

## ELISA for Detection of Soya Proteins in Meat Products

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### Abstract

Indirect competitive ELISA method for the detection of soya proteins in meat products was developed. The detection limit of the method is 0.5% of the weight of added soya protein. A total of 131 meat product samples such as salamis or sausages from the Czech Republic market were investigated for the presence of soya proteins. Soya proteins were detected in 84% of the investigated samples without any declaration on the package of the product. The use of vegetable additives, namely soya in meat products in the market of the Czech Republic is very frequent and the restriction of its usage by legislation relates only to some kinds of durable products and ham (Act 264/2003 Coll.). The need for sensitive inspecting methods for soya protein detection is not only associated with the economic aspect (adulteration), but mainly with consumer health protection in case of allergy to soya proteins.

*Detection, immunochemical method, meat products, package declaration*

Addition of vegetable components to meat products is nowadays a common practice, mainly for technological and economic reasons. Plant additives participate in water absorption, emulgating properties, and the ability to form fluffy structures, temperature stability and increased total content of proteins. Soya protein markedly increases water-binding capacity, which has an adverse effect on the product durability. These characteristics have been known for a long time and are often applied in the production of meat products (Bookwalter 1978; Lusas and Riaz 1995). Vegetable proteins can also be used for the adulteration of meat products (Flores Mungula et al. 2000; Leitner et al. 2006).

The decision of the state supervision authorities whether the legislation requirements of qualitative indicators have been met is based mostly on laboratory testing. Hence, it is necessary to have a wide array of analytical methods available to ascertain adulteration or authenticity of particular food commodities, and to develop novel methods.

A number of methods exist for the detection of plant proteins in foodstuffs. Microscopic methods have been traditionally used for analysis of the materials of vegetable and animal origin and together with histochemical and immunohistochemical techniques for analysis of meat products or other ready-to-use foodstuffs (Flint 1994; Egelandsdal et al. 1991; Boutten et al. 1999; Tremlova et al. 2007). Immunochemical methods are often used under practical conditions (Macedo-Silva et al. 2000; Moriyama et al. 2005; Morishita et al. 2008; Pospiech et al. 2009) and the methods of molecular biology have been also developed (Meyer et al. 1996; Hernandez et al. 2006). Chromatography methods have been used for the detection of soya proteins, too (Castro Rubio et al. 2005; Criado et al. 2005). Moreover, novel homogenous immunoassay for soya protein determination in food samples using gold nanoparticles as labels and light scattering detection was presented (Sánchez-Martínez et al. 2009).

The purpose of the present study was to develop an indirect competitive ELISA method and to monitor the frequency of adding soya proteins to meat products and to check the consensus of the known or declared composition with the results of the analysis.

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## Materials and Methods

### Immunisation procedure and antibody preparation

For the first dose, the immunisation antigen of extracted proteins from soya isolate (Pragosoja, Czech Republic) according to Rittenburg et al. (1987) was mixed 1:1 with complete Freund's adjuvant (Sigma, USA) and administered intradermally in ten sites on the back of three-month-old New Zealand white rabbits (0.4 mg protein). Booster applications with Freund's incomplete adjuvant were made after 4 weeks, and then again after two weeks. After the last booster, the animals were bled by cardiac puncture. Sera were separated by centrifugation ( $1000 \times g$  at  $4^\circ C$  20 min) and stored at  $-20^\circ C$ . Specificity of the antibody was improved by saturation with the cross reacting antigens followed by centrifugation (Hayden 1979).

### Characterisation of samples

The heat-processed meat product samples were subject to analysis. These were mostly various sausage and salami types randomly purchased in the market chain of the Czech Republic. A total of 131 meat product samples were examined for the presence of soya proteins.

### Sample processing

Samples of 100 g of meat products were processed in a blender with 100 ml of PBS, the homogenate was centrifuged at  $10\,000 \times g$  and  $4^\circ C$  for 15 min and the supernatant was used for analysis.

### ELISA method

The indirect competitive ELISA method was prepared according to Rencova et al. (2000) and then modified for the detection of soya proteins. ELISA was conducted utilizing 100  $\mu l$  well system with the application of solid-phase soya isolate antigen followed with the addition of sample extracts and the respective polyclonal New Zealand White rabbits anti soya isolate antibody of own provenance, peroxidase anti-rabbit conjugated antibody SwAR (Sigma-Aldridge, USA), and tetramethylbenzidine (TMB) substrate (Sigma-Aldridge, USA) and measurement of final absorbance at 450 nm. Detection limit of the semi-quantitative ELISA method was 0.5% of the weight of added plant protein (Fig. 1) (VÚVeL, v.v.i. No 32/2007).

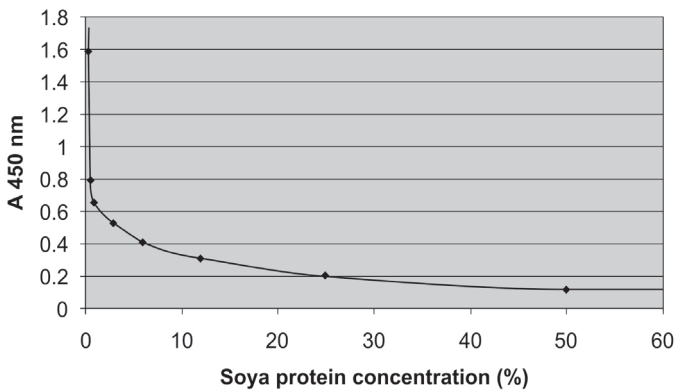


Fig.1. Standard curve for soya protein

## Results and Discussion

The indirect competitive ELISA method developed in our study showed to be sensitive and specific enough to detect meat product adulteration. It can detect added soya protein in the amount of less than 0.5% mass fraction of the plant protein added, regardless of whether the added soya was textured, isolated or concentrated. The detection limit of the method is sufficient, although more sensitive ELISA methods exist that can detect 0.07% (Castro Rubio et al. 2005) or even  $0.4 \mu g$  (0.4 ppm) per 1 g (Koppelman et al. 2004). In addition, the quantitative detection base on the conglycinin detection has been described (Moryama et al. 2005). Soya proteins belong to major food allergens (Directive 2003/89/EC). Thus, the sensitivity of the methods is mainly important for detections of threshold concentrations of allergens. The method is specific enough to detect soya proteins only. From the specificity

