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EVALUATION OF MEAT AND BLOOD MEAL RATIOS IN MIXED PROTEIN MEALS BY VIS-NIR SPECTROSCOPY

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Abstract

Only properly prepared animal by-products, protein meals can be utilized as animal feed additive. However, different protein meals are appropriate for feeding different animal species. Since the link between feeding ruminant-derived meat and bone meal (MBL) and the occurrence of bovine spongiform encephalopathy (BSE) and its human equivalent variant Creutzfeldt-Jakob disease (vCJD) has been established, it is imperative that potentially infective material is excluded from the food chain. Thus the aim of this study is to develop a spectral method which will allow to estimate the ratio of meat and blood protein meal in the final product. Consequently of the method is applicable to identify the purity meat and blood meal. During the test the products were mixed in different proportions and were examined by the spectral method. Measurements were conducted with AvaSpec 2048 spectrometer in visible (VIS) and in near infrared (NIR) wavelength range (400-1000 nm) to define the spectral differentiation of the different meal products. Significant difference can be detected in spectral reflectance between the meat and blood product in the VIS-NIR range. The blood product has a characteristic spectral property: in the range of 600 and 735 nm reflectance values are increasing following a sigmoid curve. Beside the inflection points, based on the sensitive wavelength range VKFI (R930/R600) is produced for detecting protein meal purity. Furthermore, the integral of the reflectance curve at the sensitive spectral range is found to be also a good solution for the monitoring of purity. Inflection point of meat meal and blood product are also determined. As a result, the range of wavelength can be determined, which is suitable for detecting individual characteristics of the investigated products. Using spectral examination for determining composition of the products could be quick and low energy method.

Key words: meat meal, blood meal, VIS-NIR spectrometry

INTRODUCTION

Application of protein feed with animal origin has growing importance by the development of animal husbandry. The high-protein products bring a large amount of essential amino acids to the compound feed as it is necessary. Products containing high protein and mineral substances are valuable as animal feed materials (Salminen and Rintala, 2002; Hegedűs and Sziebert, 1987; Mézes and Hausenblasz, 2010).

The measuring the chemical composition of protein meals by their optical characteristics was studied with non-destructive spectrometry by several researchers. The spectral measurement technology is based on the interaction between the sample and the visible, infrared radiation. In contrast to conventional chemical method the advantages of the spectral

analysis are non-destructive and rapid, requires minimal sample preparation, online. Reflectometry does not require reagents and solvents which reduces the cost of analysis, the amount of hazardous waste production, therefore there is no environmental impact and it promotes the determination of many physical and chemical properties of samples (Uddin and Okazaki, 2004).

Food ingredients have absorption peaks in 400-2500 nm wavelength range, therefore this range is particularly useful for determining the composition of food products (Ben-Gera and Norris, 1968; Tena et al., 2014, Bazár. 2014; Kaffka and Martin, 1985). According to a recent research NIR microscopy is one method that is suitable for examining animal protein (Pérez et al., 2009). Ben-Gera and Norris (1968) Rosenthal (1973) have already been examined fat and moisture content in meat products using near infrared technology. Cozzolino and Murray (2004) identified the difference between high quality meat product and mechanically separated meat and offal by visible (VIS) and near infrared (NIR) technique. Spectral quality of poultry meat was measured by Liu and Chen (2001). Application of spectral imaging techniques can be used to control the quality of broiler chickens (Chao et al., 2010). Tena et al. (2014) examined the differences of meat and bone meal from fishmeal by near-infrared spectroscopy. Different studies performed during the last years have demonstrated the powerful characteristics of NIR spectroscopy for the detection of meat and bone meal in feedingstuffs (Garrido et al., 2005; Murray et al. 2001).

