Detection of Plant Raw Materials in Meat Products by HPLC

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Abstract

Vaňha J., Hinková A., Sluková M., Kvasnička F. (2009): **Detection of plant raw materials in meat products by HPLC**. Czech J. Food Sci., **27**: 234–239.

The Czech legislation (Decrees No. 326/2001, 202/2003 and 651/2004 of the law No. 110/1997 as amended) regulates the requirements for the selected meat products with regard to the contents of individual ingredients. However, the methods of the determination of compliance with these regulations are not closely specified. The study presented here deals with the development and verification of analytical methods suitable for the detection of the material of plant origin. Due to the high variability in the contents in meat products of these ingredients, various markers were observed (isoflavones, phytic acid, galactooligosaccharides). For the purpose of detection, substances commonly used in food processing industries were taken into account such as soy flour, wheat flour, soy isolate, HAM 60 preparation. The values gained by measuring the given markers were subsequently converted to reflect the amount of the plant based substance added. Out of 18 products commonly available in shops, only 7 filfilled the legal criteria.

Keywords: HPLC; isoflavones; galactooligosaccharides; phytic acid; meat products; adulteration; authenticity

In the case of meat products, adulteration may generally be accomplished by deliberately substituting ingredients, replacing higher quality meat by that of lower quality, such as low fat muscle tissue by tissues with higher fat contents, offal meat or skin and, last but not least, by so called mechanically recovered meat or substituting meat altogether with a non-meat substance, usually in the form of plant based flour. All of the above mentioned are very feasible in reality as different kinds of meat are usually used in the production of meat products, and from the technological point of view non-meat ingredients are essential for the enhancement of the technological qualities of the final products (texture, water binding capacity).

In most cases, the addition of vegetable material up to 4% is possible, nevertheless, 4% is the limit value in terms of sensory acceptance and this amount is applicable only to a very narrow range of meat products.

The substances which can be considered as meat product additives are most commonly various modifications of soy. The exclusive place that soy has among other legumes is given by the chemical composition of its seeds. Soy seeds are an edible and low-cost source of protein. They contain from 15% up to 25% of oil, composed mainly of linoleic acid esters (50%), oleic acid esters (25–30%), and linolenic acid esters (2–10%). Small contents of stearic, palmitic, and arachidonic acids are also present (Mauricio *et al.* 2003). As for saccharides, saccharose and indigestible oligosaccharides rafinose and stachyose can be found. Significant amounts of vitamins B and E and minerals such as calcium, magnesium, and iron are also present. Soy can be added to meat products in the forms of soy isolate, soy concentrate, or soy flour. All these soy products differ mainly in the technology and purity of their processing, but also in the levels of protein content. To mention some other

vegetable substitutes, wheat is used frequently, in some cases being one of the regular ingredients of the recipe. Less common are additives such as pea, lupine, chick pea, amaranth, all of them in the form of flour, and potato starch (Komandi & Dworschak 1988).

As mentioned above, the addition of small amounts of plant based ingredients enhances the technological quality of the product thus being beneficial for the final consumer as it results in the product being more compact and acceptable on the bite. One of the other benefits is the increased viscosity of the final matter, which in turn benefits the yield as it causes a smaller weight loss during the heat treatment, and at the same time makes some of the plant proteins within the matter form structures resembling those of meat. Not to be omitted, the addition of non-meat ingredients means using less of the expensive materials, making the final products cheaper, which is probably the explanation of the wide occurrence of plant based ingredients in all meat products (Gayer 2002).

However, the inclusion of plant ingredients in meat products does not only brings advantages but carries certain risks as well. From the technological point of view, high amounts of plant raw materials cause a higher water binding, resulting in a shorter preservation period, even shorter shelf life, the deterioration of meat products is more frequent due to the higher content of water creating a favourable environment for the growth of majority of microorganisms. In such cases where a low quality plant ingredient is used, the final products may suffer from its scent and influence on the taste, neither of these being desirable for either the producer or consumer. From the consumer's point of view, one of the most important risks involved is the sensitivity of specific groups of people (allergy sufferers) to specific components in their diet (Smith 1990).

It is known that during the thermal processing some allergens change their structure, undergo hydrolysis, enzymatic degradation or thermal inactivation; however, these processes do not apply for example to the heat resistant allergens.

