Histochemical detection of pea protein

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Abstract

The meat industry uses vegetable proteins as meat substitutes to reduce the price of its products. Some vegetable proteins, however, have allergenic effects. Efforts are therefore being made to replace such allergenic proteins with other vegetable proteins that are not classified as allergens by the legislation. The most commonly used include pea protein, though this may also cause allergic reactions. Pea protein can be detected by various methods, one of which is microscopy. Detection using this method is based on the identification of structures characteristic of peas. We examined samples of pea flour and protein and model samples of poultry meat with their addition. Samples were processed by microscopic methods, and PAS Calleja staining was chosen as the most suitable. In all samples the addition was detected. To obtain more accurate results, it is necessary to develop and standardize an immunohistochemical staining protocol in order to distinguish between pea and soya proteins, because the particles of these proteins are very similar.

Histochemistry, microscopy, pea flour, plant proteins

Introduction

The current consumer's food choices are influenced by product price. This is one of the reasons why the meat industry uses cheaper raw materials as meat substitutes (Modi et al. 2003). Various protein preparations, e.g. proteins of animal origin (blood plasma, collagen, milk protein) and vegetable origin (soybean, peas), are added to meat products (Kameník et al. 2014). These preparations are added to meat products for various reasons. They may serve to stabilize emulsions, as the dissolved proteins have both hydrophilic and lipophilic groups that act as emulsifiers. They also increase the protein content in products and affect the taste and consistency of products (Kameník 2012).

One possible additive to meat products is pea flour. It may be added to gluten-free products to improve texture and flavour and to increase nutritional value. The low glycemic index of legumes also offers potential benefits for diabetics. Legume fibre increases the total dietary fibre in foods without affecting their sensory properties (Patterson et al. 2010).

Some of these raw materials have allergenic effects (soya, egg or milk proteins) which implies the need for their declaration on consumer packaging (Decree No. 113/2005; Regulation (EC) No. 1169/2011; Kameník et al. 2014). The food industry is therefore trying to replace them with other vegetable proteins that are not included on the list of allergenic substances. The most frequently used include pea protein which can be encountered in certain types of sausages, as reported by Tömösközi et al. (2001). Like other legumes, pea protein contains storage proteins that can cause allergic reactions, from mild skin reactions to life-threatening anaphylactic reactions. Vicilin and convicilin are considered the main pea allergens (Sanchez-Monge et al. 2004). Due to their close relationship, legumes are potentially cross-reactive, and this may further increase the risk of allergic reactions (Verma et al. 2013). Cross-reactions with other plant species such as the peanut have, however, also been described. It is therefore important for consumers to have access to accurate information on the content of pea protein with respect to cross-allergenicity.

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One of the noteworthy possible detection methods published in scientific journals is the analysis of the polyphenols that are characteristic of certain legumes. The High Performance Liquid Chromatography (HPLC) can detect the addition of as little as 0.1% soya protein in a meat product. Lupine can also be detected in the same way, but pea detection has not been accomplished reliably (Mellenthin and Galensa 1999).

Microscopic methods are some of the oldest analytical methods, though they still remain useful in the detection of food components thanks mainly to their simple design and the ability to distinguish and identify individual basic ingredients in foods. The most commonly used are histochemical methods. There are currently a large number of options for the processing and preparation of samples and for investigative techniques, from classical techniques to techniques that employ state-of-the-art equipment, microscopy (optical, light, electron or atomic force) and others. Imaging techniques offer some of the best approaches to the evaluation of food structure (Kaláb et al. 1995). According to Tremlová (2013), microscopic methods can be used to demonstrate the addition of vegetable proteinaceous admixtures if they are present in the product at a suitable size for light microscopy.

