



# Classification of dry-cured hams according to the maturation time using near infrared spectra and artificial neural networks

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## ABSTRACT

An attempt to classify dry-cured hams according to the maturation time on the basis of near infrared (NIR) spectra was studied. The study comprised 128 samples of *biceps femoris* (BF) muscle from dry-cured hams matured for 10 ( $n = 32$ ), 12 ( $n = 32$ ), 14 ( $n = 32$ ) or 16 months ( $n = 32$ ). Samples were minced and scanned in the wavelength range from 400 to 2500 nm using spectrometer NIR System model 6500 (Silver Spring, MD, USA). Spectral data were used for i) splitting of samples into the training and test set using 2D Kohonen artificial neural networks (ANN) and for ii) construction of classification models using counter-propagation ANN (CP-ANN). Different models were tested, and the one selected was based on the lowest percentage of misclassified test samples (external validation). Overall correctness of the classification was 79.7%, which demonstrates practical relevance of using NIR spectroscopy and ANN for dry-cured ham processing control.

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## 1. Introduction

Dry-cured ham “*Kraški pršut*” is a traditional Slovenian meat product protected with EU designation of geographical indication (Commission implementing regulation, 2012). This protection implies that certain *consortium* rules should be respected in regard to raw material, processing losses, chemical and sensory properties. Processing of dry-cured ham “*Kraški pršut*” consists of dry salting, absence of smoking and long maturation period. Regarding the latter, the *consortium* rules require a minimum maturation period of 12 months. During seasoning, proteins and lipids undergo intense proteolysis and lipolysis processes and these changes affect the flavour and texture of dry-cured ham, resulting in sensorial quality appreciated by consumers. Chemical and sensory changes occurring through the process are strongly dependent on the duration of the ripening process which has been demonstrated in different dry-cured meat products (Benedini, Parolari, Toscani, & Virgili, 2012; Buscailhon & Monin, 1994; Toldra & Flores, 1998 and Virgili, Saccani, Gabba, Tanzi, & Soresi Bordini, 2007). From the practical point of view it would be interesting to develop cheap and rapid method capable of detecting ham ripening stage. In the case of “*Kraški pršut*” such approach would be useful for verification purposes (e.g. to detect

if the requirement of 12 months of maturation was respected) or to recognize longer maturation associated with higher quality label. Near infrared (NIR) spectroscopy is one of the techniques which have the potential for such purposes. Its usefulness has already been proven for the prediction of chemical and physical characteristics of meat and meat products and for various classification purposes (for review see Prevolnik, Čandek-Potokar, & Škorjanc, 2004 and Prieto, Roehe, Lavín, Batten, & Andrés, 2009). NIR spectral information demands multivariate data analysis due to its complexity (Pérez-Marín, Garrido-Varo, & Guerrero, 2007). Artificial intelligent methods are often applied for the classification since their primary target is to distinguish objects or groups or populations. Their advantages are in ability to handle with non-linear data, highly correlated variables and potential for identification of problems or classification (Cartwright, 2008). Artificial neural networks (ANN) were lately tested for many problems in meat production and technology such as carcass classification, quality control of raw material, meat processing, meat spoilage or freshness and shelf-life evaluation, detecting off-flavours, authenticity assessment, etc. (for review see Prevolnik, Škorjanc, Čandek-Potokar, & Novič, 2011). To our knowledge there is no literature data on the prediction of maturation time in any of dry-cured meat products based on NIR spectral information. Therefore the present study aimed to test if dry-cured hams can be classified into different maturation classes (10, 12, 14 and 16 months) on the basis of spectral information by means of ANN.

