Determination of Soya Protein in Model Meat Products Using Image Analysis

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Abstract

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The addition of plant proteins into meat products is nowadays a commonly used practice especially for the technological and economical reasons. Their properties have been known and used in meat products production for a long time. In the past, wheat protein or flour had been used most frequently, however, in these days they are being replaced by soya protein which has much more favourable properties in its use. Considering the possible misuse of raw materials of plant origin for the adulteration of meat products, the existence of highly sensitive and accurate procedures for their detection is needed including the determination of their content. Soya protein can be detected using various methods. In our work, an immunohistochemical method was used with image analysis for the quantification of soya protein. Model meat products with the addition of known amounts of soya protein in various forms were made for this experiment.

Keywords: soya protein; image analysis; quantification; immunohistochemistry

Currently, we can find various plant protein additives destined for meat products in the food industry. Among the most common additives of this type, there are various kinds of flour, starch, fiber, and various types of plant proteins. Their use is limited by currently valid legislation not only because of the prevention of consumer deceiving but also because of the possible influence on the consumers health. For this reason, the issue is of high attention not only in our country but worldwide as well.

The decisions, made by the official authorities taking into consideration whether the legislation requirements on the food quality parameters are met, are based on laboratory investigations. Apparently, it is necessary to have a wide range of analytical methods available to prove adulteration or authenticity of individual food commodities as well as to develop new methods. There are a variety of methods for the detection and determination of individual food ingredients – chemical, physical-chemical, immunological and, today especially developed, molecular-biological methods (Benešová *et al.* 1999).

The detection of proteins of plant origin is often done by means of various forms of ELISA (enzyme-linked immunosorbent assay) method, followed by e.g. CIE method (Counter Immune

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Electrophoresis). Regarding the soya protein, ELISA is the most suitable method – it is able to detect 1.5% of soya protein regardless of the application form in which this plant additive has been used. Immunodiffusion methods are suitable to determine gluten (Benešová *et al.* 1999).

Brehmer *et al.* (1999) used the ELISA method for quantitative detection of several types of plant proteins (soya, pea, gluten) in meat products both cooked and uncooked. They considered this method to be sensitive and suitable for common application. The detection limit for soya and pea proteins reported by them is 0.05–0.1% and for gluten 0.025% and 0.5%.

Among other authors, Sánchez-Martínez *et al.* (2009) used light scattering detection for the determination of soya protein. The analytical method was applied to the analysis of fruit juice and "nonmilk yoghurt" samples. The detection limit was 65 ng/ml (0.0000065%).

Belloque *et al.* (2002) summarised the methods suitable for soya protein analysis in meat products. For this purpose, the most common methods are microscopic, electrophoresic, immunologic, and chromatographic. Soya protein determination in meat products is often connected with some problems related to the ingredients and matrices (type of meat, meat quality, soya protein source, presence of other ingredients besides meat, etc.) and processing of meat products; and although the analytical methods try to overcome these problems, no method has been available up to now for the determination of soya protein quantity in all types of meat products.

One of the possible methods of soya and wheat additives detection is microscopic detection. For the detection of individual components, morphological criteria are used in combination with other techniques for image contrast acquisition – physical (for example polarisation microscopy), chemical (staining methods), and their combinations (Tremlová 2003).

With respect to the growing requirements on the food safety and quality, the need for accurate, objective, and rapid measurements of their characteristics and features is growing as well. Computer image analysis represents one of the possible alternatives as it is an automated, non-destructive, and cost-effective method for meeting the above mentioned requirements (Brosnan *et al.* 2004).

Image analysis belongs to rapid, objective and quantitative methods using image capturing for obtaining information. This method is suitable not only for food industry (detection of sensorial, technological, and qualitative values in various food commodities) but also for medicine, criminalists, and chemical industry.

Image analysis is a method helping to describe quantitatively and specify image information acquired using macroscopic or microscopic capturing. The aim is to gain numerical data which allow for a detailed comparison of different samples, accurate processing of the acquired information, and various ways of acquired results statement. The input unit for image capturing directly in the digital form is a camcorder or a camera; however, classic photos after digitalisation can be processed as well. A significant advantage is the possibility of combination and comparison of the objects in the currently scanned section with the objects saved earlier. The data found can be evaluated using the statistical methods.

One of the advantages of the analyser is automatic measuring and computing of all objects which we have been chosen in advance, for example according to the parameters for colour and brightness. The size and area of the objects chosen can, of course, also be detected using manual localisation. The objects measured in this way can be subsequently labelled with the data found. The masurement can be done in pixels or in any unit set in advance (Druckmüller & Štarha 2007).

The aim of the work was to confirm the possibility of image analysis application as a method for the quantitative determination of soya protein in model meat products.

