Identification of free and covalently bound amino acids by near infrared spectroscopy in food proteins

Mária Váradi, Árpád Tóth and Gyöngyi Hajós
Central Food Research Institute, Budapest H-1022, Herman Ou 15, Hungary.

Introduction

On the basis of our preliminary study we have stated that there was a chance to investigate the process of enzymatic peptide modification (EPM) by near infrared (NIR) spectroscopy.[1] In the case of a model system such as casein we could differentiate the hydrolyzates, EPM products and free amino acids.

According to the current understanding of the science of nutrition, fortification of the proteins with the limiting free amino acids for food uses is not recommended above a critical level for toxicity reasons.

The aim of our present work is to develop a NIR method for quantitative determination of free amino acids in the mixture of EPM products.

Materials and methods

The samples analysed were as follows:

Protein samples

Commercial milk powder and a fraction of casein were used.

Amino acids

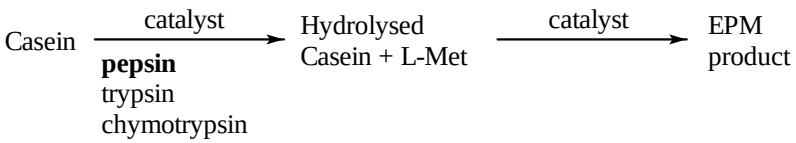
L-Methionine was used in pure form for making dilution series and L-methionine ethyl ester hydrochloride as a reactive molecule form to prepare methionine-enriched protein products.

Mixtures

A set of mixtures containing methionine of 0–4% was prepared by adding L-methionine to milk powder.

EPM products

In a second set, casein was enzymatically prehydrolyzed by α-chymotrypsin and pepsin as catalysts, respectively. Methionine-enriched protein was produced by the EPM process where hydrolysates were used as substrates in the enzymatic peptide modification reactions:



Determination of the quantity of methionine

In the milk powder mixtures methionine concentration was adjusted between 0–4%, in the case of EPM products methionine was separated by thin-layer chromatography. Merck DC Kieselgel 60 plates were used. The running system was phenol–water solvent. The R_f values were determined from 20 parallel separations.

NIR determination

The samples were scanned on a NIRSystems spectrophotometer model 6250 equipped with PbS detectors. NSAS, ISI and in-house software packages were used to acquire and evaluate spectra. Spectra consisted of 700 wavelengths in steps of 2 nm intervals from 1100–2500 nm. Each spectrum was recorded as an average of 50 scans. The mean spectra were treated by multiplicative scatter correction (MSC) method with 2OFD algorithm using a segment size of 2 nm and a 12 nm gap between segments to resolve overlapping bands and remove linear background shifts. PCA was performed to compute PC scores and loading spectra.[2] The loading spectra can be used to determine which wavelength regions are most important in the different principal components (factors).[3] PCR was used to develop calibration models. Multivariate discriminant analysis (MDA) was performed using scores for grouping objects and selecting sample subsets of EPM products.[4]

Results and discussions

In the mixtures samples we tried to determine the low concentration of pure L-methionine in the milk powder. Figure 1 shows the 2OFD spectra of milk powder and pure amino acid.

In the characteristic spectra there are three fingerprint regions where the main difference between the components can be detected. The figure contains the tentative assignment of the main absorbers. The S–H stretch can be seen at 1694 nm. Figure 2 illustrates the increase of S–H band activity with increase in the concentration of methionine.

Because of the low concentration of L-Met in the mixtures PCA transformation was used and loading spectra were analysed. The loading vector of factor 3 shows the similar shape as absorption

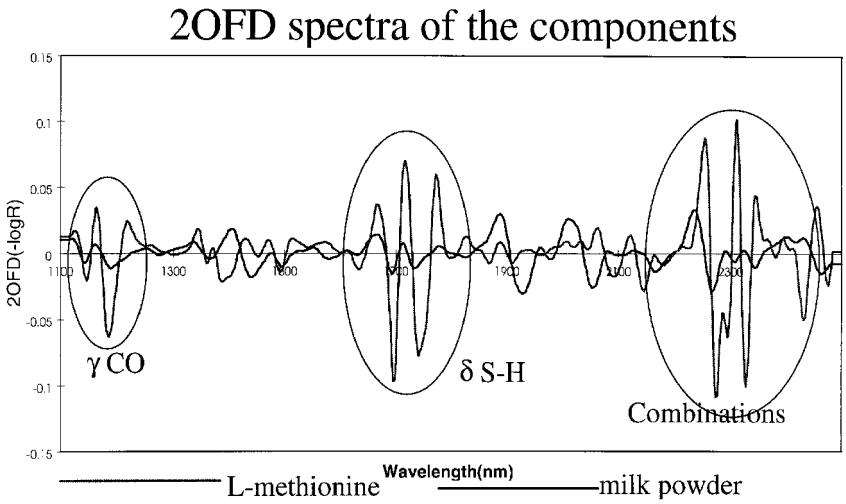


Figure 1. Second derivative spectra of milk powder and L-methionine.

