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# Chemical composition of dry ham "*Kraški pršut*" predicted by NIR spectroscopy

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**Abstract.** Dry ham "*Kraški pršut*" is a Slovenian traditional meat product with protected geographical designation. This protection implies that certain *consortium* constrains and control of dry-cured ham quality should be respected. For regular checks NIR spectroscopy offers an interesting alternative to conventional chemical methods. The aim of the present study was to test the ability of NIR spectroscopy to predict several chemical constituents of "*Kraški pršut*". Proximate analysis (moisture, salt, protein, non-protein nitrogen, intramuscular fat, free amino acids) was performed in muscles *biceps femoris* (n=135) and *semimembranosus* (n=135) of the final product. The quality of predictive models was assessed on the basis of the coefficients of determination ( $R^2_{CV}$ ) of cross-validation and residual predictive deviation (RPD, i.e. the ratio between standard deviation of the reference data and standard error ( $SE_{CV}$ ) of cross-validation). Highly reliable prediction results were obtained for moisture, protein, salt content and the percentage of salt per dry matter ( $R^2_{CV} > 0.90$ , RPD  $> 3.0$ ). For intramuscular fat, free amino acids content and non-protein nitrogen reasonable calibration models were obtained ( $R^2_{CV}$  between 0.62 and 0.87, RPD between 1.6 and 2.8). Due to good prediction ability and the simplicity of measurement NIR spectroscopy offers good opportunity to replace time-consuming, expensive and/or hazardous laboratory methods.

**Keywords.** NIR spectroscopy – Ham – Chemical composition.

## La composition chimique du jambon sec "*Kraški pršut*" prédite par la spectroscopie NIR

**Résumé.** "*Kraški pršut*" est un jambon sec traditionnel slovène protégé par l'indication géographique. Cette protection implique la certification du produit et le respect de contraintes imposées par le consortium. Pour des contrôles réguliers la spectroscopie NIR offre une alternative intéressante pour remplacer les méthodes chimiques classiques. L'objectif de la présente étude était de tester la capacité de la spectroscopie NIR pour prédire plusieurs constituants chimiques du jambon sec "*Kraški pršut*". L'analyse chimique (humidité, sel, protéines, azote non protéique, gras intramusculaire, acides aminés libres) a été réalisée sur deux muscles du produit final, notamment le Biceps femoris (n = 135) et le semi-membraneux (n = 135). La qualité des modèles de calibrage a été évaluée sur la base de coefficients de détermination de la validation croisée ( $R^2_{CV}$ ) et du coefficient RPD, à savoir le rapport entre l'écart-type des données de référence et l'erreur de la validation croisée ( $SE_{CV}$ ). D'excellents résultats ont été obtenus pour la prédiction de l'humidité, des protéines, de la teneur en sel et du pourcentage de sel dans la matière sèche ( $R^2_{CV} > 0,90$ , RPD  $> 3,0$ ). La précision de la prédiction du gras intramusculaire, de la teneur en acides aminés libres et de l'azote non protéique ( $R^2_{CV}$  entre 0,62 et 0,87, RPD entre 1,6 et 2,8) était un peu inférieure mais acceptable. En raison d'une bonne précision de la prédiction et de la simplicité des mesures, la spectroscopie NIR pourrait remplacer les méthodes chimiques plus laborieuses pour le contrôle régulier du produit.

**Mots-clés.** Spectroscopie NIR – Jambon sec – Composition chimique.

## I – Introduction

"*Kraški pršut*" is a traditional Slovenian dry-cured ham of the carstic region (called Kras in Slovenian language) which belongs to the family of Mediterranean type of dry-cured ham, characterised by dry salting, absence of smoking and long maturation. The product is economically important and highly appreciated among Slovenian consumers (Čandek-Potokar *et al.*, 2004). On the national level "*Kraški pršut*" is protected by geographical designation and

certified, implying that certain *consortium* constraints should be respected in regard to green ham properties, processing losses, chemical and sensory properties of dry-cured hams.

