Accuracy of near infrared spectroscopy to predict quality of pork and pork products including samples of Krškopolje and Turopolje pigs

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Accuracy of Near Infrared Spectroscopy to Predict Quality of Pork and Pork Products Including Samples of Krškopolje and Turopolje Pigs

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Summary

Study demonstrates the preliminary results of the evaluation of pork and pork products of local Slovenian (Krškopolje) and Croatian (Turopolje) pig breeds using near infrared spectroscopy (NIRS) conducted in the frame of European Union H2020 project TREASURE. For that purpose, samples from meat and products of two local pig breeds were collected, scanned with near infrared spectroscopy apparatus and chemically analysed (for proximate composition, fatty acids composition, proteolysis index, salt content and water activity). Data obtained were added to the database of previously collected samples and prediction models were recalibrated and reassessed. In general, NIRS calibration models are considered to be fit for purpose when the requirements (chemometric parameters) for screening purposes are met. In the present study, the quality of recalibrations using the samples from local pig breeds confirmed practical applicability for majority of studied quality traits. Further efforts are needed to enlarge the database with additional samples from local pig breeds to improve the robustness of the models and to test the calibrations on the independent sets of samples (i.e. with external validation).

Key words

near infrared spectroscopy, chemical composition, quality, meat

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Introduction

One of the challenges in the frame of European Union H2020 project TREASURE (www.treasure.kis.si) concerns testing of the new methodologies for quality evaluation of pork and pork products from local pig breeds. Among them near infrared spectroscopy (NIRS) is of great interest for food science and industry since it allows the characterization of food and quality control throughout processing (Font i Furnols et al., 2015). It is based on the physical principles of energy absorption of chemical bonds at specific wavelengths (electromagnetic waves) depending on the characteristics of the matrix and has the potential for a large-scale real-time analysis in industrial conditions. In the sector of meat, NIRS has a number of possible applications from raw material to the final product. Fast and simple checks of raw material quality are needed (e.g. amount of fat and its quality or fatty acids composition), which could serve to classify fresh meat for the most suitable way of further use (fresh consumption or processing). During processing, control over chemical and quality traits is needed (e.g. the loss of water, salt content, the extent of proteolysis etc.). Quality control is particularly important for products with long maturation time (e.g. a year or more in the production of dry-cured ham) to prevent losses due to unsuitable raw material or processing conditions, to optimise production process and thus ensure high quality products with specific sensory characteristics (aroma, flavour, tenderness, etc.). Applications in quality control are relevant also for the final products, e.g. checking water activity (an indicator of microbiological stability), chemical components, proteolysis index, etc. Conventional methods of chemical analysis are mostly unsuitable to be used in industrial conditions (time-consuming, hazardous). On the other hand, novel technologies including NIRS have great potential for such purposes, but require extensive calibration process prior to the application in practise. The ability of NIRS to predict composition and quality of fresh meat and meat products (with special emphasis on dry-cured ham) has been studied in our laboratory in previous years. The results are promising (Prevolnik et al., 2010, 2011) and in accordance with other literature reports (Prieto et al., 2009; Weeranantanaphan et al., 2011; De Marchi et al., 2017a, b). In the present study, existing models developed in our laboratory were upgraded and extended with the samples of local pig breeds (collected within TREASURE project) and their predictive ability and practical application were reassessed.

Material and methods

Collection of samples and reference analyses. The material used in the present study was divided into different sets (see Table 1) which comprised previously collected samples and samples acquired from local breeds (Slovenian Krškopolje pig and Croatian Turopolje pig). Fresh meat and fat samples were taken 1-2 days after slaughter, samples of meat products were taken at the end of processing. In the laboratory, samples were first used for NIRS scanning and then vacuum packed and frozen at –20 °C for further analyses. Reference values were obtained in accredited laboratories (SIST EN 17025, 2005). The following determinations were performed in different samples sets a) fresh meat: intramuscular fat (IMF), protein, water and fatty acids (FA) composition, b) subcutaneous fat: FA composition, c) meat products: IMF, protein, water, salt, non-protein nitrogen (NPN), proteolysis index (PI) and water activity (a_w), d) dry-cured ham: IMF, protein, water, salt, NPN and PI. Water content was analysed according to ISO 6496 (1999). Determination of IMF content was performed using petrolether extraction according to SIST ISO 1443: 2001 (Soxhlet extraction with hydrolysis). Protein content was calculated from total nitrogen content which was determined respecting ISO 5983-2 (2005) international standard using the Kjeltec 2300 nitrogen analyser (Foss Analytical, Hileroed, Denmark). For the determination of NPN, the procedure described in Monin et al. (2007) was used. PI was calculated as the percentage of NPN per total nitrogen. Salt (sodium chloride) content determination was carried out as described in Monin et al. (2007). FA composition of fat and muscle tissue was determined using gas chromatography following transesterification of lipids as described in Fidler et al. (2000). Analysis of a_w was determined by the use of Aqua LAB 4TE apparatus (Decagon Devices Inc., Pullman, WA, USA) according to ISO 21807:2004.