The principle of pea identification by microscopic methods is based on the detection of characteristic structures. Layers of palisade, goblet and parenchyma cells are visible in cross-sections of the episperm. Palisade cells are prismatic in shape, 70 – 100 µm long and 10 – 15 µm wide. Goblet cells are bell-shaped, with an expanded apical portion. Parenchyma cells contain increased amounts of extracellular matrix. A pea seed consists of two hemispherical cotyledons. Their epidermal cells contain only aleurone grains, while all the remaining cells contain protein and also starch. The appearance of starch granules varies among different varieties. Yellow pea starch granules are up to 45 µm in size, and are mainly of an oval shape with various depressions and protrusions. Bean-shaped and kidney-shaped granules are also common. Large granules have concentric layering. Spherical shapes prevail in small granules. Garden pea starch granules are round in shape with a round or elongated hollow. These granules disintegrate easily into particles 5 to 15 µm in diameter; intact granules are 20 to 30 µm, occasionally up to 45 µm, in size. Pea starch differentiation can help distinguish between raw materials, because, for example, garden pea flour is not suitable for pea soup or pea porridge as its starch has minimal swelling properties. Pea flour consists mainly of cotyledon cells filled with starch and aleurone grains. The content of episperm fragments depends on whether the flour has been made from skinned or unskinned peas (Hohmann 2007).

Given the lack of reliable methods for the demonstration of the presence of pea proteins in meat products, the aim of our study was to develop a reliable histochemical method for the detection of pea protein in meat products.

Materials and Methods

The microscopic specimens were prepared from a pea-flour and protein sample and heat-treated (70 °C, 10 minutes) model samples with a 2.5% addition of pea flour and protein. Salt (2.5%) was also added to heat-treated model samples. Model samples were processed in the microscopic laboratory of the Department of Vegetable Foodstuffs Hygiene and Technology, University of Veterinary and Pharmaceutical Sciences Brno according to Standard Operating Procedures and were histochemically stained with haematoxylin-eosin (HE), PAS Calleja (PC) and Toluidine Blue (TB). Sample processing and slide preparation consisted of the following steps: freezing of samples (in the case of heat-treated model samples) / preparation of paraffin blocks (of pea-flour and protein samples), and the cutting, staining and mounting of sections. Individual sections of the prepared samples were examined and photographed under the microscope – Eclipse E200 (Nikon, JPN) EOS 1100D (Canon, JPN) – and the images processed by DSLR REMOTE Version 2.2.2.1 software (Breeze systems, UK).

Results and Discussion

On the basis of photographs of flour samples (Plate I, Fig. 1 – 3), characteristic flour structures were demonstrated in model samples with added flour, such as palisade and

goblet cells (Plate I, Fig. 4 – 6), fragments of cotyledon cells with starch granules, and cells containing pea protein as described in the literature (Hohmann 2007). PAS-Calleja staining was chosen as the most suitable histochemical staining method for the detection of flour addition in model samples. With this staining method, the structures studied were clearly visible and distinguished according to colour at 100X magnification. The numbers of palisade cell fragments in heat-treated model samples with the addition of 2.5% pea flour and protein are shown in Table 1.

Table 1. Number of palisade cell fragments in heat-treated model samples with the addition of pea flour and protein per 1 cm^2

Staining	Samples with pea flour	Samples with pea protein
PAS Calleja	2.75 ± 1.22	0
Toluidine Blue	1.54 ± 0.83	0
Haematoxylin-eosin	1.50 ± 0.71	0

PAS-Calleja staining also proved more suitable than staining with haematoxylin-eosin or toluidine blue when samples of pea protein and samples with added pea protein were examined. Fungiform and round-shaped pea-protein grains were easily discernible at 100X magnification. Pea-protein grains are similar to soya-protein grains (Horn 1987; Tremlová 1998). Although the flour and pea protein structures were also differentiated by the other staining methods, they were more difficult to identify for a less skilled microscopist (Plate I, Fig. 7 and 8; Plate II, Fig. 9 – 12).

Conclusions

The results of our study indicate that PAS Calleja staining should be considered the most suitable histochemical staining for the detection of added pea flour as well as pea protein. This kind of staining produces results sufficiently distinguished by colour even for less experienced microscopists. Flour addition can be identified by the detection of palisade and goblet cells, which occurred in all sections, and also by the finding of starch granules. Such staining does not, however, give us clear proof of pea-protein addition, since grains of pea protein are similar in shape and appearance to soya protein grains. For this reason, it is necessary to develop a standardized immunohistochemical staining protocol that would guarantee unambiguous results.

