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High pressure applied to frozen ham at different process stages. 1. Effect on the final physicochemical parameters and on the antioxidant and proteolytic enzyme activities of dry-cured ham

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Abstract

This paper describes the effect of high pressure (400 MPa and 600 MPa) applied to frozen hams at early stages of the dry-cured ham process: green hams (GH) and hams at the end of the resting stage (ERS), on some physicochemical parameters and on antioxidant and proteolytic enzyme activities in the final product. No significant differences were observed among treatments either in the drying kinetics or in the physicochemical characteristics. However, when high-pressure was applied to frozen GH hams it produced a superficial denaturation that affected salt absorption and, consequently, the proteolysis index. The high-pressure treatment applied during the processing of previously frozen GH and ERS hams reduced the antioxidant enzyme activities slightly (superoxide dismutase, catalase and glutathione peroxidase) but did not affect the cathepsin B and the cathepsin B + L activities.

Keywords: High-pressure; Dry-cured ham; Freezing; Antioxidant enzymes; Proteolytic enzymes

1. Introduction

The application of high hydrostatic pressure on meat and meat products has been focussed mainly on studying its effect on microorganisms as a treatment to improve the microbiological safety of the final product (Aymerich, Jofré, Garriga, & Hugas, 2005; Cheftel, 1995; Garriga, Grèbol, Aymerich, Monfort, & Hugas, 2004; Moerman, 2005; Tanzi et al., 2004). Nevertheless, high-pressure treatment can also be used to develop new meat products, although some key points should be considered regarding the use of raw meat. The pressurization of pork and beef above 200 MPa has been proven to cause drastic pressure-induced colour changes, namely, lightness increase and redness decrease, which are totally undesirable in fresh meat (Carlez, Veciana-Nogues, & Cheftel, 1995; Cheah & Ledward, 1996; Cheftel & Culioli, 1997). Food enzymes can undergo reversible or irreversible pressure-induced changes resulting in partial or complete activation or inactivation (Cheftel, 1995). Several studies have reported an increase of lysosomal membrane disruption with high-pressure treatment, which results in a higher liberation of lysosomal enzymes into the cytoplasm (Homma, Ikeuchi, & Suzuki, 1994; Jung, Ghoul, & de Lamballerie-Anton, 2000; Ohmori, Shigehisa, Taji, & Hayashi, 1992). Among the several proteases contained in the lysosomes, cathepsins B and L are considered to partially account for dry-cured ham proteolysis that affects flavour and texture (Arnau, Guerrero, & Sárraga, 1998; García-Garrido, Quiles-Zafra, Tapiador, & Luque de Castro, 2000; Sárraga, Gil, & Garcıa Regueiro, 1993; Schivazappa et al., 2002; Virgili, Schivazappa, Parolari, Soresi-Bordini, & Degni, 1998). Cathepsins B and L are even active at the end of the

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dry-curing process (Sárraga et al., 1993; Toldrá, Flores, & Sanz, 1997). According to the results of Homma et al. (1994) on beef, total cathepsin activities increase with pressurization due to an increase of their content in the extract, however the relative activity of the enzymes decreases gradually with increasing pressure up to 500 MPa. Cathepsin B and L activities were the most pressure resistant enzymes since they kept around 80% of their original activity after pressurizing at 500 MPa for 5 min at 2 °C.

The oxidation of lipids is a major source of non-microbial spoilage in meat products. During the ageing process an intense lipid oxidation occurs. The normal rate of this process contributes to the development of the characteristic dry-cured flavour (Ruiz, Muriel, & Ventanas, 2002; Toldrá & Navarro, 2002). The enzymes superoxide dismutase (SOD), catalase (CAT) and glutathione peroxidase (GSHPx) constitute the in vivo system for protecting cells against oxidative mechanisms (Halliwell, Murcia, Chirico, & Arouma, 1995). Several studies on pork have reported that these antioxidant enzymes maintain their activity after the conversion of muscle to meat (Hernández, Zomeño, Ariño, & Blasco, 2004; Pradhan, Rhee, & Hernández, 2000; Young, Rosenvold, Stagsted, Nielsen, & Andersen, 2005) and also for GSHPx activity at the end of the ageing of dry-cured loin (Sárraga, Carreras, & García-Regueiro, 2002).

