Meat Science 91 (2012) 306–311

Contents lists available at SciVerse ScienceDirect

Meat Science

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

Antihypertensive effect and antioxidant activity of peptide fractions extracted from Spanish dry-cured ham

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ARTICLE INFO

Article history:
Received 14 November 2011
Received in revised form 5 February 2012
Accepted 7 February 2012

Keywords:
Dry-cured ham
Antioxidant
Antihypertensive
Bioactive peptide

ABSTRACT

This study examined the antihypertensive and antioxidant activities of water soluble fractions of a Spanish dry-cured ham extract. Antihypertensive activity of a fractionated peptide extract, by size-exclusion chromatography was determined by measuring changes in systolic blood pressure of spontaneously hypertensive rats after oral administration. Every sample exhibited antihypertensive activity, pooled fractions corresponding to 1700 Da or lower were the most antihypertensive with a decrease of 38.38 mm Hg in systolic blood pressure. In vitro experiments revealed marked in vitro angiotensin I-converting enzyme inhibitory activity in fractions corresponding to these elution volumes. Some of the fractions exhibited great 1,1-diphenyl-2-picrylhydrazyl radical-scavenging activity, ranging from 39% to 92% as well as superoxide ion extinguishing ability with values ranging from 41.67% to 50.27% of the antioxidant activity, suggesting the presence of peptides with antioxidant activity. These findings suggest that Spanish dry-cured ham contains peptides with antioxidant and antihypertensive activities.

1. Introduction

Cured products produced in Spain, represent an important part of total processed meat products, the most important is dry-cured ham. Many biochemical changes take place during the processing of Spanish dry-cured ham, leading to the unique characteristics of the product. Intense proteolysis by endogenous muscle enzymes has been reported for sarcoplasmic and myofibrillar proteins (Bellatti, Dazzi, Chizzolini, Palmia, & Parolari, 1985; Toldrá, 1998; Toldrá & Flores, 1998; Toldrá, Rico, & Flores, 1993). The result is an accumulation of peptides of different sizes (mainly small peptides) (Mora, Sentandreu, Fraser, Toldrá, & Bramley, 2009; Mora et al., 2009; Sentandreu et al., 2003) and free amino acids at the end of dry-curing (Sforza et al., 2001; Toldrá, Aristoy, & Flores, 2000), which are of great importance for their direct or indirect contribution to flavor and final quality of the product.

Apart from taste or flavor characteristics, little is known about the effect that these peptides exert on human health. Peptides can regulate different processes in the organism having a positive impact on body functions that may ultimately influence health (Kitts & Weiler, 2003). Biologically active peptides have been isolated from many food products including milk, soy and egg protein (Pihlanto & Korhonen, 2003). Health effects attributed to food-derived peptides include antimicrobial properties, blood pressure lowering (ACE inhibitory) effect, cholesterol lowering ability, antithrombotic and antioxidative effects, enhancement of mineral absorption, immunomodulatory effects and opioid activities (Hartman & Meisel, 2007). These bioactive peptides are inactive within the parent protein but can be released during enzymatic hydrolysis of the proteins and exert biological activity (Korhonen, 2009).

The most studied peptides are angiotensin converting enzyme (ACE) inhibitory ones. ACE plays an important role in the regulation of blood pressure, since ACE converts an inactive angiotensin-I to angiotensin-II which is a potent vasoconstrictor thus leading to an increase in blood pressure. For this reason, substances inhibiting ACE activity should reduce the systolic blood pressure (Houston, 2002). ACE-inhibitory peptides derived from the hydrolysis of pork meat proteins (Arihara, Nakashima, Mukai, Ishikawa, & Itoh, 2001; Katayama et al., 2003) or derived from in vitro digestion of pork meat (Escudero, Sentandreu, Arihara, & Toldrá, 2010) have been reported. Also, ACE-inhibitory activities in extracts of dry-cured ham have been studied (Arihara & Ohata, 2008). Moreover, several dipeptides derived from the action of muscle dipeptidylpeptidases during the dry-curing of Spanish dry-cured ham have been found to inhibit ACE in some cases to over 90% of its activity (Sentandreu & Toldrá, 2007a). However, there is no information about in vivo antihypertensive properties of peptide extracts from dry-cured ham.