NIR spectra acquisition. Samples were scanned with spectrophotometer NIR System model 6500 (Silver Spring, MD, USA) in a wavelength range from 400 to 2500 nm. Prior to scanning, samples were homogenized except for fat samples which were scanned intact. Samples were placed in a rectangular quartz cup (47×57 mm^2) in about 3 mm thick layer, covered by paper disc and inserted directly in NIRS apparatus. For each sample one scanning was performed. Absorbance data were collected every 2 nm as log 1/R (R – reflectance).

Chemometric analysis was performed using WinISI II software. Calibration models for selected quality traits were developed on the data points of NIR spectrum (wavelength range 1100-2400 nm) using modified partial least squares regression, the mathematical treatment 1-4-4-1 (i.e. first derivative of the log 1/R, where the derivative is calculated over the gap of 4 spectral points) and "SNV and Detrend" option for the correction of scatter effects in the spectra (WinISI II Manual, 2000). Other options were also tested, but described combination was selected for presentation as it yielded to the best results in general. Samples for which the difference between actual and predicted values exceeded three standard deviations were considered as outliers. The number of PLS factors was limited to 16, but depending on the model, 1-9 PLS factors were used (based on the decline of errors). Developed calibration models were evaluated by means of cross-validation (using 4 subsets). The results are presented as standard error of calibration (se_C) and cross-validation (se_{CV}) and coefficient of determination of calibration (R^2_C) and cross-validation (R^2_{CV}). RPD (the ratio between standard deviation of the reference values and se_{CV}) was calculated as an additional indicator of models quality (suggested by Kennedy et al., 1996; Andrés et al., 2008).

Results and discussion

Table 1 summarises basic information on datasets (number of analysed samples, mean, standard deviation and variation

Table 1. Basic statistics for selected chemical and quality characteristics

	n	Mean	Standard deviation	Range (min-max)
Fresh meat - LD muscle				
Intramuscular fat, %	75	1.99	1.14	0.62 – 7.30
Protein, %	72	22.83	1.38	19.13 – 25.50
Water, %	71	73.58	1.22	70.30 – 76.10
Fresh meat – different muscles				
Intramuscular fat, %	117	2.84	1.97	0.62 – 11.5
Protein, %	125	22.08	1.71	18.20 – 25.50
Water, %	130	73.79	1.52	68.46 – 76.90
Fat tissue - subcutaneous				
SFA, g/100g fat	56	40.8	1.90	37.8 – 46.1
MUFA, g/100g fat	56	44.6	2.2	40.5 – 40.5
PUFA, g/100g fat	56	14.6	1.7	10.1 – 18.3
n–3 PUFA, g/100g fat	56	1.10	0.20	0.6 – 1.5
n–6 PUFA, g/100g fat	56	13.4	1.6	9.4 – 16.8
n–6/n–3 PUFA	56	12.4	1.2	10.7 – 15.5
Fat tissue - LD intramuscular fat)				
SFA, g/100g fat	56	39.2	2.1	35.2 – 43.7
MUFA, g/100g fat	56	48.1	2.6	41.2 – 53.0
PUFA, g/100g fat	56	12.7	3.2	6.6 – 22.2
n–3 PUFA, g/100g fat	56	0.83	0.25	0.46 – 1.70
n–6 PUFA, g/100g fat	56	11.9	3.0	6.0 – 20.49
n–6/n–3 PUFA	56	14.5	1.8	11.3 – 19.8
Meat products (pancetta, dry-neck, salami)				
Water, %	74	29.8	9.8	13.5 – 69.4
Intramuscular fat, %	74	37.9	14.9	2.1 – 68.9
Protein, %	74	25.7	6.8	10.8 – 43.8
Salt, %	66	5.2	1.2	3.6 – 9.8
Non-protein nitrogen, %	60	0.41	0.09	0.27 – 0.62
Proteolysis index, %	60	11.1	0.4	7.1 – 22.8
Water activity	131	0.888	0.022	0.822 – 0.955
Dry-cured ham				
Water, %	153	53.8	5.9	38.7 – 63.2
Intramuscular fat, %	132	5.2	2.5	2.1 – 17.7
Protein, %	154	32.2	5.4	25.0 – 47.9
Salt, %	157	6.6	1.3	3.1 – 10.2
Non-protein nitrogen, %	153	1.07	0.29	0.57 – 1.91
Proteolysis index, %	149	20.7	6.5	7.6 – 40.0