For regular checks near infrared (NIR) spectroscopy offers an interesting alternative over the conventional methods. Since the method enables fast and simple determination of many parameters simultaneously, it could effectively replace lengthy and expensive analyses which are less suitable to be used on a large scale and in particular for assays that are healthy and environmentally damaging (e.g. determination of intramuscular fat content based on hazardous organic solvent extraction). High potential of NIR spectroscopy for the prediction of chemical composition and the quality of raw meat was demonstrated in many previously published studies (for review see Prevolnik *et al.*, 2004; Prieto *et al.*, 2009). The literature reports relating to the application of NIR spectroscopy for the analyses of meat products are not abundant; the majority of them demonstrate good prediction ability (for review see Prieto *et al.*, 2009). The lack of relevant studies is in particular evident in case of dry-cured ham. The few published studies on dry-cured ham were focused mainly on the prediction of sensory characteristics of dry-cured ham (Cruz Ortiz *et al.*, 2006; García-Rey *et al.*, 2005).

Due to the usefulness of NIR spectroscopy for mass analyses in the quality control of dry-cured ham the aim of the present study was to test the ability of the method to predict several chemical constituents of “*Kraški pršut*” dry-cured ham as this would be of high importance for the practice *i.e.* for regular checks in the production of certified product.

## II – Materials and methods

### 1. Sampling and chemical analysis

The research was carried out on 135 dry-cured hams which were processed according to the rules of *consortium* for “*Kraški pršut*”. The analysis is based on pooled data for two muscles, *biceps femoris* and *semimembranosus*. Prior to the chemical analyses, samples were trimmed of superficial fat tissue, cut in small pieces, frozen in liquid nitrogen, grinded to fine dust using a laboratory mill (IKA M120, IKA Werke, Staufen, Germany) and stored in plastic tubes at -20°C until further use. Chemical determinations (moisture, protein, intramuscular fat, non-protein nitrogen, salt and free amino acid content) were carried out in replicates.

For the determination of moisture content (ISO 6496, 1999), 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 (salt) content determination (Monin *et al.*, 1997), 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 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). Additionally, salt content was expressed as the percentage of moisture or dry matter.

Protein content was calculated from total nitrogen content (ISO 5983-2, 2005) using the Kjeltec 2300 nitrogen analyser (Foss Analytical, Hileroed, Denmark). The organic matter in the samples was degraded by heating with concentrated sulphuric(VI) acid in the presence of catalysts. After the addition of base (NaOH) the resulting ammonia gas was dissolved in boric acid solution and titrated with hydrochloric acid. The total nitrogen content was calculated from the amount of the hydrochloric acid used for the titration. To obtain total protein content total nitrogen content was multiplied with 6.25.

For determination of non-protein nitrogen, 2.5 g of sample was homogenised in 25 ml of distilled water and centrifuged (Monin *et al.*, 1997). Afterwards, 10 ml of 20% trichloroacetic acid was added, stirred well and let to stabilise for 60 min at room temperature. After the centrifugation,

the supernatant was filtered and 15 ml of it used for determination of nitrogen in the same way as described for total nitrogen (ISO 5983-2, 2005). Additionally, non-protein nitrogen was expressed as a percentage of total nitrogen (proteolysis index).

Intramuscular fat content (ISO 1443, 2001) was determined using Büchi Extraction System B-811 (Büchi Labortechnik AG, Flawil, Switzerland). The samples were boiled with dilute hydrochloric acid to free the occluded and bound lipid fractions; the resulting mass was filtered and dried, the fat retained on the filter was extracted with light petroleum. The resulting fat was expressed as a percentage of fat in the sample.

The content of free amino acids was determined according to ISO 13903 (2005) adapted for dry-cured ham (internal laboratory protocol). Free amino acids were extracted with dilute hydrochloric acid. Co-extracted nitrogenous non-amino acid macromolecules were precipitated by adding sulfosalicylic acid and removed by filtration. The pH of the filtered solution was adjusted to 2.20. FAA were separated by ion exchange chromatography and determined by reaction with ninhydrin with photometric detection at 440 nm (for proline) and 570 nm (for other free amino acids) using Agilent 1200 series HPLC apparatus (Agilent technologies, Waldbronn, Germany) equipped with sodium cation exchange column 8  $\mu$ m, 3.0×250 mm (Pickering Laboratories, Mountain View, CA, USA), Pinnacle PCX post column derivatization instrument (Pickering Laboratories, Mountain View, CA, USA) and Agilent 1200 series Diode array and multiple wavelength detector (Agilent technologies, Waldbronn, Germany).