Antioxidant activity deficiency has also been involved in the occurrence of hypertension and other diseases such as cancer, diabetes, neurodegenerative disorders and aging (Ames, Shinegaga, & Hagen, 1993). Reactive oxygen species (ROS) such as superoxide anion radical (O_2^-), hydrogen peroxide and hydroxyl radicals (−OH) are physiological metabolites derived from respiration in aerobic organisms.

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doi:10.1016/j.meatsci.2012.02.008

Uncontrolled generated ROS are very unstable, and react rapidly with other substances including DNA, membrane lipids and proteins (Jae-Young, Pyo Pam, Eun-Kyung, & Chang-Bum, 2009), thus leading to the above diseases. Thus, research has investigated natural antioxidants in food that may protect the body against free radicals and retard many chronic diseases (Liu, Chen, & Lin, 2005).

Protein hydrolysates from different sources have been reported to possess antioxidant activity (Sakanaka, Tachibana, Ishihara, & Juneja, 2004; Torruco-Uco, Chel-Guerrero, Martinez-Ayala, Davila-Ortiz, & Betancur-Ancona, 2009) and many peptides with antioxidant activity generated from the hydrolysis of various proteins have been reported, such as sunflower (Ren, Zheng, Liu, & Liu, 2010), soy protein (Chen, Muramoto, & Yamauchi, 1995) and alfalfa leaf protein (Xie, Huang, Xu, & Jin, 2008). However, there is little information about antioxidant peptides generated in meat products including dry-cured ham.

In the present study Spanish dry-cured ham was studied as a natural source of antihypertensive peptides by measuring in vitro ACE inhibitory activity and changes in systolic blood pressure (SBP) in spontaneously hypertensive rats (SHR) after oral administration of peptide fractions extracted from dry-cured ham. The antioxidant activity, by DPPH radical-scavenging activity and superoxide ion extinguishing ability of the peptide fractions was also studied. Spanish dry-cured ham as a natural source of antihypertensive and antioxidant peptides is of interest because these peptides could help to counteract the adverse action of NaCl in this product, thus helping to maintain a satisfactory blood pressure and good health.

2. Materials and methods

2.1. Reagents

1,1-Diphenyl-2-picrylhydrazyl (DPPH) and xanthine oxidase from bovine milk were purchased from Sigma Chemical Co. (St. Louis, MO, USA). 2-Methyl-6-p-methoxy phenyl ethynyl-imidazopyridine (MPEC) was purchased from ATTO Corp. (Tokyo, Japan). Hypoxanthine was purchased from Wako Chem. Industries Ltd. (Osaka, Japan). Angiotensin-converting enzyme (from rabbit lung) was purchased from Sigma Chemical Co. (St. Louis, MO., U.S.A.). Abz-Gly-*p*-nitro-phe-pro-OH trifluoroacetate salt was obtained from Bachen AG. (Bubendorf, Switzerland). Other chemicals and reagents used were of analytical grade.

2.2. Spanish dry-cured ham preparation

Three Spanish dry-cured hams were produced using meat from 6-month old pig (Landrace × Large White). Hams were bled and prepared according to traditional procedures consisting pre-salting, for potassium nitrate incorporation, salting, where hams were completely covered with solid salt and placed in a cold room (1–3 °C and 80–90% relative humidity), post-salting, where the salted hams were kept at low temperatures (3–5 °C) and with relative humidities in the range of 75–85% for 60 days and finally, the ripening–drying at 14–20 °C and lower relative humidity (until 70%). The total length of the curing process was 11 months.

2.3. Extraction and deproteinization

50 g of *Biceps femoris* muscle from the processed Spanish dry-cured hams were minced and homogenized with 200 mL of 0.01 N HCl in a stomacher (IUL Instrument, Barcelona, Spain) for 8 min. The homogenate was centrifuged (12000 *g* for 20 min at 4 °C) and, after filtering through glass wool, the supernatant was deproteinized by adding 3 volumes of ethanol and maintaining the sample for 20 min at 4 °C. Then, the sample was centrifuged again (12000 *g* for 20 min at 4 °C) and the supernatant was dried in a rotary evaporator. The dried deproteinized extract was dissolved in 25 mL of 0.01 N HCl, filtered through a 0.45 μm nylon membrane filter (Millipore, Bedford, MA) and stored at −20 °C until use.