SFA – saturated fatty acids, MUFA – mono-unsaturated fatty acids, PUFA – poly-unsaturated fatty acids.

range of reference values) for chemical and quality traits of meat and meat products. Combined sets of samples consisted of commercial and local breeds providing a broad variation range in analysed traits, which is advantageous for the development of robust models.

Results of calibrations are presented in Tables 2-5. The most important statistical indicator of the accuracy of NIRS models is the error of cross-validation: the lower the error, the higher the quality of a model. For the comparison of models (for different constituents that span different variation range or different sample types/groups) R^2_{CV} and RDP are useful indicators because they are independent of absolute values/range of variable and represent relative measures of models quality (for both, higher values are beneficial). The parameter RPD is particularly useful as it considers the error of validation in view of the variation of the reference values. According to Williams (2001, 2008), for practical applicability of models, RPD values should be more than 2.0 for rough screening, more than 3.0 for screening purposes, more than 5.0 for quality control and more than 8.0 for analytical purposes.

Fresh meat and fat. Chemical constituents (Table 2) were more reliably predicted when sample set consisted of various pork muscles as compared to the specific LD (*m. longissimus dorsi*) sample set. Best results were obtained for IMF. Reliable calibrations were obtained on both sample groups ($R^2_{CV} > 0.95$, RPD > 4.6). Developed models could be applicable for quality control. Predictive ability was lower for water and protein and consistent with the requirements for rough screening (RPD > 2), with regard to the set of different pork muscles. Models for water and protein content developed on LD muscle only, presented an accuracy that is too low for practical use, which can be ascribed to low variability of these constituents within pork LD. Predictive ability of FA composition of fat tissue was satisfactory (Table 3). Statistical parameters showed accuracy that is good enough for (rough) screening purposes (except for ratio n-6/n-3 PUFA). These results are of special practical importance as the samples were scanned intact. In muscle tissue, prediction of FA groups was far less accurate, which is probably due to low amounts of fat, the average percentage of IMF in LD being about 2%.

Meat products. Results on calibration models for prediction of chemical and quality traits in different meat products (salami, pancetta, dry neck) are mainly promising (Table 4). The highest accuracy of calibrations was reached for water followed by IMF and protein where statistical parameters showed the applicability for quality control (and even for analytical purposes in case of water). Acceptable results that met the requirements for screening purposes, were obtained for a_w and salt content, whereas models for NPN and consequently also for IP were under the limit of practical applicability (RPD < 2.0).

Table 2. Predictive ability of chemical composition of fresh meat using NIRS

	Pork LD muscle					Pork – different muscles				
	Calibration		Cross-validation			Calibration		Cross-validation		
Constituent (%)	R^2_C	se_C	R^2_{CV}	se_{CV}	RPD	R^2_C	se_C	R^2_{CV}	se_{CV}	RPD
Intramuscular fat	0.99	0.14	0.95	0.25	4.6	0.98	0.23	0.97	0.30	6.6
Water	0.90	0.39	0.63	0.75	1.8	0.91	0.45	0.82	0.65	2.6
Protein	0.45	0.92	0.28	1.05	1.2	0.92	0.48	0.81	0.73	2.1

LD – *longissimus dorsi,* se_C – standard error of calibration, se_{CV} – standard error of cross-validation, R^2_C – coefficient of determination of calibration, R^2_{CV} – coefficient of determination of cross-validation, RPD – ratio between standard deviation of the reference values and se_{CV}.