## 2. NIR spectra acquisition and spectral data analysis

Minced samples of analysed muscles were separately put in rectangular quartz cup (47×57 mm<sup>2</sup>) about 3 mm thick, covered by paper disc and placed directly in NIRS apparatus. For each sample one scanning was performed. The samples were scanned with spectrophotometer NIR System model 6500 (Silver Spring, MD, USA) in a wavelength range from 400 to 2500 nm. Absorbance data were collected every 2 nm as  $\log 1/R$ , where  $R$  represents reflectance.

Spectral data processing was performed using WinISI II software. Calibration models were developed using modified partial least squares regression with internal cross-validation. Samples for which the difference between actual and predicted values exceeded 3 standard deviations (SD) were considered as outliers.

The mathematical treatment applied was 1 4 4 1, where the first number indicates the order of the derivative (1 is the first derivative of the  $\log 1/R$ ), the second number is the gap in nm over which the derivative is calculated, the third and fourth number refer to the first and the second smoothing. The "SNV and Detrend" option was used to correct scatter effects in the spectra. Within development of calibration models we tested spectral range (visible spectrum ranging from 400 to 1100 nm, NIR spectrum ranging from 1100 to 2500 nm and the whole spectrum ranging from 400 to 2500 nm). The number of PLS factors was limited to 16, but the actual number of PLS factors was defined separately for every single calibration model respecting the fall of cross-validation errors. The quality of calibration models was assessed through standard error of calibration ( $SE_C$ ), coefficient of determination in calibration ( $R_C$ ), standard error of cross-validation ( $SE_{CV}$ ) and coefficient of determination in cross-validation ( $R_{CV}$ ). Models' performance was additionally evaluated using residual predictive deviation (RPD) which was calculated as the ratio between the SD of reference data and  $SE_{CV}$ .

### III – Results and discussion

#### 1. Material

Basic statistics of analysed chemical constituents in *biceps femoris* and *semimebranosus* muscles is presented in Table 1. Using two muscles we obtained broad range of variability which is of great importance for the development of calibration models.

#### 2. Prediction of chemical composition

In the present study the same sample set was used to develop calibration models and to validate models. Namely, our previous studies (Čandek-Potokar *et al.*, 2006; Prevolnik *et al.*, 2009) showed that external validation (prediction) on the independent set of samples yielded comparable results as the cross-validation. In the present study prediction results are presented as statistical parameters of calibration ( $R_C$  and  $SE_C$ ) and cross-validation ( $R_{CV}$  and  $SE_{CV}$ ). The parameter RPD (the ratio  $SD/SE_{CV}$ ) was also applied as an indicator of models' quality. Namely, RPD evaluates the prediction errors in view of the SD of the reference data and should be over three for accurate predictions (Andrés *et al.*, 2008; Kennedy *et al.*, 1996). Lower RPD values can be attributed either to a narrow variation range of the reference values (giving small SD) or to large NIR prediction error compared to SD of the reference values. Moreover, RPD enables to compare models' quality for the constituents/traits with different variation range where the prediction errors cannot be directly compared.

**Table 1. Basic statistics for chemical constituents of dry ham (two muscles)**

	Mean±SD	Range
Moisture, g/kg	537±51.4	435–652
Salt content, g/kg	71.2±8.19	44.8–94.1
Salt per DM, %	15.7±2.96	8.8–22.2
Salt per moisture, %	13.3±1.49	9.1–17.4
Protein, g/kg	34.2±5.08	26.0–43.9
NPN, g/kg	12.2±1.10	7.0–14.7
Proteolysis index, %	22.8±4.32	13.3–31.1
IMF, g/kg	38±14.0	16–88
FAA, mg/100g DM	7140±895	5237–9507

DM – dry matter; SD – standard deviation; IMF – intramuscular fat;  
NPN – non-protein nitrogen; FAA – free amino acids.