Since the link between feeding ruminant-derived meat and bone meal (MBL) and the occurrence of bovine spongiform encephalopathy (BSE) and its human equivalent variant Creutzfeldt-Jakob disease (vCJD) has been established, it is imperative that potentially infective material is excluded from the food chain (Phillips et al., 2000). Thus different kinds of protein meals are appropriate for different animal species as nutrients. In the EU meat meal can be utilized only for pets, although blood meal can be a nutrient for animals – excluding ruminant species – raising for human consumption. Therefore from quality assurance point of view it is important to maintain the production control as accurate as possible and which provide data within a short time. It is important to avoid accidental cross contamination of the products. In several cases meat and blood meal produced on the same technological line, therefore mixing of the products can happen in various proportions during the shift of production. Thus the aim of this study is to develop a spectral method which will allow to estimate the ratio of meat and blood protein meal in the final product. Consequently of the method is applicable to identify the purity meat and blood meal.

MATERIAL AND METHOD

Production of different protein meals are often produced in a common technological and production chain, so it is very important to assess the purity of the protein meals. Therefore the spectral assessment of meat and blood meal is examined in this study. Meat meal and blood meal were mixed in different ratios, then the spectral investigation was carried out by Avantes AvaSpec 2048 spectrometer at 400 – 1000 nm wavelength interval with 0.6 nm spectral resolution.

The number of meat and blood meal samples were 14-14 in three replications. Average and standard deviation of prepared spectral curves were calculated. In order to representative examination for different amount of components, it was needed samples, which had at least 0.5 kg weight. When the dryness of original protein meal samples were not suitable for spectral analysis, samples had to be dried since the water content can modify the reflectance values (Nagy et al., 2014b, 2015). The applied drying method is the gravimetric method, where samples are dried to a constant weight at 105°C. During drying, not only the moisture content of the samples are vaporized, but also other volatile components (Csapó and Csapóné Kiss, 2003). Based on the weight ratios of meat meal and blood meal, mixing sequence was set in laboratory conditions in order to model the mixing rates of the two examined protein meals during the shift of the production process. Meat and blood meal laboratory samples (as follows: MBL samples) were generated in the following proportions: 100-0%, 99-1%, 97,5-2,5%, 95-5%, 90-10%, 80-20%, 70-30%, 50-50%, 30-70%, 20-80%, 10-90%, 5-95%, 2,5-97,5%, 1-99% and 0-100%, where the mixed samples were analysed by spectrometer in VIS and NIR wavelength range. After examination spectral purity identifying index was created based on the reflectance curves of different mixing samples.

Based on the spectral profiles, the standard deviation of reflectance curves of MBL samples was calculated for each groups. Based on the standard deviation curves, spectral wavelength ranges, sensitive for protein meal purity were chosen. Then, based on the sensitive ranges, different simple ratio indices and reflectance based spectral indices were created for the spectral evaluation of purity and the rate of different protein meals.

On the basis of the purity sensitive spectral range, the application of curve integration is also assessed as a tool for determining meal purity and identifying the protein meal ratio in a mixture. The rate of decrease can be described numerically by determining the area under the curve. The area under the curve can be calculated by curve integration.

Besides creating spectral indices, inflexion point of reflectance curve was examined. Nevertheless, inflexion point is effective the analysis of spectra curves, because it can characterise the certain material. An example for infection point is the Red Edge Position (REP), which is useful in vegetation analysis. REP is correlated with the chlorophyll content of leaves (Broge and Mortesen 2002; Mutanga and Skidmore 2004, Nagy et al, 2014a, Tsai and Philpot, 1998).

Inflexion point can be useful for identifying the mixing ratio. By definition, inflections occur at wavelengths where the second derivative equals zero and the corresponding first derivative is at a local maximum or minimum.

RESULTS AND DISCUSSION

For modelling of meal purity and mixing rates, samples with various blood and meat meal ratios were created and measured spectrally. Based on the spectral characteristics of the mixed samples in the range of 400 and 510 nm, the reflectance values are generally low (0-10%). Thereafter the reflectance of the samples were increased. The reflectance growth is more intense in the case of pure meat meal than in the case of samples containing blood product (Fig. 1.). As it was identified earlier, lighter colour of meat meal cause higher reflectance values than blood product. 20% reflectance value of pure meat meal is reached at 628 nm, however, in case of the 50-50% blood and meat meal mixed sample the reflectance reached 20% at 725 nm.

