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PRKAG3 and *CAST* genetic polymorphisms and quality traits of dry-cured hams—III. Associations in Slovenian dry-cured ham *Kraški pršut* and their dependence on processing

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ABSTRACT

The aim of the present study, the third in a series of three papers, is to show the effects of *PRKAG3* and *CAST* gene polymorphisms on the quality traits of the Slovenian dry-cured ham "*Kraški pršut*" and their interaction with ham producers. Significant interaction of polymorphisms with producer in the case of salt content, lipid oxidation (*PRKAG3 Ile199Val*), proteolysis index (*CAST Arg249Lys*) and pastiness (*CAST Ser638Arg*) indicated that genotype manifestation was reliant on the manufacturing practice. *PRKAG3 Ile199Val* polymorphism affected several physico-chemical, rheological and sensory traits. The *Ile/Ile* genotype yielded less salty and softer hams, indicating beneficial effects on dry-cured ham quality. The effect of *CAST* polymorphisms was less pronounced, although the observed associations with pastiness, proteolysis index and several free amino acid concentrations indicate its possible influence on proteolysis, with haplotype *CAST 249Arg/638Ser* being associated with a higher degree of proteolysis.

1. Introduction

Dry-cured ham quality is dependent on raw material traits and dry-curing procedure. Duration of individual processing step, ambient conditions (temperature, air humidity and circulation) and additives (salt) influence the course of physical and chemical changes and determine end product quality (Toldrá, 2002). Green ham traits like ham fatness, weight, pH and water holding capacity are considered equally important (Virgili & Schiwazappa, 2002) and depend on numerous pig production related factors (see Čandek-Potokar & Škrlep, 2012; Russo & Nanni Costa, 1995) including genetic ones. Recent studies on dry-cured ham, conducted within the EC 6th Framework Programme project TRUEFOOD that were simultaneously performed in France, Slovenia and Spain, highlighted some important associations of *PRKAG3* and *CAST* gene polymorphisms with green ham traits (Škrlep et al., 2010) and seasoning losses (Škrlep, Čandek-Potokar, Santé-Lhoutellier, & Gou, 2011). The effect on the quality of fresh hams differed according to the sample of pigs selected for ham production in each country, indicating a possible interaction with other genetic or environmental factors. Moreover, there are differences in dry-curing and drying procedures between countries, which could modify the manifestation of gene polymorphisms. Therefore, the final step of this joint research was to test the effect of these genetic polymorphisms on dry-cured ham properties in each country. The aim of this study, which is the third in a series of three papers, was to test the effect of three genetic polymorphisms (*PRKAG3 Ile199Val*, *CAST Arg249Lys*, and *CAST Ser638Arg*) on the quality traits of Slovenian dry-cured ham *Kraški pršut*, including chemical composition, rheological and sensory traits and taking into consideration two different producers.

2. Materials and methods

2.1. Ham processing, genotype determination and sample selection

Dry-cured hams were obtained from a pool of hams ($n=724$) that were genotyped for *PRKAG3 Ile199Val* polymorphism according to Ciobanu et al. (2001) and for both *CAST* polymorphisms (i.e. *Ser638Arg* and *Arg249Lys*) according to Ciobanu et al. (2004). The hams were processed by two dry-cured ham producers, both following the rules of the *Consortium* for dry ham *Kraški pršut*. Duration of processing was similar for both producers, however they differed in regard to the duration of salting, drying and ripening phases and rate of

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seasoning losses (for details see Čandek-Potokar & Škrlep, 2011; Škrlep, Čandek-Potokar, Santé-Lhoutellier, et al., 2011). From the pool of processed hams a subsample was selected using SURVEYSELECT procedure of SAS (SAS Institute Inc., Cary, NC) taking into account processing batch and polymorphisms *PRKAG3 Ile199Val, CASTLys 249Arg* and *CAST Ser638Arg*. The aim was to obtain a minimum of 50 samples per homozygous genotype, which yielded a final number of 135 hams (corresponding to 135 different animals). For the planned analyses (rheological measurements, chemical and sensory analyses), three 3 cm thick pieces from the central part of the dry-cured hams, containing *biceps femoris* (BF) and *semimembranosus* (SM) muscles, were taken (Fig. 1), vacuum packed and stored at −20 °C until analyses. Additionally (prior to packing and freezing) CIE (commission international de l'eclairage) L^*, a^* and b^* colour parameters were measured on SM and BF cut between samples B and C (Fig. 1) using a Minolta Chroma Meter CR-300 (Minolta Co. Ltd, Osaka, Japan) with 11 mm diameter aperture and D_{65} illuminant. Measurements were taken in five repetitions along each muscle and the mean value was used for statistical analysis.

2.2. Rheological measurements

For instrumental texture measurement, two 15 mm thick slices were cut from the ham sample (denoted as sample A in Fig. 1) and 12 subsamples from SM and BF muscles (six from each muscle, Fig. 2) were accurately cut with a scalpel into parallelepipeds (dimensions of 20 mm × 20 mm × 15 mm for length, width and height, respectively), covered with plastic film to avoid drying and stored at 4 °C for two hours before the analysis. Stress Relaxation (SR) and Texture Profile Analysis (TPA) tests were performed (according to Morales, Guerrero, Serra, & Gou, 2007), each on three samples per muscle, using Texture Analyser TA.XTPlus (Stable Micro Systems Ltd., Surrey, UK) with a 50 kg load cell and a 50 mm diameter compression plate. Average of the three measurements was used for statistical analysis.

