Methods of Detecting Plant Raw Materials in Meat Products – a Review

Jan VAŇHA and František KVASNIČKA

Department of Food Preservation and Meat Technology, Faculty of Food and Biochemical, Institute of Chemical Technology Prague, Prague, Czech Republic

Abstract

Vaňha J., Kvasnička F. (2011): **Methods of detecting plant raw materials in meat products – a review**. Czech J. Food Sci., **29**: 299–307.

The detection of plant raw materials in meat products is a way to estimate the actual amount of plant raw materials and thus monitor the meat product quality. Markers found in plant materials, that are most a frequently used in the meat processing industry, were selected. In order to prove the presence of plants in meat products, it is essential for the same markers not to be natural compounds of the raw meat as well. Such specific markers include isoflavones, oligosaccharides, phytic acid and starch. The review summarises only the methods used in the last decade.

Keywords: meat products; isoflavones; phytic acid; starch; oligosaccharides

Plant raw materials can be found in nearly all meat products. They may originate from various seasoning mixtures and spices, but also from different types of plant additives used in the meat processing. Theoretically, any plant can be used in the process, however, the key to its suitability lies in the price and taste neutrality, i.e. it must have minimum possible impact on the sensory characteristics of the final product. The lower is the price of the raw material, the greater will be the profits for the producer, as the plant compounds will be always less expensive than the meat. That is the main reason why soy is used predominantly, other options ranging from wheat, peas, and rice to potato starch.

The exclusive place that soy takes among other pulses is given by the composition of its seeds. Soy seeds are one of the cheapest sources of protein. This is the reason for soy being added to meat products in various forms such as soy isolate, soy concentrate, or soy flour. The differences between them are above all in the technology and purity of processing and also in the amounts of protein they contain.

The addition of small amounts of plant based ingredients enhances technological quality of the product and is therefore beneficial for the final consumer as it results in the product being more compact and acceptable on the bite. Another benefit is the increased viscosity of the final product, when higher viscosity in turn increases the yield by reducing the weight loss during heat treatment. Some of the plant proteins are also able to form similar structures in the products as does the meat.

The presence of plant materials can be encountered in heat treated or fermented meat products, and also in meat products heat treated for a long term. Their contents vary, but maximum proportion to be added is roughly 4%, because anything above this level would have a negative impact on the sensory quality of the final product.

Every plant contains certain amounts of substances specific for the given plant group. In the case of legumes the contents of isoflavones, phytic acid, and galacto-oligosaccharides are significant while in cereals, for example, isoflavones occur in negligible amounts. By tracing these indicators, the plant presence can be detected. Prior to the detection, however, their absence in raw flesh of livestock has to be established. This condition be-

ing met, any presence in the meat product can only originate from either the spices used or intentional plant compounds additions. The description of the methods of detection of the above mentioned markers, namely isoflavones, phytic acid, galactooligosccharides, and starch, follows below.

ISOFLAVONES

Isoflavones belong to a group of phytoestrogens, or polyphenol substances synthesised by plants. They are a group of flavonoid substances with various biological effects. The most common ones are daidzein (7.4'-dihydroxyisoflavone), genistein (5.7.4'-trihydroxyisoflavone), formononetin (7-hydroxy-4'-methoxyisoflavone), biochanin A (5.7-dihydroxy-4'-metoxyisoflavone), and coumestrol (3,9-dihydroxy-6-benzofurano[3,2-c]chromenone) (Franke *et al.* 1994; Velíšek 1999).

In plants, these are usually found in the form of glucosides such as 7-β-D-glucoside or their acetyl 6''-*O*-acetyl malonyl 6''-*O*-malonyl derivatives (Morton *et al.* 1999).

Chromatographic methods

Choice of a specific chromatographic method to be used depends on the sensitivity required and the complexity of the biological matrix. Selecting the correct method is crucial due to its impact on the time spent in working up the simplex prior to analysis, the chromatographic resolution required, and the expenses incurred.

The second important issue with isoflavones analysis is whether or not to measure them in their conjugated or unconjugated form. For the most part, food contains glycosidic conjugates with the exception of fermented products.

In order to carry out quantitative measurements of isoflavones by chromatographic method, the internal standards are necessary to include the compensation of unknown losses during the procedure.

Gas chromatography (GC)

This method has been the basis of isoflavones analysis for the past 20 years, particularly because of the usually low concentrations of isoflavones. A clean up procedure is usually carried out to remove co-extracted compounds from the biological matrix that would otherwise be detrimental to the final analysis.

GC-MS detection route method was developed to determine quantitatively daidzein, genistein, glycitein, glycitin, 6''-*O*-acetyldaidzin, 6''-*O*-acetylglycitin, and 6''-*O*-acetylgenistin contents. The sample preparation of the plant or food samples starts with grinding, before the extraction with aqueous ethanol or methanol. The majority of GC-MS analyses have focused on determining the total flavonoid aglycone concentration. The sample preparation using reversed-phase SPE is also widely used for flavonoid analysis in biological samples and food. Sample preparation is based on enzymatic hydrolysis, ether extraction, acidic hydrolysis, and chromatography on DEAE-sephadex-OH^- and QAE-sephadex-AC^-. GC condition: silica capillary column (12.5 m × 0.22 mm *i.d.*, 0.25 m), initial temperature was 100°C, held for 1 min, then raised to 280°C at a rate of 30°C/minutes. Time of analysis was approximately 20 min (Prasain 2004; Wu *et al.* 2004).

