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# Application of near infrared spectroscopy to predict chemical composition of meat and meat products\*

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A b s t r a c t: Near infrared spectroscopy was evaluated as a tool for predicting main chemical constituents (intramuscular fat, protein, water content, water-to-protein ratio) of different raw meats and meat products. Muscle and meat product samples (n=294) were divided into four groups: 1) pig longissimus dorsi muscle, 2) different pork muscles, 3) different muscles of different species and 4) meat and meat products. The quality of the developed models was evaluated using coefficient of determination in calibration ( $R_c^2$ ) and prediction ( $R_p^2$ ), standard error in calibration (se<sub>o</sub>) and prediction (se<sub>p</sub>) and RPD (ratio between standard deviation of the reference data and se<sub>p</sub>). We prepared separate model for pig longissimus dorsi muscle samples and several combined models for various meats and meat products. Best prediction results were obtained for intramuscular fat content ( $R_p^2$ =0.94-0.99; RPD=4.1-10.1), followed by water content ( $R_p^2$ =0.67-0.96; RPD=1.2-5.0). Prediction of protein content was also very good ( $R_p^2$ =0.87-0.96; RPD=2.7-4.5), except in a separate sample set of pig longissimus dorsi muscles, which was probably due to narrow variation range. Water-to-protein ratio was also predicted satisfactory accuracy ( $R_p^2$ =0.50-0.91; RPD=1.4-3.1). Developed models proved remarkable ability of near infrared spectroscopy for the prediction of chemical composition of raw meats and meat products. NIR spectroscopy.

Introduction

Chemical composition of meat is important for its nutritional value, technological, and sensory quality. Existing chemical procedures are very exhaustive so there is an interest for fast and reliable methods with the potential for industrial use. In recent years, spectral information is increasingly used for rapid analyses of food. Many studies are available in the literature showing near infrared (NIR) spectroscopy as a promising method for the analyses of meat (for review see Prevolnik et al., 2004; Prieto et al, 2009) in particular the chemical composition of raw meat by NIR spectroscopy (for review see Prevolnik et al., 2004; Prieto et al., 2009). On the other hand, studies dealing with the prediction of chemical composition of meat products are rare (Ortiz-Somovilla et al., 2007; Gaitán-Jurado et al., 2008; Collell et al., 2010), but the results also show high potential. Advantages of NIR spectroscopy compared to the conventional chemical methods are its speed, simplicity and the possibility to determine a large number of different parameters simultaneously. Thus the method would be especially useful and interesting where analyses on large scale are needed or in case of conventional methods which are harmful to health or environment. The limitations of the potential are related mainly to the laborious calibrations needed for each purpose.

In the present research we want to present the results of the tests of NIR spectroscopy application for the prediction of main chemical constituents (fat, water, protein) in an extensive set of different raw meats and meat products. It was of our interest to find out if model for separate muscles (within muscle models) or combined models for various meats and/ or meat products are more suitable for practical application.

### Material and methods

### Collection of samples

Material used in the current study consisted of 294 samples of raw meat and meat products. Samples

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were divided into four groups: i) pork *m. longissumus dorsi* (LD) muscle, ii) pork (different parts), iii) meat of various *species* and iiii) meat and meat products. The first group comprised 76-82 pig LD samples, the second group consisted of 146-163 samples of different pig muscles (LD, ST- *m. semitendinosus*, SM- *m. semimembranosus*, BF- *m. biceps femoris*), the third group consisted of 237-258 samples (LD, BF, TB-*triceps brachii* of various *species* (pig, cattle, poultry, lamb, rabbit) and the fourth group (n=278-294) comprised samples of raw meat and meat products (sausage, hot dog, dry cured ham).

# *Chemical analysis of intramuscular fat, water and protein*

The analyses of intramuscular fat (IMF), water and protein content were performed in the laboratory of the Agricultural Institute of Slovenia using accredited methods (*SIST EN 17025*, 2005). Prior to the chemical analyses, the samples of meat and meat products were trimmed of superficial fat tissue and minced in a blender. Samples were stored until use at  $-20^{\circ}$ C. All chemical analyses were carried out in replicates.

Determination of IMF was performed by using petrolether extraction according to *SIST ISO 1443:2001* (also known as Soxhlet extraction with hydrolysis). IMF content was expressed as a percentage on a fresh weight basis.