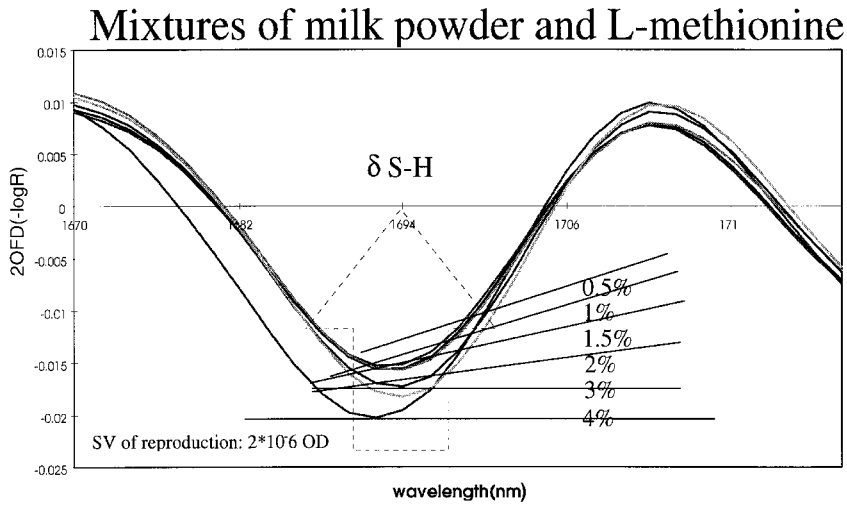


Figure 2. Expanded region of part of the second derivative spectra of mixtures of milk powder and L-methionine.

spectra of pure methionine. The third and the first factors were separated with this technique. The PCA score plot of the observations using the first and third PCs are shown in Figure 3. PCA can also be used for multivariate outlier detection and the point 0.5%, signed by the arrow, is an outlier. This object was removed from the calibration set.

After PCA transformation MLR was performed to develop a PCR calibration model. A test set validation model was used to test the generality of the model for the seven test mixtures. The

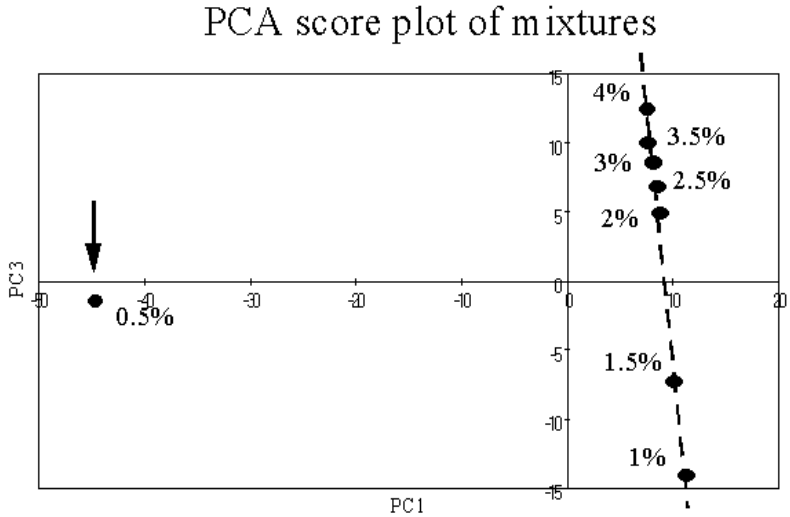


Figure 3. PCA scores plot for PC1 and PC3.

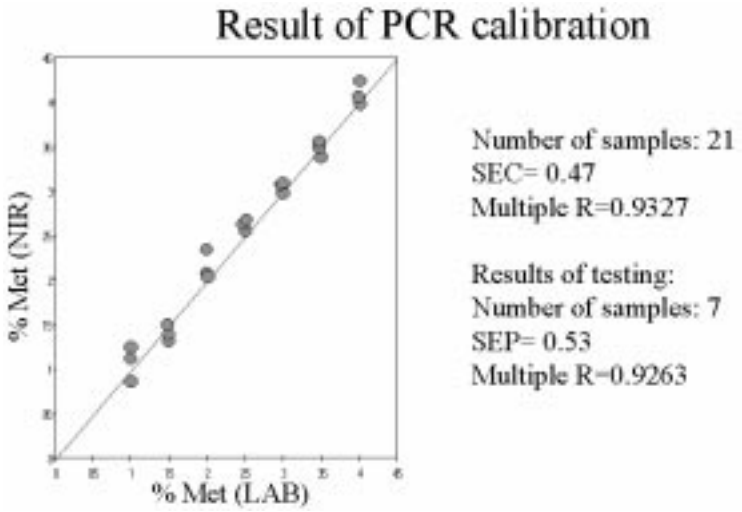


Figure 4. Calibration plot for methionine in milk powder.

prediction results are presented in Figure 4. Here the results are given as *SEC* and *SEP* values for the methionine determination.