The presence of plant matter can be traced in the heat processed meat products, fermented meat products, and long-life heat processed meat products. Each plant contains certain amounts of substances specific for their particular plant family. In the case of estrogenic flavonoids, analytically significant values are measured in pulses, in comparison to cereals, which contain negligible amounts of the same substances. The presence of such specific substances in the meat products can indicate the use of plant material as a substitute of meat. The essential requirement is the absence of these markers in raw meat. Upon complying with this condition, it is apparent that any traces in the final meat product originate from the spices used in the process or from a deliberate addition of plant mixtures to the product.

At the moment, most commonly used plant based additives are made from soy, wheat and rice flours, but there is a wide range of materials that can be generally used the specific type of additive is determined by the technological nature of the final product (Ambrosiadis & Vareltzis 1998).

Isoflavones are a class of flavonoids which incur various biological impacts. Their incidence is very common in legumes (*Fabaceae* or *Leguminosae* plant family). Most abundant ones are daidzein (7,4'-dihydroxyisoflavone), genistein (4',5,7-trihydroxyisoflavone), formononetin (7-hydroxy-4'-methoxyisoflavone), biochanin A (5,7-dihydroxy-4'-metoxyizoflavon), and coumestrol. In plants they are mostly present in the form of 6''-*O*-acetyl or malonyl 6''-*O*-malonyl derivates (Franke *et al.* 1994; Morton *et al.* 1999).

Galactooligosaccharides (RFO – raffinose family oligosaccharides) are represented by stachyose, raffinose, verbascose, and ajugose. These form a class of indigestible oligosaccharides which cannot be hydrolysed or absorbed in the small intestine and therefore act similarly as the soluble dietary fibre (Cáceres *et al.* 2004). Oligosaccharides are natural compounds found in many types of fruit and vegetable, milk and honey (Velíšek 2002).

Phytic acid known as inositol hexakisphosphate (IP6) is an ester of myo-inositol and phosphoric acid. Formally, inositol belongs among cyclitols (carbocyclic polyols) (Raboy 2003). In the form of phytin, it is the principal storage of phosphorus and microelements for plants in their germinating stage. Phytic acid represents approximately 50–80% of phosphorus in the seeds of cereals, oil plants, and pulses. The amount of phytic acid is reduced by heat processing (Reddy *et al.* 1982). If a pressure higher then atmospheric is applied, the losses are even greater (Duhan *et al.* 2002).

The contents of all markers in the plants are dependent on many factors such as the species, growth conditions, or fertilisation and processing methods.

MATERIAL AND METHODS

The indicators observed were primarily the contents of isoflavones (daidzein and genistein), RFO (stachyose, raffinose and verbascose) and phytic acid. First, the contents of these substances in the plant additives were determined. Subsequently, model samples were created for the purpose of verification of the method. These were prepared as mixtures of raw meat and the particular additive using the proportion ratios of 1, 2, 4, and 5% of soy flour. Finally, 27 samples of actual meat products purchased in retail shops were analysed, using the standards purchased from Sigma-Aldrich Co., USA and other chemicals procured at Penta CR.

Food samples. The following plant materials were observed: coarse soy flour, fine low fat soy flour, pea flour, chickpea flour, rice flour, soy isolate, and HAM 60 preparation (commercial mixture of additives).

Extraction procedures. The methods of extraction as described below were taken from the literature (Graf & Eaton 1990; Frias *et al*. 1996; Skoglund *et al*. 1997). These were modified with respect to the samples used while the suitable concentrations of HCl, BHT, and ethanol had to be found.

Isoflavones and RFO – 1 g of the raw plant material or 5 g of the meat product was homogenised. The extracting solution consisted of 50 ml 80% ethanol (v/v) containing 0.05% BHT (butylatedhydroxytoluene). The sample was refluxed at 100°C for 1 hour. The extract was cooled to the room temperature and centrifuged at 3000 g for 10 minutes. The clear supernatant was filtered through a PTFE micro filter (0.2 µm pore size).

Phytic acid sample weight – 0.5 g of the raw plant material or 2 g the meat product – was homogenised. The extracting solution consisted of 20 ml 0.8M HCl. The sample was refluxed at 100°C for 30 minutes. The extract was cooled to the room temperature and centrifuged at 3000 g for 10 minutes. The clear supernatant was filtered through a PTFE micro filter (0.2 µm pore size) and evaporated. Prior to analysis, the sample was diluted appropriately.

Analytical methods. The literature listed at the end of this article served also as a source of the analytical methods applied throughout the experiment. Certain modifications of the procedures described there were carried out when adjusting the ratio of the mobile phase components and flow rate.