Determination of water content was performed according to the *ISO 6496* (1999). Shortly, 5g of 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 water in the sample.

Protein content was calculated from total nitrogen content which was determined according to *ISO 5983-2* (2005) international standard using Kjeltec 2300 nitrogen analyser (Foss Analytical, Hileroed, Denmark). Shortly, 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 titration.

Additionally, the water-to-protein ratio (WP ratio) was calculated.

### NIR spectra acquisition

Fresh samples of about 100 g were homogenised in a blender for at least 30 s in order to obtain a homogenous mixture. Samples were then put in rectangular quartz cup ( $47 \times 57$ 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. 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 l/R, where *R* represents reflectance.

## Spectral data analysis

Spectral data processing was performed by using WinISI II software (2000) with the purpose of developing calibration models for IMF, protein, water content and WP ratio prediction from numerous spectral data points and the reference information. Samples were divided into calibration subset (used to develop models) and prediction subset (used for independent testing or independent validation of models) using WinISI II option Make and use scores. Spectra were first reduced to independent sources of variation (scores) to replace the spectra using PL1 option (an algorithm that reduces spectral data to scores and fine-tunes them for a single constituent) on the basis of global H, which was set to 3. After that we selected about two thirds of the samples into the calibration set on the basis of the neighbourhood concept. The remaining samples were used as an independent prediction set. Splitting of samples was performed for each group of samples separately a) pig LD muscle, b) different pig muscles, c) different muscles of different species and d) meat and meat products. Basic statistics for calibration and prediction sample sets (number of samples, means and standard deviations) are presented in Table 1. The actual number of samples used for a single calibration model could be seen in Figures 1-4.

Calibration models for the prediction of IMF, protein, water and WP ratio were developed using modified partial least squares regression on the calibration sets of samples. 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. Samples for which the difference between actual and predicted values exceeded three standard deviations were considered as outliers. For a single calibration model from none to seven samples were removed as outliers. The number of PLS factors was limited to 16, but the actual number of PLS factors was defined for every single calibration model according to the decline of errors. Developed calibration models were further evaluated on an independent (prediction) set

 Table 1. Calibration and prediction sample sets used for the development and validation of NIR calibration models

Sample type/	Sample set/	n	Mean ± standard deviation/			
Vrsta uzorka	Set uzoraka	$\frac{11}{1000000000000000000000000000000000$			vijacija	
			IMF/IMM	Protein/ Protein	Water/Voda	WP ratio/ Odnos VP
<i>Pig LD muscle/</i> LD mišić svinje	<i>Calibration/</i> Kalibracija	52-58	1.94±1.15	22.60±1.25	3.75±1.07	3.25±0.17
	<i>Prediction/</i> Određivanje	22-26	1.69±0.77	22.82±0.94	73.88±0.47	3.24±0.14
<i>Different pig muscles/</i> Različiti mišići svinje	<i>Calibration/</i> Kalibracija	100-113	2.96±2.06	21.85±1.62	73.91±1.50	3.40±0.28
	<i>Prediction/</i> Određivanje	46-54	3.41±2.52	21.29±1.75	73.84±1.11	3.49±0.28
Different muscles of different species/ Različiti mišići različitih životinjskih vrsta	<i>Calibration/</i> Kalibracija	160-172	3.63±2.75	21.41±1.85	73.84±1.65	3.47±0.30
	Prediction/ Određivanje	77-86	3.52±2.60	21.11±1.74	74.06±1.61	3.51±0.28
<i>Meat and meat products/</i> Meso i proizvodi od mesa	<i>Calibration/</i> Kalibracija	188-196	5.59±7.42	21.26±3.05	71.75±8.62	3.39±0.59
	Prediction/ Određivanje	90-98	6.24±8.68	20.67±2.61	72.02±6.88	3.55±0.46

**Tabela 1.** Setovi uzoraka za kalibraciju i određivanje koji su korišćeni za razvoj i validaciju modela kalibracije u bliskoj infracrvenoj spektroskopiji

LD – *longissimus dorsi* muscle/mišić *longissimus dorsi*; IMF – intramuscular fat content/IMM sadržaj intramuskularne masti; WP ratio – water-to-protein ratio/Odnos vode i proteina