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Plate I
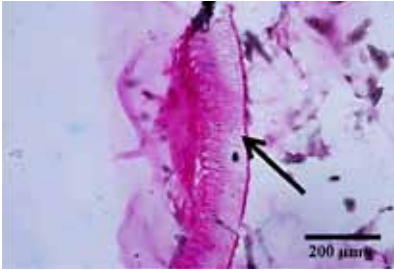Javůrková Z.. et al.: Histochemical ... pp. 3-6
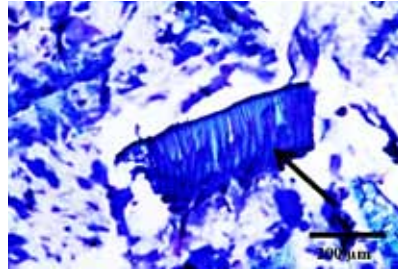

Fig. 1. Palisade cells in pea flour, PC

Fig. 2. Palisade cells in pea flour, TB

Fig. 3. Palisade cells in pea flour, HE

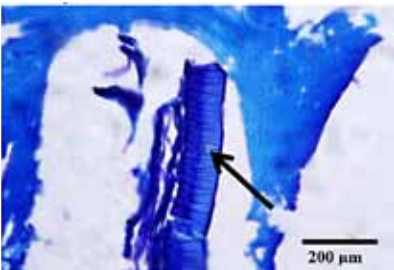Fig. 4. Palisade cells in a heat-treated model sample, PC

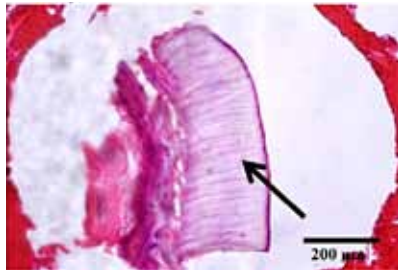Fig. 5. Palisade cells in a heat-treated model sample, TB

Fig. 6. Palisade cells in a heat-treated model sample, HE
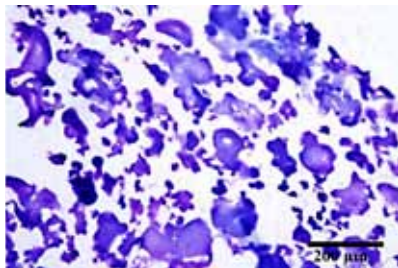

Fig. 7. Pea protein, PC

Fig. 8. Pea protein, TB
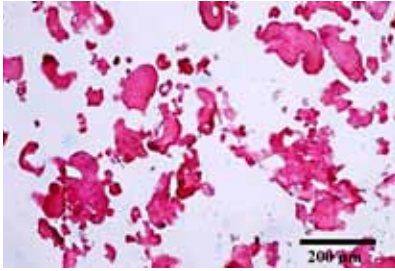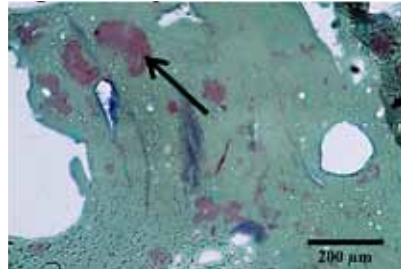
Plate II

Fig. 9. Pea protein, HE

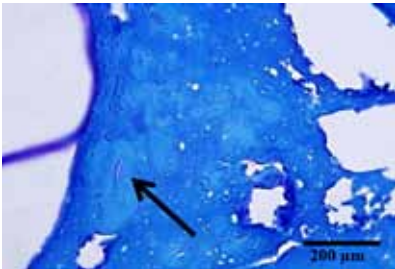Fig. 10. Pea protein in a heat-treated model sample, PC

Fig. 11. Pea protein in a heat-treated model sample, TB

Fig. 12. Pea protein in a heat-treated model sample, HE