testing weak cross-reactivity is seen to other leguminous plants as beans, lentil and peas. Cross-reactivity has been eliminated using saturation (immunoabsorption) of antibody with the respective cross-reacting antigens, followed by centrifugation (Hayden 1979). Cross reactivity did not occur when testing other additives commonly used in meat product recipes such as casein, ovalbumin or wheat flour.

A total of 131 samples of different kinds of heat-treated meat products, largely of sausage and soft salami types were analysed. Among these 131 investigated samples, the soya protein was detected with certainty in 110 (84%) samples, the results were dubious in 5 cases (3.9%) and soya protein was not detected in 16 (12.1%) cases (data not shown).

In 49 products with declared composition by the manufacturer examined for soya protein were only general data (plant protein) in 16 (32.7%) cases. Soya was declared in 21 (42.9%) cases, no plant protein was declared in 12 (24.5%) samples. Plant protein declared generally on the products was confirmed by the ELISA method in all 16 samples. Declared soya was confirmed in 17 (82.2%) of 21 samples. In spite of no plant protein declaration the presence of soya was detected in 10 (72%) of 12 products. Two products were in agreement with the declared composition. The declared presence of soya protein was not confirmed in four cases (Table 1).

The group of investigated samples included packaged products or products packaged on the consumer's request. According to the legislation (Directive 2003/89/EC, Act 120/2008 Coll., Regulation No. 101/2007 Coll.), the presence of potential allergen must be declared on the consumers' packages, either on the foodstuff cover or on the exterior of the packing or its parts. In case of unpackaged foodstuffs or if it is not possible to declare the date on the exterior cover of the packing, it must be a part of documentation that accompanies the foodstuff in the shop. However, it does not follow from the legislation how the consumer should be informed if a foodstuff is packaged in the shop.

Our results showed that the declared soya proteins were not in agreement with the detected results, i.e. soya protein was detected in 84% samples without any declaration (neither general) on the product.

In conclusion, our study shows that the use of vegetable additives, namely soya in meat products in the market of the Czech Republic is very frequent and the restriction of its usage by legislation relates only to the use of plant protein in some kinds of durable products and ham (Act 264/2003 Coll.). The need for sensitive methods for plant protein detection is not only associated with the economic aspect (adulteration), but also another important aspect: the protection of consumers' health threatened by allergy to plant protein. Consumers often encounter potential allergenic components namely in the cases of meat products packaged in the shops without being informed despite valid legislation (Directive 2003/89/EC).

### **ELISA metoda pro detekci sójových proteinů v masných výrobcích**

Byla vyvinuta nepřímá kompetitivní ELISA metoda pro detekci sójových proteinů. Detekční limit metody je 0.5% hmotnostního přídatku sójového proteinu. Celkem 131 vzorků masných výrobků salámů a párků z české tržní sítě bylo vyšetřeno na přítomnost sójových proteinů. Sója byla detekována v 84% výrobků bez jakékoliv deklarace na obalu výrobku. Používání rostlinných proteinů zejména sóji do masných výrobků v České republice je velmi časté a omezení jejího používání legislativou je dané jen u některých trvanlivých masných výrobků a šunky (Vyhláška 264/2003 Sb.). Potřeba citlivých kontrolních metod pro detekci sójových proteinů není důležitá pouze z důvodů ekonomických (falšování), ale především z důvodu ochrany zdraví spotřebitele v případě alergie na sójové proteiny.

Table 1. Results obtained by the examination of meat products with declared composition

Sample No.	Sample name	Declared		ELISA
		Generally (plant proteins)	Specifically - soy	
3	Liver sausage		+	-
10	Soft salami		+	-
13	Bratislava salami		+	+
15	Chicken salami		+	+
16	Cheap salami		+	+
17	Minisalami		+	+
18	Cheap sausage		+	-
19	Chicken sausage	+		+
20	Delicious sausage	+		+
21	Bratislava salami		+	-
22	Knackers		+	+
24	Turkey delicious sausage	+		+
25	Knackers	+		+
26	Spa salami	+		+
27	Slovak salami	+		+
28	Chicken cocktail sausage		+	+
29	Hungarian sausage	+		+
30	Sausage		+	+
32	Bratislava sausage			+
33	Klaus sausage		+	+
34	Strip sausage		+	+
35	Soft sausage		+	+
37	Chicken salami		+	+
45	Cheese sausage	+		+
46	Knackers	+		+
47	Bangers	+		+
48	Wieners	+		+
49	Sausage		+	+
50	Delicious sausage	+		+
52	Cheap salami			+
53	Sausage Extra			+
54	Cheese sausage			-
55	Chicken sausage	+		+
56	Spis sausage			+
57	Tramp sausage			+
59	Bratislava salami		+	+
60	Ham sausage			-
61	Czech Knackers			+
62	Knackers	+		+
63	Turkey hot-dog	+		+
64	Eso sausage			+
65	Strip sausage			+
101	Minisalami		+	+
107	Bavaria chopped meat		+	+
115	Gladiator		+	+
116	Knackers	+		+
120	Franks			+
121	Mushroom delicious sausage		+	+
123	Highlander			+

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