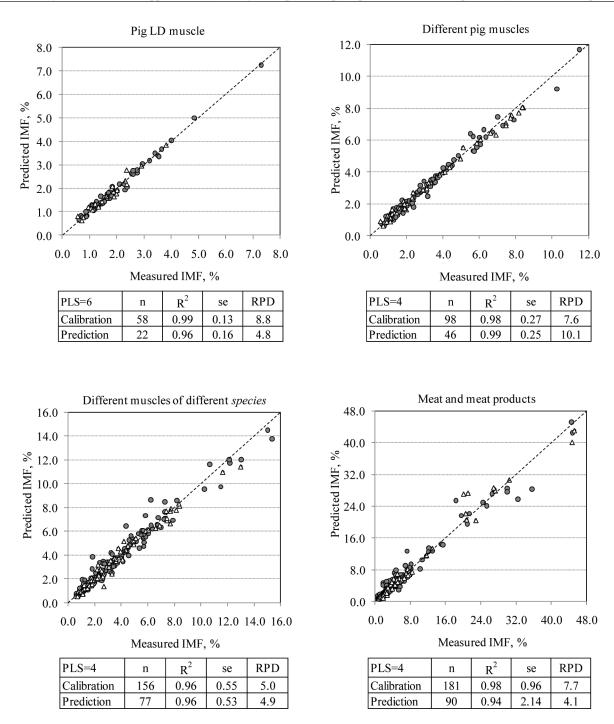
of samples. Therefore, the results are presented as standard error of calibration (se<sub>c</sub>), coefficient of determination in calibration ( $R^2_c$ ), standard error of external prediction (se<sub>p</sub>) and coefficient of determination of external prediction ( $R^2_p$ ). Additionally, the parameter RPD (residual predictive deviation) was calculated **as an** indicator of models' quality. RPD represents the ratio between the se<sub>c</sub> (or se<sub>p</sub>) and standard deviation (sD) of reference data in the calibration (or prediction) sample set.

#### **Results and discussion**

In the present study the results are presented as statistical parameters of calibration ( $R_{\rm C}^2$  and  $se_{\rm C}$ ) and external prediction ( $R_{\rm P}^2$  and  $se_{\rm p}$ ). In case when prediction variables span different variation range like different constituents or different sample types/groups, which is also the case of our study, then the errors cannot be directly compared. The errors of prediction should be considered in view of the variation of the reference values. Since the emphasis of our research was to test different sample types/groups with different variation range of four chemical constituents, the parameter RPD (the ratio sD/se) was additionally applied as an indicator of models' quality, as suggested by *Kennedy et al.*, 1996; *Andrés et al.*, 2008; *Prieto et al.*, 2008. For accurate/reliable predictions, it has been suggested that RPD should exceed three (*Kennedy et al.*, 1996; *Andrés et al.*, 2008; *Prieto et al.*, 2008). 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 (*Kennedy et al.*, 1996; *Andrés et al.*, 2008; *Prieto et al.*, 2008).

#### Prediction of IMF content

The results of the prediction of IMF content (Figure 1) were highly reliable in all four sample types/groups.  $R_{p}^{2}$  were over 0.96 and  $RPD_{p}$  over 4.1. However, the most accurate prediction of IMF content was observed in the group of different pig muscles ( $R_p^2=0.99$ ,  $RPD_p=10.1$ ). The accuracy of the common model for meat and meat products was a little lower, but only in case of higher (> 20%) IMF content (*i.e.* mainly for meat products). High quality IMF calibration models obtained in our study are in accordance with many previously published data (Tøgersen et al., 2003; Windham et al., 2003; Prevolnik et al., 2005; Prieto et al., 2006). The reasons for somewhat lower accuracy to predict IMF content in meat products with common model could be high variability of IMF content in different meat products and insufficient/small number of samples with high IMF content. However, calibration model