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## 2. Materials and methods

### 2.1. Ham processing and sampling

The study comprised 128 dry-cured hams taken from regular production in three commercial ham processing facilities (members of consortium for Kraški pršut). The production of hams was carried out respecting the rules of *consortium* for Kraški pršut. In short, the hams from commercial crossbred pigs were trimmed into a prescribed shape and put to salting for 2–3 weeks at 2–4 °C. According to the rules only sea salt is allowed as a conservation additive. After the salting, the hams were washed and left to rest in controlled atmosphere (at 4–6 °C and 70–85% relative humidity) for 10 weeks. Following the resting period the hams were dried (14–20 °C and 60–80% RH) until the required weight loss had been attained. Thereafter the open surface of the hams was coated with a mixture of pork leaf fat, rice flower and spices (to permit ripening while preventing further desiccation) and left to ripen. Hams were sampled after 10 ( $n = 32$ ), 12 ( $n = 32$ ), 14 ( $n = 32$ ) and 16 months ( $n = 32$ ) of seasoning in all three dry-cured ham processing facilities. Thereafter the hams were boned and sampled from the central part of the dry-cured hams containing *biceps femoris* and *semimembranosus* muscles according to Škrlep et al. (2012).

### 2.2. NIR spectra acquisition

*Biceps femoris* muscle samples (2 cm thick slice) were trimmed of superficial fat tissue, cut in small pieces, frozen in liquid nitrogen and grinded to fine dust using a laboratory mill (IKA M120, IKA Werke, Staufen, Germany). Homogenized ham samples (app. 50 g) were put in rectangular quartz cup ( $47 \times 57 \text{ mm}^2$ ) about 3 mm thick, covered by paper disc and placed directly in NIRS apparatus NIR System model 6500 (Silver Spring, MD, USA). For each sample one scanning was performed in a wavelength range from 400 to 2500 nm. Absorbance data were collected every 2 nm as  $\log 1/R$ , where  $R$  represents the reflectance.

### 2.3. Dry-cured ham chemical composition

Several chemical constituents (moisture, intramuscular fat, protein, non-protein nitrogen, salt content, the percentage of salt per dry mater and proteolysis index) of dry-cured ham muscle *biceps femoris* were determined by means of internal NIR spectroscopy calibration models published in Prevornik, Škrlep, Janeš, Velikonja-Bolta, Škorjanc & Čandek-Potokar (2011). Data are presented in Table 1.

### 2.4. Chemometric analysis

Chemometric analysis was performed using ANN software developed at the National Institute for Chemistry (Ljubljana, Slovenia), written in FORTRAN for IBM-compatible PCs and a Windows operating system. In the present study, unsupervised Kohonen ANN and supervised counter-propagation (CP) ANN were applied. Although both types of ANN are comprehensively described in the literature (Dayhof, 1990; Hecht-Nielsen, 1987; Zupan, 1994 and Zupan, Novič, & Ruisanchez, 1997), a short explanation of them is given in the next paragraphs.

In the case of unsupervised learning strategy, only the description of objects are needed, i.e. the independent variables for the input vectors. The properties are not given, so the map obtained shows only the relationship between the independent variables of the objects, regardless of their property that may be known, but is not represented in object vectors. The main goal of Kohonen ANN is to project or map objects from  $m$ -dimensional into 2-dimensional space on the basis of input data (similarity among objects). Thus Kohonen ANN is most frequently applied for visualization and clustering purposes (Zupan, 1994).

**Table 1**

Chemical composition of dry-cured hams (means and standard deviations).

Constituent <sup>a</sup>	Maturation time (months)			
	10	12	14	16
<sup>1</sup> Moisture, g/kg	592 ± 16.1	567 ± 21.8	582 ± 15.1	564 ± 22.8
<sup>2</sup> Salt, g/kg	70.3 ± 10.38	76.7 ± 10.18	77.0 ± 8.30	78.1 ± 8.38
<sup>3</sup> Salt per dry mater, %	17.2 ± 2.03	17.7 ± 2.11	18.4 ± 1.67	17.9 ± 1.60
<sup>4</sup> Protein, g/kg	27.8 ± 1.07	29.5 ± 1.64	29.6 ± 1.07	30.1 ± 1.58
<sup>5</sup> Non-protein nitrogen, g/kg	11.6 ± 0.51	11.8 ± 1.50	12.7 ± 1.00	12.6 ± 1.45
<sup>6</sup> Proteolysis index, %	27.1 ± 1.05	26.1 ± 3.14	27.8 ± 1.75	27.2 ± 2.55
<sup>7</sup> Intramuscular fat, g/kg	42 ± 7.3	43 ± 20.3	30 ± 9.0	40 ± 13.7