The objectives of this research work were, firstly, to evaluate the effect of pressurization of frozen hams at early stages of ham elaboration on the final product characteristics (e.g. flavour and texture); and secondly, to study the effect on some muscle enzyme activities, which could contribute to changes in texture and/or flavour attributes. In order to avoid the pressure-induced colour changes of meat the hams were treated frozen. No previous studies have been found on the effect of high-pressure treatment of whole hams at early stages of the process. In the present paper, the pressurization effect on the proteolytic activity of cathepsins B and L, on the antioxidant activities (i.e. SOD, CAT, GSHPx) and on some physicochemical parameters is studied. The pressurization effect on sensory attributes and on colour characteristics is presented in a second paper (Serra et al., 2006).

2. Materials and methods

2.1. Experimental design

This study was carried out in two separate experiments, with 30 hams per test obtained from 15 commercial carcasses. The two hams from each carcass were weighed and the ultimate pH (pH_{24SM}), with a pH penetration electrode (Crison 52-32) and a portable pH meter (Crison PH 25, Crison Instruments, S.A., Alella, Spain), and the electrical conductivity (EC_{24SM}), with a Pork Quality Meter (PQM-I, Intek GmbH, Aichach, Germany), at 24 h post-mortem in the *semimembranosus* muscle were recorded. The two hams from each pair were randomly assigned to different treatments to block the carcass effect. The experimental design is shown in Table 1.

Table 1
Experimental design

Pressurization process-point					
Experiment 1 – green ham (GH)			Experiment 2 – end of the resting stage (ERS)		
Treatments			Treatments		
Control	400-MPa	600-MPa	Control	400-MPa	600-MPa
1–5[a]	1–5	–	16–20[b]	16–20	–
6–10[a]	–	6–10	21–25[b]	–	21–25
–	11–15[a]	11–15	–	26–30[b]	26–30

[a] Carcass numbers of experiment 1 (ham pairs: 1–15).
[b] Carcass numbers of experiment 2 (ham pairs: 16–30).

In order to avoid the pressure-induced colour changes in raw meat, the hams were treated frozen as described below. Two dry-cured ham process-points were chosen for the pressurization treatment. The first selected point (Experiment 1) was the green ham (GH), which is of interest from an industrial point of view because many dry-cured ham manufacturers use frozen green hams as raw material. The second point of interest (Experiment 2) was at the end of the resting stage (ERS), i.e. when the dry-cured ham is submitted to a temperature over 5 °C.

2.2. Ham processing

2.2.1. Experiment 1: Green ham process

The 30 green hams from the GH process were vacuum-packed at 24 h post-mortem in plastic bags (50 μm polyamide/100 μm polyethylene multilayer; oxygen permeability: 18 cm^3/m^2/24 h at 23 °C; CO_2 permeability: 55 cm^3/m^2/24 h at 23 °C; N_2 permeability: 4.0 cm^3/m^2/24 h at 23 °C; water vapour permeability: 1.8 g/m^2/24 h at 23 °C and 85% RH; Sacoliva, S.L.®, Castellar del Vallès, Spain). The packed hams were immediately frozen at −20 °C (air speed: 2.5 m/s) and kept at −20 °C until the pressurization treatment. The GH hams were kept covered with dry ice for 48 h before the pressurization was carried out (see Section 2.2.3 for high-pressure treatment details). The plastic bags used for packaging protected the hams from freezer burns caused by dry ice and prevented the contact of hams with the water during the pressurization. The reason for using dry ice was to maintain the hams at very low temperature, in order to minimize the thawing of the external part. Besides the adiabatic heating due to pressurization, the temperature of the frozen ham increases during the time lag between the start of the pressurization cycle and the pressure build up (i.e. sample charging, vessel sealing and vessel filling with pressure medium) and also, as a result of being immersed in the water. This point was especially critical in the GH ham process (green hams), since pressurization could cause important colour changes. After the pressurization treatment, all the hams were left to thaw at 3 ± 1 °C for 4 days. Subsequently, the hams were salted with 50 g NaCl and 0.6 g $NaNO_2$ per kilogram of ham and