Immunofluorescent detection of soya protein in frankfurters

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Abstract

This work aimed to verify the conformity between the detection of soya protein using the immunofluorescence method and the manufacturer’s declaration of the presence of soya protein in frankfurters purchased from the retail network. Thirty-two samples were tested. These samples were selected with a view to the presence of soya protein declared by the producer on the packaging. The method was validated by comparing positive results produced by the method under investigation with the details given on the packaging of the meat product. Soya protein was detected in 92.8% of samples in which the producer stated the presence of vegetable protein on the packaging and in 94.4% of samples in which the producer stated the presence of soya protein. The results indicate that the immunofluorescence method is suitable for demonstrating the presence of soya protein in meat products.

Allergens, fluorescence methods, foodstuff, immunohistochemistry, meat

Introduction

Soya proteins can be used in the food industry thanks to their excellent nutritional and technological properties and health benefits (L’Hocine and Boye 2007; Singh et al. 2008). In the case of meat products, soya proteins are widely used in emulsified products in view of their unique functional properties, such as their binding capacity with water and fat, the formation of texture and their emulsifying ability, and organoleptic properties such as appearance, cohesion and sliceability (Belloque et al. 2002). Soya proteins also help improve technological processes used in the production of meat products and reduce production costs (Criado et al. 2005). In addition to this, consumers’ demands for healthier and safer products support the use of soya protein in processed meat products (Castro-Rubio et al. 2005). On the other hand, however, soya is, along with cow’s milk, eggs, wheat, peanuts, other nuts, fish, crustaceans and molluscs, responsible for around 90% of food allergies, and is also one of the foodstuffs that can cause anaphylaxis (Sicherer and Sampson 2000). Estimates of the threshold values for inducing an undesirable reaction among people allergic to soya protein vary greatly, and values between 0.0013 and 500 mg have been published (Becker et al. 2004; Ballmer-Weber et al. 2007). The clinical symptoms of soya allergies include digestive problems, respiratory illnesses (colds, asthma), dermatological reactions (rashes, atopic eczema), and IgE-mediated systemic reactions (Batista et al. 2007). There are three basic approaches to the management of food allergies: restricting consumption of the allergen, medical treatment, and consumption of foodstuffs with a low content of the allergen or with a modified allergen. The only option for consumers, however, remains the strict exclusion of the given allergen from the diet. Analytic methods for the determination of soya protein in meat products have been developed to avoid consumers being deceived and to ensure the protection of allergic consumers. The majority of these methods are based on electrophoresis, immunochemistry or chromatography. The main problem involved with the use of electrophoresis is the presence of interference relating to other constituents of the meat product. What’s more, even when electrophoretic methods might prove useful in qualitative studies,
the measurement of bound soya is extremely difficult, particularly at low concentrations. In cases in which it has proven possible to perform a determination of soya protein, this has always been in raw products – this technique has not been used successfully on cooked products (Belloque et al. 2002). High-performance liquid chromatography (HPLC) has also been used to detect soya in meat products. Separation is based on reversed-phase and anion exchange chromatography regimes. Neither of these methods, however, has enabled the unambiguous separation of soya protein, and quantitative evaluation has not been possible. Moreover, these methods have been used on samples prepared in model experiments by the addition of soya protein to raw meat; they have never been used to detect soya protein in commercially produced products or in model products after cooking (Ashoor and Stiles 1987; Parris and Gillespie 1988; Belloque et al. 2002). The aim of this study was, therefore, to develop a method suitable for the determination of soya protein in meat products.

Materials and Methods

The material studied were 32 frankfurters purchased on the retail market, in which the presence of soya protein was evaluated. The detection method selected was immunofluorescence microscopy, as a method more sensitive and more selective than light microscopy. As soon as possible after being purchased on the retail network, the samples were cut into 1 x 1 x 0.5 cm cubes and worked in the form of cryocuts. The cuts produced were transferred to Superfrost Plus slides. Nine slices cut to 50 µm were prepared for the study of each sample. To achieve better fixation of the slice, the samples were first treated with acetone. This was followed by washing in PBS, the blocking of non-specific binding with normal goat serum and the application of primary anti-soy rabbit antibody (Sigma Aldrich). The samples were left overnight in a freezing chamber and again rinsed in PBS the next day. Biotinylated secondary antibody was then applied to the samples, followed by further rinsing in PBS. Finally, fluorochrome was applied to the samples. Three fluorochromes were used to highlight the soya protein in the samples – Texas Red, Fluorescein Streptavidin and AMCA. The immunohistochemical approach according to Pospiech (2011) was used for the control samples. The slices were then mounted and examined in a Leica type -11307072057/BZ: 09 fluorescence microscope and further processed using the LAS AF program. The images obtained were saved in TIFF format. The results were then compared with the details on soya protein given by the producer on the packaging of meat product. One sample in which the presence of soya protein had been demonstrated by the fluorescence method was stained in a traditional immunohistochemical manner and photographed using a light microscope for the purposes of photographic documentation and illustration of the better clarity achieved by immunofluorescence as opposed to traditional immunohistochemistry.