Isoflavones were determined by HPLC using the modular chromatograph (pump P580, UVD detector 170 S, autosampler GINA 50); column Separon SGX C18 (4 × 250 mm, 10 µm; Tessek Prague); detection UV 249 and 259 nm; temperature 30°C; mobile phase 30% acetonitrile and 70% mixture of water and acetic acid (99:1, v/v) with a flow rate of 1 ml/min; loop 20 µl and the time of analysis 20 minutes.

HPLC system (micropump LPC 3001) with refractometric detection was also used for the determination of RFO. The analysis took place on the column Separon SGX C18 (4 × 250 mm, precolumn 150 mm, 5 mm; Tessek Praha), under the conditions of ambient temperature, demineralised water as the mobile phase with the flow rate of 0.3 ml/min, loop 20 µl, and time of analysis 15 minutes.

For the determination of phytic acid content, the HPLC technique (pump GS 50, thermostat STH 585, autosampler 324 Dionex USA) with triple pulsed amperometric detection (PAD) was chosen. The conditions of analysis were as follows: column CarboPak 1 (2 × 250 mm), ambient temperature, mobile phase 16–200mM NaOH, flow rate 0.25 ml/min, loop 15 µl, time of analysis 60 minutes.

RESULTS AND DISCUSSION

Eighteen samples of meat products, 3 samples of fresh meat, and 7 samples of plant additives were analysed. Both isoflavones were found in all plant samples except wheat and rice flours. In all additives, a higher amount of genistein was found as compared to daidzein; this fact is in line with the relevant literature. The highest levels of genistein and daidzein were found in coarse soy flour (725 mg/kg and 578 mg/kg, respectively). Very significant amounts of genistein and daidzein were also observed in soy isolate (426 mg/kg and 306 mg/kg, respectively). In comparison, no isoflavones were traced in wheat or rice flours. The detected amounts of isoflavones in the selected meat products are illustrated in Figure 1. Some of the long-life meat products and also some of those containing single type of meat did not correspond to declaring them as the products with no addition of the plant material.

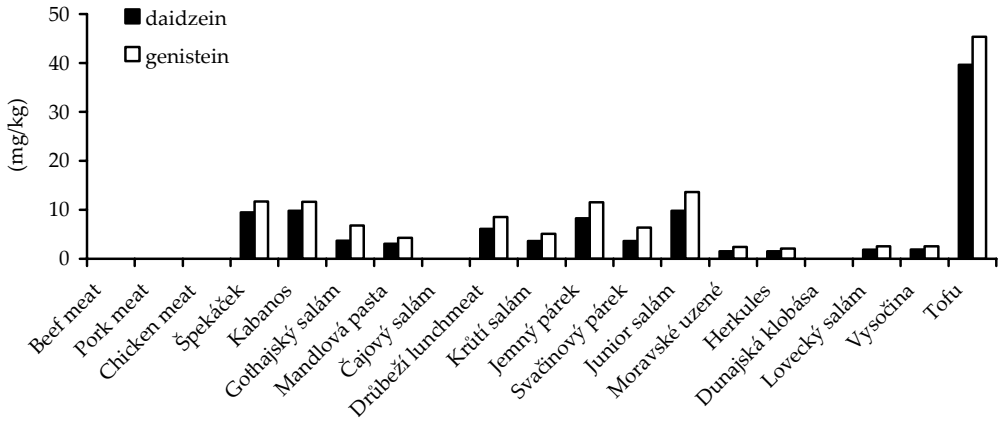

Figure 1. Content of isoflavones in fresh meat and meat products

RFO contents in the observed products were as follows: the highest amount of raffinose was detected in chickpea flour (8.3 g/kg), while the highest content of stachyose was found in soy products (38.7 g/kg), and of verbascose in pea flour (6.3 g/kg). Neglegible amounts were then traced in rice flour and HAM 60 preparation. The determined contents of stachyose, raffinose, and verbascose are represented in Figure 2. Similarly, the same products, which failed the isoflavones test, did not fall within the criteria in this test.

The content of phytic acid was observed in all plant raw materials in analytically significant concentrations in the range of 1.1–12.3 g/kg. The values traced in the meat products are summarised in Figure 3. From all the graphs presented, it is clear that the highest contents of the markers taken into account were present in the lower cost products in which they may be used. However, the values found in some of the long-life products and those composed of a single meat type were not acceptable (e.g. Poličan, Herkules, Debrecínka). The characterisation of the individual methods is shown in Table 1.