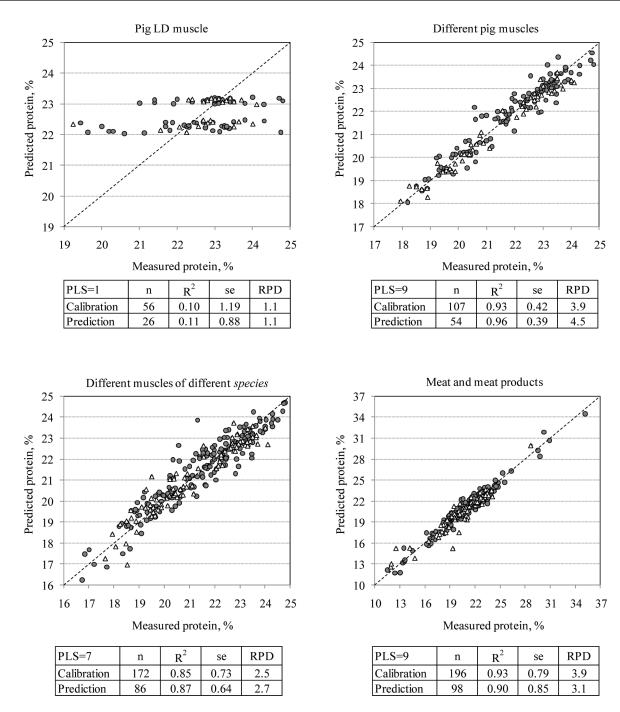


• calibration/kalibracija;  $\Delta$  prediction/određivanje; ----- fictive line/fiktivna linija(x = y);

LD - longissimus dorsi muscle/mišić longissimus dorsi; IMF - intramuscular fat content/IMM sadržaj intramuskularne masti; PLS - number of PLS factors used to develop calibration model/broj PLS faktora korišćenih za razvoj modela kalibracije; se - standard error/ standardna greška; R<sup>2</sup> - coefficient of determination/koeficijent determinacije; RPD - residual predictive deviation (the ratio between standard deviation of the reference data and standard error)/rezidualno odstupanje (odnos između standardne devijacije referentnih podataka i standardne greške)

**Figure 1.** Prediction of IMF content in different sample sets using NIR spectroscopy (spectrum 400–2500 nm)

Slika 1. Određivanje sadržaja IMM u različitim setovima uzoraka korišćenjem bliske infracrvene spektroskopije (NIR) (spektar 400–2500 nm)

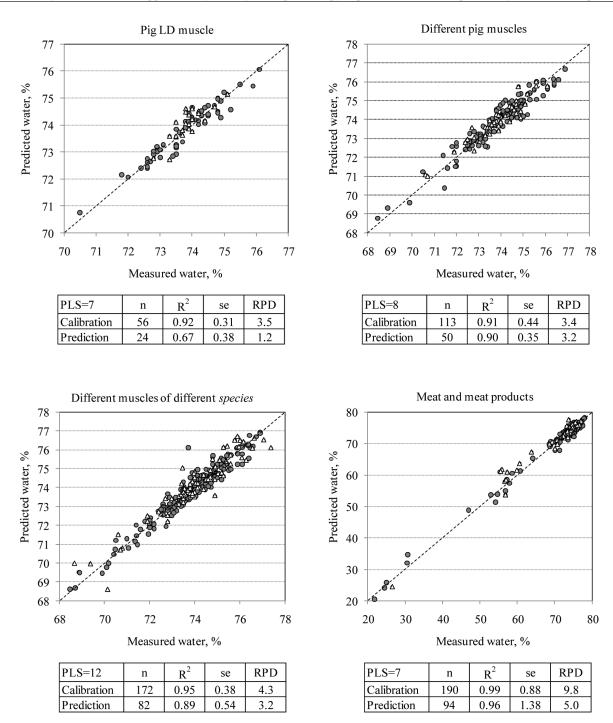


• calibration/kalibracija;  $\Delta$  prediction/određivanje; ----- fictive line/fiktivna linija (x = y);

LD – *longissimus dorsi* muscle/mišić *longissimus dorsi*; PLS – number of PLS factors used to develop calibration model/broj PLS faktora korišćenih za razvoj modela kalibracije; se – standard error/standardna greška; R<sup>2</sup> – coefficient of determination/koeficijent determinacije; RPD – residual predictive deviation (the ratio between standard deviation of the reference data and standard error)/ rezidualno odstupanje (odnos između standardne devijacije referentnih podataka i standardne greške)