<sup>a</sup> Assessed with NIR spectroscopy using internal calibration models (Prevornik, Škrlep, et al., 2011) with the following chemometric parameters:

<sup>1</sup>  $n = 131$ ,  $se_c = 4.5$ ,  $R^2_c = 0.89$ ,  $se_{cv} = 5.0$ ,  $R^2_{cv} = 0.86$

<sup>2</sup>  $n = 130$ ,  $se_c = 1.23$ ,  $R^2_c = 0.97$ ,  $se_{cv} = 1.44$ ,  $R^2_{cv} = 0.96$

<sup>3</sup>  $n = 129$ ,  $se_c = 0.42$ ,  $R^2_c = 0.92$ ,  $se_{cv} = 0.46$ ,  $R^2_{cv} = 0.90$

<sup>4</sup>  $n = 131$ ,  $se_c = 0.49$ ,  $R^2_c = 0.81$ ,  $se_{cv} = 0.55$ ,  $R^2_{cv} = 0.77$

<sup>5</sup>  $n = 131$ ,  $se_c = 0.28$ ,  $R^2_c = 0.88$ ,  $se_{cv} = 0.36$ ,  $R^2_{cv} = 0.80$

<sup>6</sup>  $n = 130$ ,  $se_c = 0.67$ ,  $R^2_c = 0.78$ ,  $se_{cv} = 0.83$ ,  $R^2_{cv} = 0.67$

<sup>7</sup>  $n = 128$ ,  $se_c = 3.00$ ,  $R^2_c = 0.88$ ,  $se_{cv} = 3.16$ ,  $R^2_{cv} = 0.87$ .

The CP-ANN is based on two-steps learning procedure. The first step corresponds to the mapping of objects in the input layer (also called Kohonen layer) and is identical to the Kohonen learning procedure. The second step of the learning is supervised, which means that for the learning procedure the response or target value is required for each input. This input-target pairs are the input to the neural network, which is after being trained for certain amounts of epochs, capable of the prediction of the unknown samples. Every object excites one single neuron. The algorithm modifies the weight of the neuron with the weights most similar to the input signal and smoothes the map by making modulated changes to neurons in a defined “neighbourhood” of that one. These corrections of weights are made around the neuron position in the Kohonen and output layer (Zupan, 1994).

### 2.5. Selection of sample sets

Composition of the training and the test sets should guarantee that these sets are scattered over the similar descriptor spaces and the training set is a representative set of the whole data set and Kohonen ANN are able to select a representative training set and a test set similar to it (Gramatica, Pilutti, & Papa, 2004).

For chemometric analysis, absorbance data collected every 2 nm ( $n = 1032$ ) were compressed into 262 data points by averaging four sequential original measurements. Each sample is represented as a combination of 262 input variables or descriptors. For this unsupervised step only input variables are needed. The distribution of samples ( $n = 128$ ) in the top map of the Kohonen ANN was used to divide samples into the training and test set. Splitting of the samples was performed for each of four maturity classes separately (10, 12, 14 and 16 months). Samples of certain maturity class ( $n = 32$ ) were trained using  $4 \times 4$  Kohonen net for 100 epochs. After the training, each sample obtained the location in the Kohonen map, i.e. in a rectangular grid of neurons. Among the samples sited on the same neuron, the one with the shortest distance to the neuron was selected for the training set, while the remaining sample(s) was (were) placed in the test set. For each maturity class 16 samples were selected for training set and the others were placed into the test set. The final training set ( $n = 64$ ) was used for the development of calibration models and test set ( $n = 64$ ) for independent external validation of models.

### 2.6. Development and validation of models

In the present study, the CP-ANN was employed as a classification model. Different models were prepared varying the net size, i.e. the

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