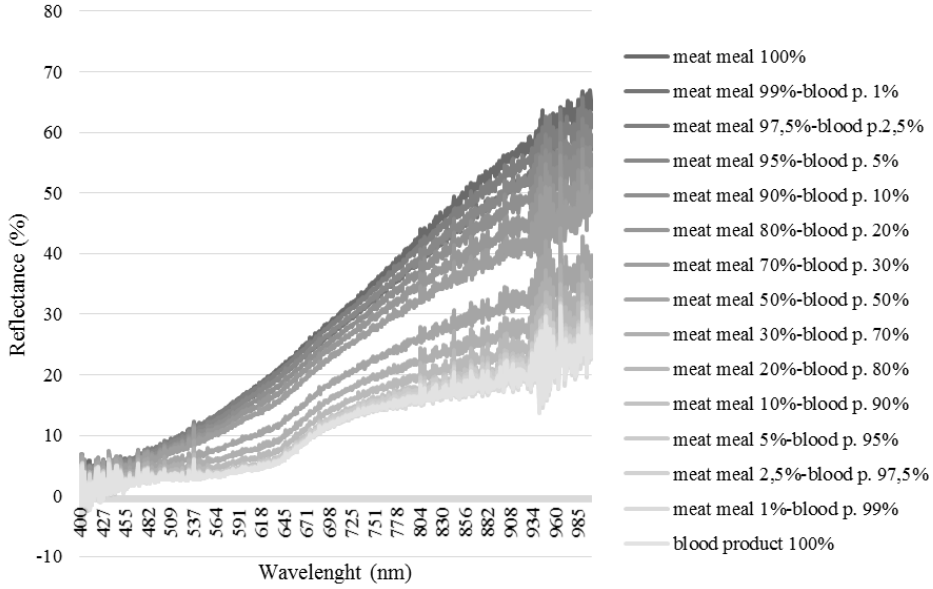

Fig. 1. Reflectance values of mixed meat meal and blood product

Based on the most sensitive wavelengths (600 nm and 930 nm) a simple ration index: Protein Meal Mixing Index (PMMI) = (R930/R600) was created. Based on the result of linear regression, a strong significant ($p<0.05$) correlation was detected between PMMI and the ratio of mixing. There is negative linear correlation between the index values and the ratio of meat meal, and in the case of blood product positive linear regression is identified. In order to measure the mixing ratio, estimation equations were created. To validate the estimation equations, two-sample t-test was used. Between the measured and the existing mixing ratios were not significant differences ($p=0.586$), thus the estimation equations are reliable applicable for detecting the mixing ratios.

R_{930}/R_{600} blood product $y=0.5285x – 1.6274$; $R^2 = 0.8969$; $P=0.006$

R_{930}/R_{600} meat meal $y = -0.5285x + 2.6274$; $R^2 = 0.8969$; $P=0.006$

y is the rate of blood or meat meal

x is the value of PMMI index

Calculation of inflexion point has high priority in our investigation. In the case meat and blood meal mixture, spectral feature are also changing, thus the position of inflexion point must varies. Analysing the spectral features of the samples, inflection point of the reflectance values is moving from 687 nm to 699 nm between 100% and 50% blood product ratios in mixed samples. After that, the inflection point of meat meal sample moves to 721 nm, from 50-50% mixing condition to 100% meat meal. Location of inflection point is clearly identified and there is statistical difference among the inflection points of protein meal samples. Thus, based on the inflexion points in red region, the purity and the rate of different protein meals can be identified and separated. Based on the results of linear regression, a significant ($p<0.05$) strong correlation was detected between the inflection points and the mixing ratios. This linear correlation was positive in the case of meat meal and it was negative in the case of blood product. Estimation equations were created for meat meal and blood product:

Meat meal ratio = $0.0351x - 24.186$; $R^2 = 0.953$, $p=0.000$

Blood product = $-0.0351x + 25.186$ $R^2 = 0.953$, $p=0.000$

x: inflection point

In order to validate the estimation equations, two-sample t-test was used. There were not significant differences ($p=0.761$) between the measured and the estimated mixing ratios thus the estimation equations are applicable for detecting the mixing ratios.