Figure 2. Prediction of protein content in different sample sets using NIR spectroscopy (spectrum 400–2500 nm)
 Slika 2. Određivanje sadržaja proteina u različitim setovima uzoraka korišćenjem bliske infracrvene

spektroskopije (NIR) (spektar 400-2500 nm)

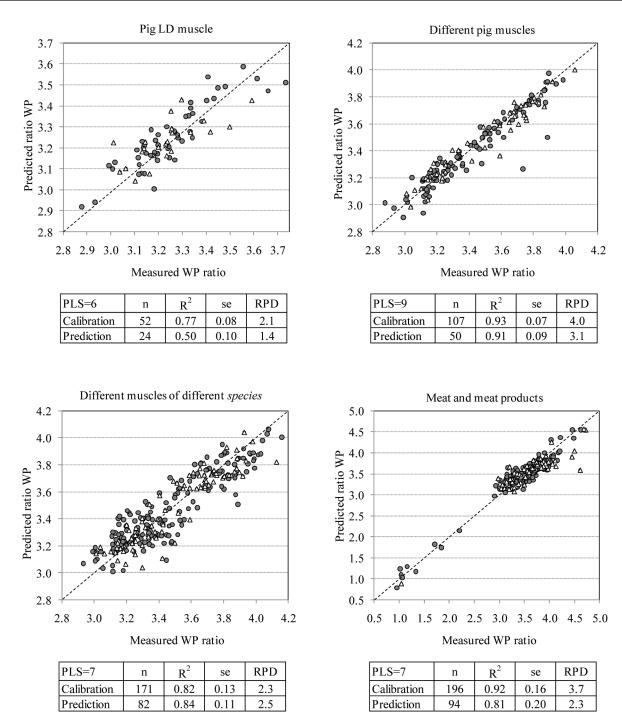


• calibration/kalibracija;  $\Delta$  prediction/određivanje; ----- fictive line/fiktivna linija (x = y);

LD - longissimus dorsi muscle/mišić longissimus dorsi; PLS – number of PLS factors used to develop calibration model/broj PLS faktora korišćenih za razvoj modela kalibracije; se – standard error/standardna greška; R<sup>2</sup> – coefficient of determination/koeficijent determinacije; RPD – residual predictive deviation (the ratio between standard deviation of the reference data and standard error)/ rezidualno odstupanje (odnos između standardne devijacije referentnih podataka i standardne greške)

Figure 3. Prediction of water content in different sample sets using NIR spectroscopy (spectrum 400–2500 nm)

Slika 3. Određivanje sadržaja vode u različitim setovima uzoraka korišćenjem bliske infracrvene spektroskopije (NIR) (spektar 400–2500 nm)



• calibration/kalibracija;  $\Delta$  prediction/određivanje; ----- fictive line/fiktivna linija (x = y);

LD - longissimus dorsi muscle/mišić longissimus dorsi; WP ratio – water-to-protein ratio/VP – odnos vode i proteina; PLS – number of PLS factors used to develop calibration model/broj PLS faktora korišćenih za razvoj modela kalibracije; se – standard error/standardna greška; R<sup>2</sup> – coefficient of determination/koeficijent determinacije; RPD – residual predictive deviation (the ratio between standard deviation of the reference data and standard error)/rezidualno odstupanje (odnos između standardne devijacije referentnih podataka i standardne greške)

Figure 4. Prediction of WP ratio in different sample sets using NIR spectroscopy (spectrum 400–2500 nm)

Slika 4. Određivanje odnosa vode i proteina u različitim setovima uzoraka korišćenjem bliske infracrvene spektroskopije (NIR) (spektar 400–2500 nm)

solely for meat products (n=40, data not shown) gave also very accurate predictions of IMF content ( $R_p^2=0.97$ , RPD<sub>p</sub>=6.1).