MATERIAL AND METHODS

Material. For the purpose of quantitative determination of soya protein, a group of model meat products was prepared. These products were produced in the common way and soya protein was added in three different forms in defined quantities. The sample set included the following commercial meat products: cooked sausage Kabanos, dry cooked sausage Vysočina, and raw fermented meat product Čajovka. Soya protein was added in the form of isolate (Supro 500 E IP Non-GM, Solae Company, Geneva, Switzerland), concentrate (Alpha 8 IP, Solae Company, Geneva, Switzerland), and in textured form (PragoSoja s.r.o., Prague, Czech Republic), in concentrations 0.1%, 0.5%, 1.5%, and 3.0%.

Immunohistochemical staining. A process based on avidin–biotin complex for immunohistochemical detection of soya was used for immunohistochemical staining. DAB (3,3'diaminobenzidine) was used as the visualisation agent. Modified Calleja's staining was selected for the background staining as more suitable. In contrast to toluidine blue stain, it reaches a better contrast for image analysis because in the case of soya protein, the brown colour of DAB chromogene changes into black by toluidine blue stain and, at the same time, the typical microstructure is distorted (Pospiech *et al.* 2009).

Image analysis. Subsequently, six sections were examined from each sample. The whole area of each section was scanned by means of a microscope (Jenaval, VEB Carl Zeiss Jena, Germany) with the magnification of 25.6×. From the photos captured, five photos were chosen for a set using random number method, and this set was consequently evaluated using a program for image analysis (Adaptive Contrast Control, vers. 6.0, SOFO, Prague, Czech Republic).

At first, the area of soya protein (brown colour) was measured, next the area of the background was subtracted, and after that the measured brown area of the samples with 0% of soya protein added (control) was deducted. We reached the following values for the controls: Kabanos 2.9%, Vysočina 4.10%, and Čajovka 3.10%. The results obtained (average values) were compared with the known concentrations. Subsequently, the results were compared using the correlation coefficient. These statistical computations were done rationally using the statistical program Unistat Vers. 5.6 from UNISTAT Ltd. (London, UK).

Two-dimensional parameters were converted into three-dimensional ones using the probability geometry methods according to Delesse's relation in agreement with the International Society of Stereology (from now on ISS) recommendation.

RESULTS AND DISCUSSION

The determination of the individual components in the meat products is usually based on chemical methods which are precise but also time-consuming. Therefore, our work tried to find an alternative to these methods.

Model samples included known and precisely weighted amounts of soya protein additive. These data stated in the recipes were compared to the results of the image analysis. Using the statistical processing of the results, we reached a very good coefficient of correlation. The lowest correlation coefficient of 0.81 was found with the Vysočina SI (Vysočina with soya isolate) sample for Kabanos ST (Kabanos with soya texturate) it was 0.91, for Kabanos SK (Kabanos with soya concentrate) 0.96, for Čajovka SI (Čajovka with soya isolate) 0.98, and for other samples it was 1.00 (Table 1).

Benešová *et al.* (1999) informed that the ELISA method is able to detect 1.5% of soya protein regardless of the form it was added in. In the experiments carried out in our laboratory using immunohostochemical staining, we succeeded in quantification much lower quantity of soya protein, that is the amount of 0.1%.

On the other hand, Brehmer *et al.* (1999) using the ELISA method detected even a smaller amount than was the amount 0.1% of the quantified additive stated here.

More sensitive methods than that by Brehmer *et al.* (1999) were described by Sánchez-Martínez *et al.* (2009). These methods were used only for liquid foods.

Table 1. Image analysis results (v/v %) for cooked sausage Kabanos, Vysočina and raw spreadable product Čajovka

Concentrations prepared (% w/w)	Kabanos			Vysočina			Čajovka		
	Si	ST	SK	Si	ST	SK	SI	ST	SK
0.10	0.13	0.33	0.24	0.04	0.26	0.02	0.18	0.40	0.22
0.50	0.40	0.85	1.32	0.16	0.38	0.14	0.88	0.75	0.80
1.50	0.94	1.58	1.66	0.78	1.46	0.77	2.01	1.59	1.19
3.00	1.83	1.75	2.97	0.66	3.24	1.69	2.93	2.84	2.87
Correlative coefficient	1.00	0.91	0.96	0.81	1.00	1.00	0.98	1.00	0.98

SI – with soya isolate; ST – with soya texturate; SK – with soya concentrate

CONCLUSION

The results presented here proved the hypothesis about the possible relationship between the concentrations of soya protein used and the image analysis. The measurements of the basic micrometric parameters were performed and manifested the relationship between the volume of soya protein and the volume of the sample. In a subsequent work, the transformation of these volume parameters into weight parameters will be performed using optical parameters of the soya protein model sample in a neutral gel, for example in acrylamide. This transformation relationship can be then brought into direct correlation with the result of chemical determination of soya protein.

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