The application of curve integration is also assessed as a tool for purity evaluation. In order to better evaluation of curve integrals, linear models of the purity sensitive spectral curves of the mixture samples were fitted between 600 and 735 nm. Strong significant correlations

(0.997>R2>0.977) is detected between the measured spectral profiles and the fitted models. The model lines (y=ax-b) represent the spectral curves in 600-735nm. The general forms of the spectral model is the following:

The range of interpretation contains real numbers – f(x)∈R. However, in order to determine the mixing ratio, only the area under the model line should be calculated in the range of 600-735 nm. This is obtained by subtracting the area of model line calculated at 735 nm from the area calculated at 600 nm. Accordingly, the area of the model line between the range of 600 and 735 nm can be calculated by the following simplified integration equation:

$$T = \int_{600}^{735} ax - bdx = \left[a\frac{x^2}{2} - bx\right]_{600}^{735} = a\frac{735^2}{2} - 735b - \left(a\frac{600^2}{2} - 600b\right)$$

$$= 90112.5a - 135b$$

where:
a: slope of the model
b: constant of the model

Based on the above, model lines of mixed samples between 600 and 735 nm were integrated (Table 1.).

Table 1.

The spectral model curves and the estimated area under the line between 600-735 nm

	Spectral model line	Calculated area
meat 100%	y = 0.0822x - 46.174	3320
meat 99% - blood 1%	y = 0.1123x - 52.048	3093
meat 97.5% - blood 2.5%	y = 0.1185x - 54.944	3261
meat 95% - blood5%	y = 0.1159x - 54.255	3119
meat 90% - blood10%	y = 0.1115x - 52.692	2934
meat 80% - blood20%	y = 0.1095x - 52.531	2776
meat 70% - blood30%	y = 0.1066x - 52.021	2583
meat 50% - blood50%	y = 0.0964x - 49.313	2030
blood 70% - meat 30%	y = 0.0935x - 49.824	1699
blood 80% - meat 20%	y = 0.09x - 49.192	1469
blood 90% - meat 10%	y = 0.0872x - 48.645	1291
blood 95% - meat 5%	y = 0.0866x - 48.553	1249
blood 97.,5% - meat 2.5%	y = 0.0834x - 46.823	1194
blood 99% - meat 1%	y = 0.0793x - 44.33	1161
blood 100 %	y = 0.123x - 57.506	1173

In order to analyse the statistical differences between the area values, multivariate variance analysis was calculated. Based on the results of the variance analysis, the areas are statistically different and trend like changes is observed thus the mixing condition can be distinguished based on integration.

Regression analysis were carried out between protein meal and calculated areas. Based on analysis, strong linear correlation was detected between the areas and the mixing conditions. Based on the results, linear estimation algorithms were set.

Blood meal rate = 0.0005x-0.5225; R^2=0.994; P=0.000

Meat meal rate = -0.0005x+1.5225; R^2=0.994; P=0.000

x: area of the model curve in 600-735 nm

These algorithms were validated by paired T-test. There is no significant difference between the estimated and the measured protein meal ratios ($P_{blood\ product}$=0.657; $P_{meat\ meal}$=0.758).

CONCLUSIONS

During the research, spectral detecting and monitoring identification systems were developed which could be useful for separation and purity identification of different by-products. Spectral indices and inflexion point based estimated algorithms can be useful for determining purity and mixing ratio of meat meal and blood product fast and accurate way. Thus, calibrated spectral data can provide fast defining purity level of examined protein meals in laboratory condition. The results support the spectral based automatic separation method of meat and blood meal products in the future. Based on the results, investigated wavelength can utilize to design sensors, which can integrated into the production line for real time analysis of produced protein meals. Spectral indices have to recalibrate in the case of using other products.

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