Results and Discussion

Soya protein was detected in 30 samples of the total of 32 meat products in which the presence of vegetable or soya protein was declared by the producer on the product packaging on the basis of its specific morphology and also with the use of fluorescence, which was achieved by an immunohistochemical process and staining with fluorochromes. Immunohistochemical approaches are generally based on a reaction between the allergen and the appropriate labelled antibody. The binding of the labelled antibody was evaluated under a fluorescence microscope with an appropriate filter for each fluorochrome. The experiment was based on the formation of fluorescence staining which signals a positive reaction between the antigen and the antibody. Initial staining took place with the use of the fluorochromes Texas Red, Fluorescein Streptavidin and AMCA. Complications arose, however, with the fluorochromes AMCA and Fluorescein Streptavidin during detection because of the marked fluorescence of the background. For that reason, samples were examined with the use of the fluorochrome Texas Red alone, with which the problem did not arise. (Plate V, Fig. 1) is a microphotograph of soya protein which is differentiated in colour from the black background represented by muscle tissue and other constituents of the meat product and can, therefore, be differentiated from the meat protein which does not fluoresce and is black. For the sake of comparison, (Plate V, Fig. 2) shows soya
protein photographed without the use of a fluorescent cube using a traditional immunohistochemical method. Soya protein could also be detected in the slides on the basis of its specific morphological structure and brown colouring. Soya protein has a characteristic shape comprised of annular, crescent-shaped or circular particles (Horn 1987).

We compared the results we obtained with the details given by the producer on the product packaging. The values relating to soya protein obtained are given in Table 1. Electrophoresis methods or HPLC methods can be used to detect soya protein in meat products, though either these methods cannot be used for determination in cooked meat products (Belloque et al. 2002) or this has not yet been attempted (Aschoor and Stiles 1987; Parris and Gillespie 1988; Belloque et al. 2002). As can be seen from Table 1, the fluorescent immunohistochemistry method appears suitable for the determination of soya protein in frankfurters. We detected soya protein in 13 of the 14 samples for which the producer declared the presence of vegetable protein. One product was negative for soya protein, which may have been caused by the use of a vegetable protein other than soya in the product. Eighteen products for which the producer directly stated the presence of soya protein were also investigated. Soya protein was demonstrated in 17 of these products. The presence of soya protein was not demonstrated in one of these samples, which may have been because it had been stated on the product packaging for purely preventative reasons to protect the producer in a situation in which, for example, a production process may have occurred, or perhaps because the binding sites on the soya protein had been deactivated during the course of the technological production process.

<table>
<thead>
<tr>
<th>Protein</th>
<th>Vegetable Declared by the producer</th>
<th>Soya protein detected</th>
<th>Soya Declared by the producer</th>
<th>Soya protein detected</th>
</tr>
</thead>
<tbody>
<tr>
<td>Number of samples</td>
<td>14</td>
<td>13</td>
<td>18</td>
<td>17</td>
</tr>
<tr>
<td>Percentage</td>
<td>100</td>
<td>92.8</td>
<td>100</td>
<td>94.4</td>
</tr>
</tbody>
</table>

Table 1. The presence of soya protein in small meat products

Conclusions

For the purposes of our investigation, we modified the histological paraffin slices method with immunohistochemical highlighting of soya protein with fluorochrome. Instead of the traditional paraffin blocks, we used cryocuts for improved antigen-antibody binding. With the use of this method, it was not necessary to process the samples using the time-consuming paraffin slice technique, and this reduced the time taken by the procedure. Three fluorochromes were used, of which the most suitable was Texas Red in view of the absence of any background fluorescence. This immunofluorescence method for the detection of soya protein was verified by a study of 32 frankfurters purchased on the retail network. The results we obtained were compared with the details given on the product packaging, where soya protein was stated on 18 packages and unspecified vegetable protein in 14 products. It was not possible to determine the type of protein added to the product from this unspecific labelling. We succeeded in detecting soya protein in 30 products, of which 17 were products for which soya protein was stated on the packaging and 13 were products for which an unspecified vegetable protein was stated. The results demonstrate the possibilities for the use of this method to demonstrate the presence of soya protein in meat products.
Acknowledgements

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References


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Plate V
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Fig. 1. Soya protein in green – Texas Red (400 x magnification)

Fig. 2. Soya protein in brown – immunohistochemistry (400 x magnification)