## Prediction of protein content

The results for the prediction of protein content (Figure 2) were acceptable for all sample groups  $(R_{p}^{2}=0.87-0.96, RPD_{p}=2.7-4.5)$  except for the separate set of pig LD muscle samples where the results were completely insufficient. The best ability to predict protein content was observed in the group of different pig muscles ( $R_p^2=0.96$ ,  $RPD_p=4.5$ ). The reason for inability of NIR spectroscopy to predict protein content in pig LD muscle was most likely due to the narrow variation range of protein content within pig LD muscle which was reported also for bovine muscles (Prieto et al., 2006; Ripoll et al., 2008). It may however partly be also due to the method (Kjeldahl method vs. NIR measurement). Namely, protein content was calculated on the assumption that all nitrogen in the sample appears in protein, although a part (≈13%, *Čandek-Potokar* et al., 2002) of nitrogen is in form of non-protein nitrogen. The lack of strong correlations between spectral information and protein content of meat was already noted before. Several authors reported similar results, *i.e.* poor prediction ability of NIR spectroscopy to predict protein or at least inferior prediction results compared to IMF prediction (Tøgersen et al., 1999; Cozzolino and Murray, 2002; Tøgersen et al., 2003; Ripoll et al., 2008; De Marchi et al., 2007). Published reports on the prediction of protein content within LD muscle are rare. We found a study of Chan et al. (2002) with moderately better results ( $R_c^2=0.69$ ,  $se_p=0.0042$ ) compared to those obtained in the present study. Ripoll et al. (2008) reported basically the same results for beef LD muscle  $(R_{p}^{2}=0.16 \text{ and } RPD=1.09)$  as obtained in our study.

# Prediction of water content

The prediction of water content (Figure 3) proved to be highly reliable in all sample groups with  $R_p^2$  ranging from 0.89 to 0.96 and  $RPD_p$  over 3.2. The best ability to predict water content was observed with common model for meat and meat products ( $R_p^2=0.96$ ,  $RPD_p=5.0$ ). We can observe very accurate predictions also in case of extreme water contents especially very low content (<40%; mainly in meat products). The ability of NIR spectroscopy to predict water was very similar as in case of IMF content prediction. In meat, the content of water is the inverse proportion with IMF content which was predicted very well. For this reason good prediction results in case of water constant consti-

tuent of meat which is associated with narrow variation range (especially in case of single muscle) and thus with lower prediction ability compared to water and IMF content. The results for the prediction of water (or dry matter) that can be found in the literature vary from very good (*Tøgersen et al.*, 2003; *Viljoen et al.*, 2005; *Ortiz-Somovilla et al.*, 2007; *Viljoen et al.*, 2007; *Gaitán-Jurado et al.*, 2008; *Collell et al.*, 2010) to moderate (*Cozzolino et al.*, 2000; *Cozzolino and Murray*, 2002; *Andrés et al.*, 2007) and even deficient (*Abeni and Bergoglio*, 2001; *Cozzolino et al.*, 2002).

### Prediction of WP ratio

The predictions of WP ratio (Figure 4) using NIR spectroscopy in different sample groups were moderate to acceptable with  $R_{P}^{2}$  ranging from 0.50 to 0.91 and RPD<sub>p</sub> between 1.4 and 3.1. Due to deficient ability of NIR spectroscopy to predict protein content in separate set of pig LD muscle samples the results were poor also in case of prediction of WP ratio within LD muscle. Reliable prediction results (*i.e.* where RPD exceeds three) were obtained only for the group of different pig muscles ( $R_{p}^{2}=0.91$ ,  $RPD_{p}=3.1$ ). WP ratio has practical importance as an indicator of technological quality, since water (60%) is bound to proteins in meat. The ability of meat to retain water is an important property in most processed meat products. Namely, the loss of water from meat can significantly affect the sensory quality (and also with this the weight). WP ratio is especially associated with the losses of water during cooking. Negative relationship between WP ratio and cooking losses has been suggested by Monin et al. (1986). The water is probably lost due to heat induced protein denaturation during cooking of the meat, which causes less water to be entrapped within the protein structures held by capillary forces. No example/attempt of predicting WP ratio using NIR spectroscopy was found in the literature.