As regards the prediction ability of NIR spectroscopy (Table 2), the results show, that there was a negligible difference in the prediction of chemical constituents based on NIR or the whole spectral range. Visible spectrum was also tested, but the results are not presented as the models showed considerably lower prediction accuracy. On the whole,  $R_{CV}$  for different chemical constituents ranged from 0.65 to 0.96 and RPD from 1.7 to 5.0. Highly reliable results were obtained for salt content and the percentage of salt per moisture or dry matter for which the  $R_{CV}$  were over 0.90 and RPD exceeded three which is indicative of highly reliable predictive models. Satisfactory results were obtained also for moisture, non-protein nitrogen and intramuscular fat content ( $R_{CV}$ =0.80–0.90, RPD=2.2–2.8), while for other constituents (protein, proteolysis index and free amino acids content) moderate results were obtained ( $R_{CV}$ =0.65–0.80, RPD=1.7–2.0).

Good calibration and cross-validation results in NIR (and consequently in the whole) spectral range can be explained by high correlations (up to  $\pm 0.80$ ) between assessed chemical constituents and the absorbance data (data not shown) in wavelength range from 1100 to 2400 nm. In the dry-cured ham water represents 43-65% of the total fresh matter. Absorbance peaks of O-H bonds at 1450 and 1940 nm (Shenk *et al.* 1992) explain satisfactory NIR predictability for water content. Good performance of NIR spectroscopy to predict fat content is due to the strong absorption of C-H bonds in the NIR region at 1000 to 1400, 1700 and 2200-2400 nm (Shenk *et al.* 1992). Regarding protein, specific absorbance of N-H bonds could be found in the NIR region from 1460 to 1570 nm and from 2000 to 2180 nm (Shenk *et al.* 1992). In the case of proteins, it should be mentioned that protein content was calculated on the assumption that all nitrogen in the sample appears in protein, although a part (27%) of nitrogen is in form of non-protein nitrogen. High correlation coefficients between average spectrum and salt content led to good prediction results although NIR spectroscopy is known to be unable to detect inorganic substances unless they are bound to organic substance (Van Kempen, 2001). It is likely that in dry-cured ham salt content is indirectly predicted from other compounds (e.g. correlation coefficient between salt and water content amounts to 0.53). Moreover, NaCl itself shows no absorbance in the NIR region, but the presence of dissolved salts gives rise to the wavelength shifts in the spectrum. This phenomenon has been used to assess the content of NaCl in meat products (Downey and Hildrum, 2004).

**Table 2. Prediction of chemical composition using NIR spectroscopy in two dry ham muscles (*biceps femoris* and *semimembranosus*)**

