Meat Science 91 (2012) 378–381

Contents lists available at SciVerse ScienceDirect

Meat Science

journal homepage: www.elsevier.com/locate/meatsci

Evolution of nitrate and nitrite during the processing of dry-cured ham with partial replacement of NaCl by other chloride salts

Mónica Armenteros [1], María-Concepción Aristoy, Fidel Toldrá *

Instituto de Agroquímica y Tecnología de Alimentos (CSIC), Av. Agustín Escardino 7, 46980 Valencia, Spain

ARTICLE INFO

Article history:
Received 27 January 2012
Received in revised form 9 February 2012
Accepted 10 February 2012

Keywords:
Nitrate
Nitrite
Dry-cured ham
Low sodium
Low salt
Sodium chloride

ABSTRACT

Nitrate and nitrite are commonly added to dry-cured ham to provide protection against pathogen microorganisms, especially *Clostridium botulinum*. Both nitrate and nitrite were monitored with ion chromatography in dry-cured hams salted with different NaCl formulations (NaCl partially replaced by KCl and/or $CaCl_2$, and $MgCl_2$). Nitrate, that is more stable than nitrite, diffuses into the ham and acts as a reservoir for nitrite generation. A correct nitrate and nitrite penetration was detected from the surface to the inner zones of the hams throughout its processing, independently of the salt formulation. Nitrate and nitrite achieved similar concentrations, around 37 and 2.2 ppm, respectively in the inner zones of the ham for the three assayed salt formulations at the end of the process, which are in compliance with European regulations.

1. Introduction

Potassium and sodium salts of nitrate (E251 and E252) and nitrite (E249 and E250) together with sodium chloride (salt) are commonly used as curing agents in dry-cured ham. The addition of nitrificant salts (nitrates and nitrites) to dry-cured ham reinforces the preservative effect of salt by inhibiting the growth of *Clostridium botulinum* and thereby the formation of its neurotoxic botulinum toxin. Nitrite, which is the active form, also contributes to the development of dry-cured ham flavour and to the formation of the characteristic reddish colour in the final product that is well known by consumers. Moreover, nitrite exerts its antioxidant activity, retarding the development of rancidity and off-flavours during storage (Toldrá, Aristoy, & Flores, 2009). However, under certain circumstances nitrite can react with secondary amines to form nitrosamines, compounds that have shown to be carcinogenic in a variety of animal studies (Pegg & Shahidi, 2000). Nitrate is more stable and less toxic than nitrite, and acts as a reservoir for nitrite generation into the ham through the action of the microbial enzyme nitrate reductase (Toldrá, 2006, 2007).

The correct nitrate diffusion inside the ham is essential for appropriate nitrite generation to exert its preservative effect. The monitoring of nitrite and nitrate content of dry-cured ham is thus recommended to keep their levels in a range to comply with legal regulations and safety.

The presence of salt together with the nitrificant salts contributes to the preservative effect, and to the generation of a desirable red colour and typical dry-cured ham flavour (Toldrá & Flores, 1998). Nowadays, there is a trend to reduce the salt content in foods because an excessive sodium intake contributes to raised blood pressure in salt susceptible consumers (He & MacGregor, 2010; Toldrá & Reig, 2011). Recent studies have been performed to reduce the salt content in dry-cured ham by either reducing the addition of sodium chloride (Andrés, Cava, Ventanas, Muriel, & Ruíz, 2004) or more recently by partially replacing sodium chloride by other chloride salts (Armenteros, Aristoy, Barat, & Toldrá, 2012).

The purpose of this study was to evaluate the effect of different salt formulations (NaCl partially replaced by KCl, $CaCl_2$, and $MgCl_2$) on the penetration of nitrate and nitrite during the processing of dry-cured ham.