2.4. Size-exclusion chromatography

5 mL aliquots of each deproteinized extract from three different Spanish dry-cured ham extracts were subjected to size-exclusion chromatography to fractionate the peptides according to their molecular mass. For this purpose, a Sephadex G25 column (2.5 × 65 cm, Amersham Biosciences, Uppsala, Sweden), previously equilibrated with 0.01 N HCl, was employed. The separation was performed at 4 °C using 0.01 N HCl as eluent, at a flow rate of 15 mL/h. The first 195 mL was discarded, and then 5 mL fractions were collected using an automatic fraction collector and further monitored by ultraviolet (UV) absorption at 214 nm (Agilent 8453 UV spectrophotometer, Agilent Technologies, Palo Alto, CA). Fractions eluting from 200 mL to 450 mL (fractions 40 to 90) and fractions eluting from 505 to 625 mL (fractions 101 to 125) were collected and lyophilized. Then each fraction was redissolved in 2 mL distilled water and 0.5 mL of each fraction was separated and stored at −20 °C until use for ACE inhibitory activity and antioxidant activity assay.

For the antihypertensive in vivo assay, fractions corresponding to elution volumes from 200 mL to 320 mL, 1.5 mL each, were pooled (sample 1). The same procedure was followed for fractions corresponding to elution volumes from 325 mL to 450 mL (sample 2) and fractions corresponding to elution volumes from 505 mL to 625 mL (sample 3). Then, samples 1, 2 and 3 were submitted to solid phase extraction using an Oasis® HLB cartridge (35 cm^3, Waters, Ireland) in which the respective peptides were retained and then eluted with 50% methanol. The eluate was lyophilized and constituted the starting material for the antihypertensive activity assay.

2.5. Assay of ACE inhibitory activity

The ACE inhibitory activity of the collected fractions from three different ham samples was measured according to Sentandreu and Toldrá (2006). This assay is based on the ability of ACE to hydrolyze the internally quenched fluorescent substrate *o*-aminobenzoylglycyl-*p*-nitro-*L*-phenylalanyl-*L*-proline (Abz-Gly-Phe-(NO_2)-Pro). A sample solution (50 μL of each fraction) was mixed with 50 μL of 150 mM Tris-base buffer (pH 8.3) containing 3 mU/mL of ACE, then the mixture was preincubated for 10 min at 37 °C. The reaction was initiated by the addition of 200 μL of 150 mM Tris-base buffer (pH 8.3) containing 1.125 M NaCl and 10 mM Abz-Gly-Phe-(NO_2)-Pro, which was preincubated for 10 min at 37 °C. The reaction mixture was then incubated for 45 min at 37 °C. The generation of fluorescence due to the release of *o*-aminobenzoylglycine (Abz-Gly) by the action of ACE was measured using excitation and emission wavelengths of 355 and 405 nm, respectively. ACE inhibition of each collected fraction is expressed as a percentage.

2.6. Antihypertensive activity

2.6.1. Animal preparation

Male spontaneously hypertensive rats (SHR), 10 week old, were purchased from Charles River Japan Inc. (Yokohama, Japan). The SHR were housed in cages in a controlled environment (12 h light–dark cycle and temperature and humidity of 24 °C and 50 to 60%, respectively). The SHR were fed with a standard laboratory diet (CE-2; Clea Japan, Inc., Tokyo, Japan), and tap water was freely available.

2.6.2. Dosing of the animal

Single oral administration of the lyophilized samples 1, 2 and 3 dissolved in distilled water at a concentration of 0.834 mg/mL for sample 1, 0.27 mg/mL for sample 2 and 1.59 mg/mL for sample 3 was performed using SHR 15 to 28 week old (330 to 395 g body weight).