaCl aqueous solutions were prepared to obtain concentrations of 0.25%, 0.5% and 2% of NaCl in the final mixture ME-FT (w/w).

Each of the individual ME-FT models was carried out in triplicate (pork pieces from different animals being used in each case).

Circular plastic containers (9 cm of diameter) were filled (0.5 cm of thickness) with each FT mixture (with or without NaCl), ME and ME-FT. The binding temperatures were established after carrying out the procedure described in Section 2.1.1.

2.1.3. Pork model system (PMS)

The model systems (approximately 1 kg in weight) were made from two meat portions obtained from deboned ham, which had internal surfaces, originally touching or close to the femur (*quadriceps femoris*, *semimembranosus*, *biceps femoris*). These were considered as the meat binding surfaces. A FT mixture was spread on these meat surfaces using a palette knife. Thus, a continuous layer (around 2 mm of thickness) of FT mixture on the meat surfaces was formed. Previously, these meat surfaces were subjected to one of the followed pre-treatments:

(U) Samples without additional treatment.
(I) Samples were immersed 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 fluid was removed with a filter paper.
(SS) A mixture of salts (NaCl with 200 ppm of KNO_3 and 100 ppm of $NaNO_2$) was evenly spread on the meat surface. After 10 min setting time, the surfaces were washed with the same saline aqueous solution (3%, w/v) then the excess liquid was removed using a filter paper.

Meat surfaces with the same pre-treatment were covered with a FT mixture, following the procedure described above to form a continuous layer of this binding agent. Then, the meat surfaces with FT were immediately placed in contact to obtain a meat-FT-meat structure (binding area). The FT mixture was prepared following the conditions selected by following the procedure described in Sections 2.1.1 and 2.1.2.

The pork model systems were packed using two different procedures:

(A) in plastic containers at atmospheric pressure.
(V) in plastic bags (Cryovac®) in a high-vacuum machine (750/400 model, Vapta, SL, Spain) until a vacuum of 20 kPa was reached.

Each of the individual PMS was prepared in triplicate (a ham from a different animal was used in each case).

2.1.4. Restructured fresh deboned hams (RDH)

The distal meat portions and external or hanging flaps were removed from the deboned pork hams to obtain homogeneous pieces of a similar cross-sectional area, obtaining deboned pork portions weighing about 3 kg. The uncovered muscle surfaces resulting from the deboning process were pre-treated by procedures U, I and SS. To restructure the fresh deboned hams, the FT mixture (F/T, 10/1, v/v) was spread using a palette knife onto muscle surfaces uncovered during the deboning process. The pork hams were then restructured by placing parallel meat surfaces in contact and were immediately packed using one of three different procedures (A, V and vS). The pork deboned hams were packed as described in procedure A (plastic containers at atmospheric pressure) or V (in plastic bags in a high-vacuum machine) were stored at 6 °C during the binding time. At the end of the binding period, the restructured deboned hams (RDH) were removed from the plastic bags and the pieces were covered with a dry mixture of salts (salting). 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 manufacture and the amount used was equivalent to 2% of the weight of the pork model complex (Santos, De la Hoz, Cambero, Cabeza, & Ordóñez, 2008). The salting period lasted 0.75 days per kg of restructured ham.

During the vS packing procedure, simultaneous binding and salting treatments were carried out. These consisted in introducing the fresh deboned ham treated with FT mixture in plastic bags and