2. Materials and methods

2.1. Processing of the hams

Forty-eight fresh hams with an average weight of 10.7 ± 0.5 kg were selected in a local slaughterhouse in the pH 5.5 to 6.0 range. All the hams were frozen in an industrial freezer at −40 °C and stored for at least 30 days at −20 °C. Then the frozen hams were thawed at 3 °C for at least 5 days, similar to the industrial process (Motilva, Toldrá, Nadal, & Flores, 1994). Three of the hams were used as a raw material control. The remaining 45 hams were randomly divided into three groups and submitted to the salting process. Salt formulations were chosen according to the results obtained in previous

* Corresponding author. Tel.: +34 963900022; fax: +34 963636301.
E-mail address: ftoldra@iata.csic.es (F. Toldrá).
[1] Current address (M. Armenteros): Production and Food Science Department, Faculty of Veterinary Science, University of Extremadura, Cáceres, Spain.

0309-1740/$ – see front matter © 2012 Elsevier Ltd. All rights reserved.
doi:10.1016/j.meatsci.2012.02.017

works with low-sodium dry-cured hams and loins (Armenteros, Aristoy, Barat, & Toldrá, 2009a; Armenteros, Aristoy, Barat, & Toldrá, 2009b; Armenteros et al., 2012). This process included a previous manipulation in which a small portion of the respective salting mixture was mixed with 150 ppm of KNO_3 and 100 ppm of $NaNO_2$ and applied by rubbing and kneading to each ham as curing agents. Afterwards, each ham was salted by covering the free-of-skin part with the respective salt formulation, and left to rest in individual trays. Thus, hams from the first group were salted with the traditional NaCl content (100% NaCl, formulation I). The second group was salted using a mixture of NaCl and KCl at 50% each (formulation II) and the third group was salted with 55% NaCl, 25% KCl, 15% $CaCl_2$ and 5% $MgCl_2$ (formulation III). Salt contents, analysed at the end of processing in the *Biceps femoris* muscle was 2.5 g/100 g of NaCl for formulation I, 1.3 g/100 g NaCl plus 1.8 g/100 g of KCl for formulation II and 1.3 g/100 g NaCl plus 1.1 g/100 g KCl plus 0.3 g/100 g $CaCl_2$ plus 0.15 g/100 g $MgCl_2$ for formulation III (Armenteros et al., 2012). It must be noted that formulations II and III are not equal in molarity or even in ionic strength to the same weight of NaCl. Then, the trays with hams were placed in salting chambers at 3 ± 1 °C and 90% relative humidity for a total of 12 days.

After salting, hams were brushed to remove the remaining salt from their surfaces and transferred to the post-salting chamber, where complete salt homogenisation took place. The temperature was kept below 4 ± 1 °C at 75–85% relative humidity. Hams salted with formulation I (100% NaCl, considered as control), were maintained for 50 days in the post-salting stage, whereas hams salted with formulations II and III were maintained up to 80 days in order to reach similar a_w values in the inner zones to the control hams as suggested by Aliño, Grau, Fuentes, and Barat (2010). During the last stage, corresponding to the ripening period, hams were placed in air-conditioning chambers and subjected to different temperature (15–18 °C) and relative humidity (65–75%) cycles, similar to industrial processes. The weight loss of each ham was measured and recorded throughout the process. The process was finished when total weight loss of the ham reached 32–34% of the initial weight, the range of typical values achieved in the ham industry (Toldrá, 2006).

2.2. Sampling

Samples (50 g) from the muscles *Semimembranosus* and *Biceps femoris* were taken at different times during the process and subsequently kept at −80 °C until analysis. The sampling process was carried out on the raw material (0 days) and after 20 and 50 days of post-salting stages for formulations I, II and III, at 80 days in the formulations II and III, and finally at 270 days in the three formulations (I, II and III).

2.3. Methods

2.3.1. Chemical and reagents

All chemical reagents used were of HPLC/IC Trace CERT® grade. Standards of nitrite and nitrate were purchased from Fluka Chemie AG (Buchs, Switzerland). Sodium hydrogen carbonate and sodium carbonate were obtained from Panreac (Barcelona, Spain), whereas acetone and nitric acid were obtained from Scharlau (Barcelona, Spain). Standards solutions of nitrate and nitrite were prepared by appropriate dilution from 1000 mg/L stock solutions (Sigma-Aldrich, St Louis, MO, USA). De-ionized water used was of Milli-Q grade (Millipore, Billerica, MA, USA).