2.3. Sensory analysis

Sensory evaluation was carried out by a panel of six trained members. Samples were distributed randomly to different session days according to processing batch and genotype by the use of SURVEYSELECT procedure of SAS (SAS Inc., Cary, NC, USA). Five or six dry ham samples were evaluated per session, the evaluations were made separately for BF and SM. Each panel member received two ham slices (1.5 mm thick) and evaluated the following sensory traits (one- to seven-point scale with increasing intensity of sensation):

- *Marbling*: Quantity of fat infiltrations within the muscle.
- *Adhesiveness*: Degree to which the surface of the muscle sample adheres to the palate when compressing it with the tongue.

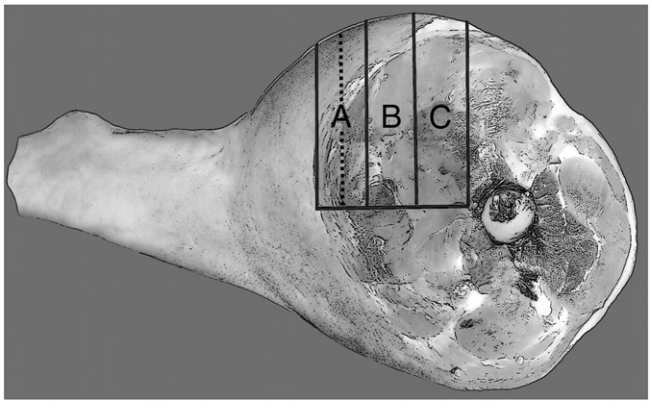

Fig. 1. Dry-cured ham medial view with sampling sites for rheological (A), sensory (B) and chemical (C) analyses.

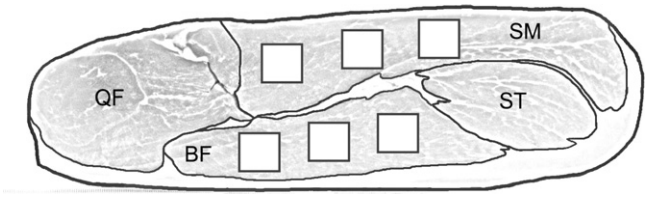

Fig. 2. Cross-section of the dry-cured ham indicating four visible muscles and the position of sampling for rheological analyses (SM — *m. semimembranosus*, ST — *m. semitendinosus*, BF — *m. biceps femoris* and QF — *m. quadriceps femoris*; places of sampling are indicated with blank squares).

- *Hardness*: Initial force necessary to compress muscle sample between molars.
- *Pastiness*: Mouth coating sensation produced by flour-water paste during mastication.
- *Crumbliness*: Ease with which the sample can be separated into smaller particles.
- *Dryness*: The intensity of saliva absorption during mastication.
- *Smell*: Typical smell of mature meat with no off smells i.e. rancid, fungi.
- *Taste*: Typical taste of mature meat, no off tastes (i.e. sour, bitter off tastes).
- *Saltiness*: Basic taste perceived due to salt.

For each descriptor, the average score of the six panellists was used in statistical analysis.

2.4. Chemical analysis

Samples were trimmed of connective and superficial fat tissue. SM and BF were separated and cut in small pieces, ground in liquid nitrogen using a laboratory mill (IKA M120, IKA Werke, Staufen, Germany) and stored in plastic tubes at −20 °C until further use. Moisture, sodium chloride, total nitrogen, non-protein nitrogen (NPN), intramuscular fat (IMF) contents and lipid oxidation (TBARS) were determined. For determination of moisture content, 5 g of the sample was mixed with equal amount of quartz sand and dried at 103 °C to a constant mass. The loss of mass was recorded and expressed as a percentage of moisture in the sample. For sodium chloride content,1 g of sample was mixed with 80 ml distilled water and boiled at 100 °C for one hour. After cooling, 2 ml of 15% potassium ferrocyanide and 2 ml of 30% zinc acetate was added for deproteinisation and diluted with distilled water to 100 ml. After filtration, the NaCl content was determined by potentiometric titration using DL53 General Purpose Titrator (Mettler Toledo, Schwarzenbach, Switzerland). Total nitrogen content was determined according to ISO 5983-2 international standard (ISO 5983–2, 2005) using the Kjeltec 2300 nitrogen analyser (Foss Analytical, Hileroed, Denmark). For determination of non-protein nitrogen (NPN), 2.5 g of sample was homogenised in 25 ml of distilled water and centrifuged. Afterwards, 10 ml of 20% trichloroacetic acid was added, stirred well and left to stabilise for 60 min at room temperature. After centrifugation, the supernatant was filtered and 15 ml used for determination of nitrogen (ISO 5983–2, 2005). Index of proteolysis (NPN%) was calculated as a ratio between NPN and total nitrogen content. Intramuscular fat content was determined according to ISO 1443 international standard (ISO 1443, 1973) using Büchi Extraction System B-811 (Büchi Labortechnik AG, Flawil, Switzerland), expressed as a percentage of fat in the sample. The extent of lipid oxidation was estimated by TBARS (thiobarbituric acid reactive substances) assay (mg of malondialdehyde per kg of dry-cured muscle samples), according to Shahidi, Rubin, Diosady, and Wood (1985).