The results demonstrate that PCR model gives good possibility to determine the quantity of free amino acid in milk powder as a carrier material.

Furthermore, we analysed the amount of incorporated amino acids. First, the methionine concentration was determined by thin-layer chromatography in the EPM samples described above. The NIR spectra of hydrolysates (0%) and EPM samples (2–8%) are generally overlapped but some obvious differences can be observed in the combination range in the Figure 5.

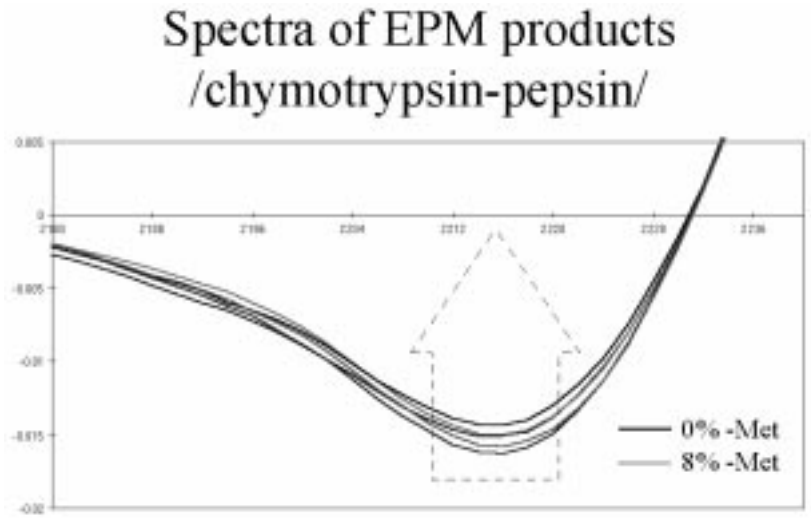


Figure 5. Partial spectra of EPM products.

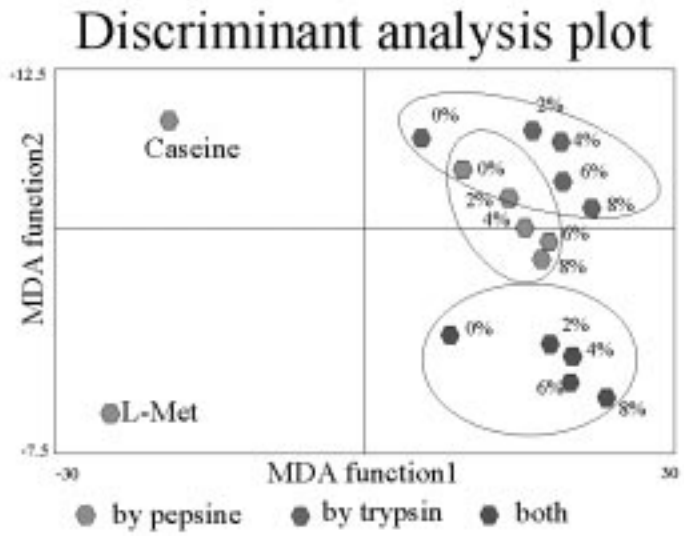


Figure 6. Discriminant analysis plot of the EPM products.

PCA was used to reveal hidden differences between the spectra. Evaluating the loadings spectra we found that the third factor is as sensitive as in the case of mixtures. Thus, the first and the third PCs were used to make a learning set for MDA. Figure 6 shows with these two factors that the MDA model is able to classify and significantly separate the group of free amino acid, the casein and the EPM products. The MDA plot of objects is especially useful for discrimination of EPM products prepared by one or two catalysts, pepsin and trypsin.

Finally, identification of the different chemical structure can also be performed. This result shows that NIR using the MDA model can be applied for studying the mechanism of reactions and for detecting the amino acids incorporated into protein molecules.

Acknowledgement

This work was supported by the Hungarian Science Research Fund (Grant No. OTKA T 017103).

References

1. M. Váradi, A. Tóth, and Gy. Hajós, *Proc. of the 6th International Conference on Near Infrared Spectroscopy*. Lorne, Australia (1994).
2. W.W. Cooley and P.R. Lohnes, *Multivariate Data Analysis*. John Wiley & Sons, New York (1971).
3. H. Martens and T. Næs, *Multivariate calibration.* John Wiley & Sons, Chichester (1989).
4. D.J. Hand, *Discrimination and Classification.* John Wiley & Sons, New York (1981).