2.3.2. Extraction

Briefly, 5 g of minced sample (*Biceps femoris* or *Semimembranosus* muscles) was homogenised 1:10 (w/v) with 50 mL Milli-Q water in a stomacher (IUL Instruments, Barcelona, Spain) for 10 min at 4 °C. Then, samples were centrifuged at 12.000 ×*g* for 20 min at 4 °C. Finally, the supernatant was filtered through glass wool and immediately frozen at −20 °C until use. Thawed samples were filtered through nylon membrane filters (0.45 μm) previously injected into the ion chromatograph (IC).

2.3.3. Chromatographic method

The method described by Gómez-Ordóñez, Alonso, and Rupérez (2010) was followed and adapted to ham with some modifications. Twenty microlitres of each sample was injected into a Metrohm Advanced compact (IC-761 model, Metrohm, Herisau, Switzerland), using an Advance Sample Processor (IC-838, Metrohm). The Sample Processor module was equipped with an ultrafiltration cell containing a cellulosic acetate membrane filter (0.2 μm) which reliably removes particles from the samples and protects the analytical column from particulate clogging. The column was a Metrosep A Supp 5 (4.0 × 250 mm) from Metrohm, operating at room temperature. An isocratic elution was adopted with 1.0 mM $NaHCO_3$ and 3.2 mM Na_2CO_3 plus 0.3% of acetone (v/v) as mobile phase at a flow-rate of 0.7 mL/min. A conductivity detector was employed and the eluate from the column was submitted to an automatic chemical suppression, previous detection, to enhance sensitivity (both facilities are included in the 761-IC module Compact chromatograph). The total run time was 35 min. Prior to the injections, the column was equilibrated for 20 min with the mobile phase. Calibration curves for nitrate and nitrite were built (in triplicate) by plotting the peak areas against 5 concentration levels of the standards solutions of NO_3^- (0.1–5 mg/L) and NO_2^- (0.01 to 2.5 mg/L) from 1000 mg/L stock solutions (Sigma). The results were expressed as mg/kg of sample (ppm).

2.3.4. Statistics

Three hams were tested for each time and formulation and three replicates of every parameter were analysed for each ham. Data are expressed as mean ± standard deviation on a wet weight basis. The influence of the different salt formulations on nitrate and nitrite content as well as the evolution of these parameters over time was done by analysis of variance (ANOVA) using the Statgraphics Plus (v 5.1). In cases where the effect was significant (p-value < 0.05) the means were compared using Fisher's least significant difference (LSD) test.

3. Results and discussion

In the figures the contents of nitrate (Fig. 1) and nitrite (Fig. 2), in both the *Semimembranosus* and *B. femoris* muscles, during ham processing are shown. There is some variability in the data, probably due to several factors such as the inherent variability of the sample mainly in terms of fat content and its distribution throughout the ham which may affect the nitrate/nitrite diffusion rates, the artisanal way of applying the curing mixture, the distribution and activity of nitrate reductase as well as the high reactivity of nitrite.

As expected, the highest levels of nitrate were found in *Semimembranosus* because this is the first muscle in contact with the curing salts. Nevertheless it is important to note that a considerable amount of nitrate had reached the *B. femoris*, at 20 days of processing, in all the hams, with no significant differences (p > 95%) between formulations. This confirms that the correct diffusion and penetration of nitrate have occurred independent of the mixture of chloride salts employed.

Also the conversion of nitrate to nitrite at this time is already high in the inner *B. femoris*. The nitrite content at 20 days was highest in hams with formulations II and III and significantly different from that of formulation I (100% NaCl) and from that found in the *Semimembranosus* at this time (Fig. 2). The nitrite is maintained at 7.5 ± 0.5 ppm during the whole post salting period (up to 50–80 days) in the three formulations. This level of nitrite helps to