Table 3. Prediction of groups of fatty acids in fat and muscle tissue using NIRS

	Fat tissue					Muscle tissue				
	Calibration		Validation			Calibration		Validation		
FA group (g/100 g fat)	R^2_C	se_C	R^2_{CV}	se_{CV}	RPD	R^2_C	se_C	R^2_{CV}	se_{CV}	RPD
SFA	0.95	0.439	0.83	0.791	2.4	0.98	0.255	0.58	1.332	1.5
MUFA	0.98	0.350	0.91	0.696	3.2	0.18	2.387	0.11	2.535	1.0
PUFA	0.97	0.315	0.89	0.568	3.1	0.78	1.508	0.53	2.209	1.4
n-3 PUFA	0.96	0.035	0.83	0.076	2.6	0.62	0.119	0.55	0.130	1.9
n-6 PUFA	0.97	0.286	0.89	0.507	3.1	0.77	1.428	0.52	2.075	1.4
n-6/n-3 PUFA	0.80	0.480	0.30	0.894	1.3	0.12	1.445	0.02	1.524	1.2

FA – fatty acid, SFA – saturated FA, MUFA – mono-unsaturated FA, PUFA – poly-unsaturated FA, se_C – standard error of calibration, se_{CV} – standard error of cross-validation, R^2_C – coefficient of determination of calibration, R^2_{CV} – coefficient of determination of cross-validation, RPD – ratio between standard deviation of the reference values and se_{CV}.

Table 4. Prediction of chemical composition of meat products using NIRS

	Calibration		Cross-validation		
Constituent	R^2_C	se_C	R^2_{CV}	se_{CV}	RPD
Water, %	0.99	0.70	0.99	0.94	10.4
Intramuscular fat, %	0.98	2.05	0.98	2.23	6.7
Protein, %	0.98	0.90	0.97	1.20	5.7
Salt, %	0.92	0.35	0.84	0.48	2.5
Non-protein nitrogen, %	0.49	0.067	0.39	0.074	1.3
Proteolysis index, %	0.66	2.10	0.63	2.17	1.7
Water activity – a_w	0.91	0.0068	0.88	0.0076	2.9

se_C – standard error of calibration, se_{CV} – standard error of cross-validation, R^2_C – coefficient of determination of calibration, R^2_{CV} – coefficient of determination of cross-validation, RPD – ratio between standard deviation of the reference values and se_{CV}.

Table 5. Prediction of chemical composition of dry-cured ham using NIRS

	Calibration		Cross-validation		
Constituent	R^2_C	se_C	R^2_{CV}	se_{CV}	RPD
Water, %	0.99	0.70	0.98	0.81	7.3
Intramuscular fat, %	0.82	1.13	0.79	1.19	2.1
Protein, %	0.99	0.62	0.98	0.74	7.3
Salt, %	0.95	0.30	0.94	0.33	3.9
Non-protein nitrogen, %	0.87	0.104	0.83	0.121	2.4
Proteolysis index, %	0.86	2.38	0.80	2.89	2.2

se_C – standard error of calibration, se_{CV} – standard error of cross-validation, R^2_C – coefficient of determination of calibration, R^2_{CV} – coefficient of determination of cross-validation, RPD – ratio between standard deviation of the reference values and se_{CV}.

In dry-cured ham, NIRS calibration models developed for quality traits showed practical applicability for most of the traits (Table 5). The highest accuracy was obtained for water and protein content, *i.e.* R^2_{CV} of 0.98 and RDP of 7.3 approaching the level of analytical precision. It is worth noting, that high predictive ability could even be seen in the case of salt content. For all the other traits (IMF, NPN, PI) presented results indicate that screening with NIRS appears possible.

Conclusion

In the present paper, existing calibration models for the prediction of different quality traits in meat and meat products were extended and upgraded with samples of two local pig breeds. In general, the quality of models denotes their practical value for quality control of fresh meat and processed pork products. Inclusion of new samples kept the accuracy of models and contributed to increased robustness. Further external validations of models, especially those for prediction of dry cured ham quality, will be undertaken using samples collected from other local European pig breeds/pork chains (*i.e.* Italian Cinta Senese, French Gascon).

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