Constituent	Spectrum nm	n	Mean $\pm$ SD	emin	emax	SE <sub>c</sub>	R <sub>c</sub>	SE <sub>cv</sub>	R <sub>cv</sub>	RPD	PLS
Moisture, g/kg	400-2500	262	538.7 $\pm$ 51.6	383.9	693.6	15.8	0.91	16.3	0.90	3.17	4
	1100-2500	258	539.7 $\pm$ 51.3	385.9	693.6	15.6	0.91	16.1	0.90	3.18	4
Salt, g/kg	400-2500	254	71.69 $\pm$ 7.68	48.66	94.72	2.45	0.90	2.55	0.89	3.01	5
	1100-2500	253	71.70 $\pm$ 7.69	48.63	94.77	2.28	0.91	2.36	0.91	3.25	5
Salt per DM, %	400-2500	257	15.81 $\pm$ 2.88	7.17	24.44	0.85	0.91	0.91	0.90	3.15	5
	1100-2500	254	15.85 $\pm$ 2.89	7.17	24.53	0.86	0.91	0.91	0.90	3.16	5
Salt per moisture, %	400-2500	254	13.33 $\pm$ 1.41	9.10	17.56	0.46	0.89	0.50	0.87	2.80	6
	1100-2500	255	13.33 $\pm$ 1.40	9.12	17.55	0.50	0.87	0.52	0.86	2.69	5
Protein, g/kg	400-2500	264	33.96 $\pm$ 5.05	18.81	49.12	1.42	0.92	1.57	0.90	3.22	7
	1100-2500	262	33.90 $\pm$ 5.01	18.86	48.94	1.46	0.91	1.58	0.90	3.18	6
NPN, g/kg	400-2500	260	12.25 $\pm$ 0.99	9.29	15.21	0.53	0.71	0.57	0.66	1.72	6
	1100-2500	258	12.25 $\pm$ 0.97	9.34	15.17	0.57	0.65	0.59	0.64	1.65	5
Proteolysis index	400-2500	262	22.91 $\pm$ 4.26	10.12	35.69	1.57	0.86	1.69	0.84	2.52	6
	1100-2500	262	22.92 $\pm$ 4.25	10.17	35.66	1.55	0.87	1.70	0.84	2.50	8
IMF, g/kg	400-2500	259	36.6 $\pm$ 12.3	0.0	73.7	7.5	0.63	7.6	0.62	1.63	3
	1100-2500	259	36.6 $\pm$ 12.3	0.0	73.6	7.6	0.62	7.7	0.61	1.60	3
FAA, mg/100g DM	400-2500	258	7120 $\pm$ 879	4484	9757	348	0.84	382	0.81	2.30	6
	1100-2500	258	7124 $\pm$ 873	4507	9742	396	0.79	410	0.78	2.13	4

DM – dry matter; NPN – non protein nitrogen; IMF – intramuscular fat content; FAA – free amino acid content; SD – standard deviation of the reference values (calculated after the elimination of outliers); emin – estimated minimum; emax – estimated maximum; SE<sub>c</sub> – standard error of calibration; R<sub>c</sub> – coefficient of determination of calibration; SE<sub>cv</sub> – coefficient of determination of cross validation; R<sub>cv</sub> – standard error of cross validation; RPD – residual predictive deviation (ratio SD/se<sub>cv</sub>); PLS – number of PLS factors.

### 3. General discussion

Our results demonstrated high potential of NIR spectroscopy to predict chemical constituents and amino acid content of dry-cured ham which is very important for the industry to fulfil the *consortium* requirements. Presently, producers use classical/wet chemistry which is lengthy, expensive, often hazardous and thus less interesting to be used on a large scale. Since NIR spectroscopy enables fast and simple determination of many parameters simultaneously, it could effectively replace regular checking of dry-cured ham chemical constituent prescribed by the *consortium*.

In the literature there are a few literature reports on meat products (Collell *et al.*, 2010; Cruz Ortiz *et al.*, 2006; Gaitán-Jurado *et al.*, 2008; García-Rey *et al.*, 2005; González-Martín *et al.*, 2009; Ortiz-Somovilla *et al.*, 2007), moreover, there is a lack of information regarding the prediction of chemical composition of dry-cured ham. Accuracy of NIR predictive models obtained cannot be directly compared for different meat products because of different matrix, different constituents' variation range, *etc.* Literature reports (Gaitán-Jurado *et al.*, 2008; Ortiz-Somovilla *et al.*, 2007) on meat products (mainly pork sausages) showed successful prediction of fat, moisture and protein with NIR spectroscopy ( $R_{CV}=0.88-0.99$ ,  $RPD=2.9-10.4$ ). Excellent prediction results were published also by Collell *et al.* (2010) for moisture content in fermented pork sausages ( $R_{CV}>0.99$ ,  $RPD>20$ ). Similar as in our study they also managed to predict salt (NaCl) content with high accuracy ( $R_{CV}=0.97$ ,  $RPD=6.2$ ). Ellekjær *et al.* (1992) reported errors in the range of 0.4 to 1.3 g/kg in the prediction of salt content in cooked sausages.

## IV – Conclusions

In the present work NIR spectroscopy proved as highly reliable method for the prediction of studied chemical constituents of dry-cured ham “*Kraški pršut*”. For eventual replacement of (conventional chemical) methods currently used in regular checking of certified products the calibration models should be extended with samples of the whole a slice of dry-cured ham containing several muscles and adjacent fat.

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