### **General discussion**

In the literature there are numerous papers testing NIR spectroscopy for the prediction of raw meat chemical composition (for review see *Prieto et al.*, 2009). Interestingly, there are only a small number of studies on meat products (*Ortiz-Somovilla et al.*, 2007; *Gaitán-Jurado et al.*, 2008; *González-Martín et al.*, 2009; *Collell et al.*, 2010). In general, our study revealed very good or even better results compared to many literature reports (*Tøgersen et al.*, 1999; *Brøndum et al.*, 2000; *Rødbotten et al.*, 2000; *Alomar et al.*, 2003; *Hoving-Bolink et al.*,

2005; Barlocco et al., 2006; Savenije et al., 2006). The results of recently published studies show for the most part very good prediction ability of NIR spectroscopy to predict chemical composition (Berzaghi et al., 2005; Viljoen et al., 2005; Prieto et al., 2006; De Marchi et al., 2007; Viljoen et al., 2007; Ortiz-Somovilla et al., 2007; Gaitán-Jurado et al., 2008). The originality of the present paper relates to the extensive mixed sample set of different raw meats and meat products. Contrary to our experiment, most of the published studies were carried out on more uniform sample material such as one meat product (Ortiz-Somovilla et al., 2007; Gaitán-Jurado et al., 2008), meat of one species (Brøndum et al., 2000; Hoving-Bolink et al., 2005) or even only one muscle (Rødbotten et al., 2000; Savenije et al., 2006; Ripoll et al., 2008). In our study very reliable results were obtained for all main chemical constituents in most of the sample groups (exceptions being water and especially protein content in pig LD muscle) which is of special importance especially if heterogeneity of samples in the applied groups is considered. There were some studies comparing different samples groups. Tøgersen et al.

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(2003) studied prediction of fat, protein and water of pork and beef batches by NIR spectroscopy. Their findings showed no important differences in the results of prediction between combined set of samples and separate sets of pork and beef which agrees with our results. According to the results obtained in the present study we can recommend the use of different models for different purposes. For the specific use (e.g. prediction of IMF content for selection purposes in pigs) within muscle model would be more suitable but only in case of sufficient variation range.

#### Conclusions

In the present study, NIR spectroscopy proved to be highly reliable for the prediction of the content of all studied chemical constituents (IMF, water, protein and WP ratio) within single muscle and combined groups of different meats and meat products. The exception was the prediction of protein content and consequently WP ratio in a separate sample set of pig LD muscle which was probably due to narrow variation range of protein content.

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# Primena bliske infracrvene spektroskopije u određivanju hemijskog sastava mesa i proizvoda od mesa

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R e z i m e: Bliska infracrvena spektroskopija je ocenjivana kao instrument za određivanje glavnih hemijskih sastojaka (intramuskularna mast, protein, sadržaj vode, odnos vode i proteina) različitih vrsta svežeg mesa i proizvoda od mesa. Uzorci mesa i proizvoda od mesa (n = 294) su podeljeni u četiri grupe: 1) mišić longissimus dorsi svinja, 2) različiti mišići svinja, 3) različiti mišići od različitih životinjskih vrsta i 4) meso i proizvodi od mesa. Kvalitet razvijenih modela je ocenjivan korišćenjem koeficijenta determinacije kalibracije ( $R_c^2$ ) i određivanja ( $R_p^2$ ), standardne greške kalibracije (se<sub>c</sub>) i određivanja (se<sub>p</sub>), kao i RPD-a (odnos između standardne devijacije referentnih podataka i se<sub>p</sub>). Pripremljen je odvojeni model za uzorke mišića longissimus dorsi svinja, kao i nekoliko kombinovanih modela za različite vrste mesa i proizvoda od mesa. Najbolji rezultati u određivanju su dobijeni za sadržaj intramuskularne masti ( $R_p^2 = 0, 94-0,99$ ; RPD = 4,1-10,1), a zatim za sadržaj vode ( $R_p^2 = 0,67-0,96$ ; RPD = 1,2-5,0). Određivanje sadržaja proteina je takođe bilo dobro ( $R_p^2 = 0,87-0,96$ ; RPD = 2,7-4,5), osim u slučaju odvojenog seta uzoraka mišića longissimus dorsi svinja, što je verovatno zbog malog intervala varijacije. Odnos vode i proteina je određivan sa zadovoljavajućom tačnošću ( $R_p^2 = 0,50-0,91$ ; RPD = 1,4-3,1). Razvijeni modeli su dokazali izuzetnu sposobnost bliske infracrvene spektroskopije u određivanju hemijskog sastava svežih mesa i proizvoda od mesa, prema tome potencijalno mogu da zamene postojeću konvencionalnu hemiju.

Ključne reči: hemijski sastav, meso, proizvodi od mesa, bliska infracrvena spektroskopija.

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