Using HPLC method, isoflavones, RFO, and phytic acid were traced in plant raw materials and meat products. Prior to analysis, optimised procedures of the hydrolysis of the samples hyprolysis were determined. The methods were verified on model samples and the final values measured were converted to reflect the amounts of individual ingredients added in the production. The presence of the plant material was declared in seven meat products of the total number of

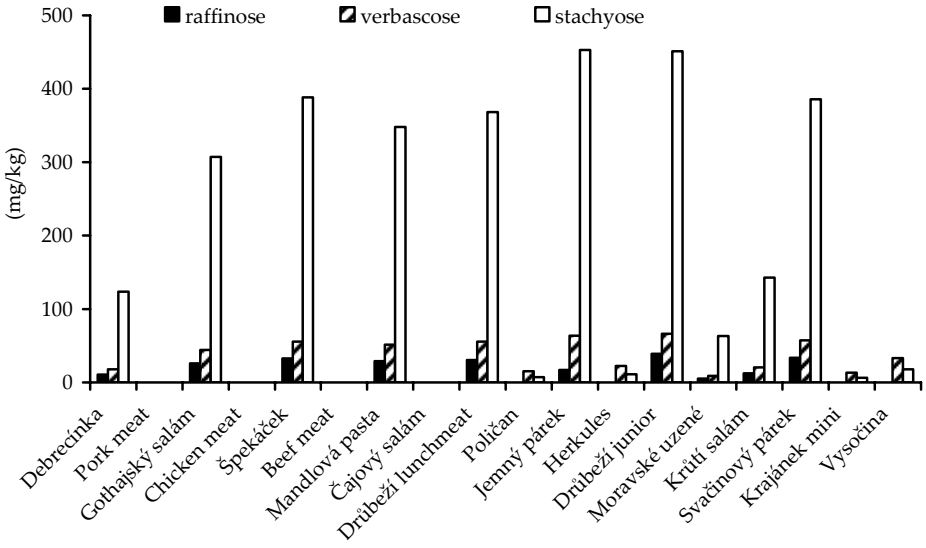

Figure 2. Content of RFO in fresh meat and meat products

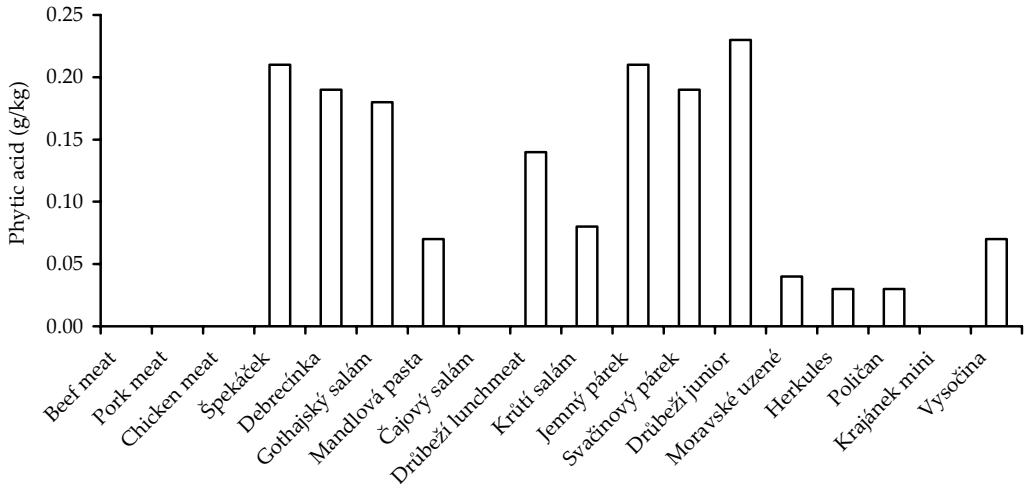

Figure 3. Content of phytic acid in fresh meat and meat products

Table 1. Characteristic of the methods

	Linearity	LOD	LOQ	Reproducibility (%)	Recovery (%)
Isoflavones	0.1–5 mg/l	0.007 mg/l	0.021 mg/l	5.93	98
RFO	0.2–320 µg/l	2.4 µg/l	8 µg/l	5.3	97
Phytic acid	0.0–2.5 mg/l	0.06 mg/l	0.2 mg/l	5.0	93

18 samples analysed. In reality, further 6 of them proved to contain plant materials. RFO tracing has proved to be the most sensitive method, however, it was applicable in such cases where significant amounts of RFO were present in the additives used. The tracing of phytic acid can therefore be named as the most universal method due to the analytically significant amounts present in all additives studied.

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Received for publication November 16, 2008
Accepted after corrections July 29, 2009

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