High Performance Liquid Chromatography (HPLC)

HPLC separation of isoflavones is generally carried out on a reversed-phase column with the mobile phase of methanol or acetonitrile and water containing small amount of an acid as modifier (acetic, trifluoracetic, or phosphoric acids). The structure of phytoestrogens and their metabolites mostly contains phenolic hydroxyl groups, which show a weak acidic nature. A modifier of the mobile phase significantly increases the detecting sensitivity by enhancing positive ion formation.

HPLC photo diode array UV detection. In this method, the way in which isoflavones are extracted from the sample matrix using hydrolysis with hydrochloric acid (HCl) and butylatedhydroxytoluene (BHT), preventing them from decomposition, is crucial. Other criteria for the success of this analysis procedure include the time it takes and the temperature it reaches. Instead of hydrolysis, methanol (MeOH), water, or dimethylsulfoxide can be used to extract the isoflavones from the sample. The selection of the suitable extraction agent has been dealt with by Luthria among others (Vaňha *et al.* 2002; Achouri *et al.* 2005; Luthria *et al.* 2007). Both gradient and isocratic elution can

be used for the purpose of the modified sample analysis. The proportions of mobile phases will vary slightly in solutions with different ratios. Acidified MeOH and water with acetic acid are usually used. The evaluation of the data gained can be carried out using internal standards (bisphenol A, dihydroxybenzaldehyde). The wave length of the detection varies between 245 nm and 254 nm (Klejdus *et al*. 2005; Rostagno *et al*. 2007).

HPLC coulometric electrode array detector. Another option for isoflavones detection is HPLC with coulometric electrode array detector. The modification of the sample slightly differs from the previous method in chemicals used and in sample purification. The electrode array detector has a potential at the first electrode of +390 mV, increasing in increments of 60 mV to the eighth electrode of +810 mV, against modified palladium electrodes (Müllner & Sontag 2000).

HPLC-MS (mass spectrometry) detection. The preparation of the sample and the extraction of isoflavones are made using the ultrasound assisted extraction device. The extraction can be carried out in acetonitrile, water, or MeOH. Acetonitrile, pure or blended with formic acid, has proved to be the most practical in this process. The author identically presents the same linear gradient of the mobile phase. This method can be used to identify glycosides, aglycone isoflavones, and acetylisoflavones. Some of the most important parameters are a suitable method and the number of extractions, length of sonication, and the solvent and temperature applied. The system was coupled on-line to a mass selective HP MSD detector using electrospray ionisation (ESI). Isoflavones were separated on C18 reversed-phase chromatographic column (150 mm × 2.1 mm, 3 m) particle size. The mass spectrometer was regularly calibrated with an ESI tuning solution. The nebuliser gas pressure was 350 kPa, the drying gas was nitrogen at the temperature of 300°C and capillary voltage was 3500 V (Klejdus 2004).

Capillary electrophoresis – CE. CE is a relatively new separation technique compared to chromatographic methods such as GC and HPLC. Basically, the separation by CE is the result of differences between electrophoretic mobilites of the charged species in electric field in small-diameter capillaries.

ED (electrochemical detection). This method has also been adapted for CE separation. However, it may be necessary to separate the electrochemical detector (ED) from the CE capillary, since high noise levels may occur when the detector is exposed to the high electric field used for CE.

The detection of isoflavones such as daidzein, genistein and biochanin A using the CE technique was studied by Peng *et al*. (2004) and Peng and Ye (2006). The samples were extracted for 2 h in 70% solution of MeOH in the ultrasound assisted extraction device. The key factors observed in both cases were the interdependence of the separation voltage on the migration time, pH, concentration of the buffer, and also the relation of the separation voltage and injection time to the detection limits of the analytes. In either case 95% recovery was reached.

GALACTOOLIGOSACCHARIDES

Galactooligosaccharides belong to a sub-group of oligosaccharides and together with monosaccharides form a group commonly known as sugars. They share many characteristics and also their sweet taste. Typical examples are stachyose, raffinose and verbascose and ajugose. Galaktooligosaccharides are stable under heat treatment for a period of 10 min, temperature of 160°C, and neutral pH.

Gas chromatography

GC-MS detection – is based on mass spectral and retention data determined on two capillary columns with different stationary phases and allows the identification and quantification of galactooligosaccharides. The samples were prepared with 80% ethanol, the solution was mixed with phenyl-D-glucoside and evaporated under vacuum. Sugar oximes were formed using hydroxylamine chloride in pyridine and heated. After the reaction, the samples were persilylated using hexamethyldisilazane (HMDS) and trifluoroacetic acid and centrifuged. GC-MS analyses were carried out using the same capillary columns, installed in a chromatograph with quadruple mass detector working in electron ionisation (EI) mode at 70 eV. Helium was used as carrier gas, and injections were made in the split mode (Sanz *et al*. 2004).

High Performance Liquid Chromatography

HPLC-RID refraction index detection; the separation of galacto-oligosaccharides by HPLC involves

the extraction of the analyte prior to the analysis together with centrifugation and microfiltration. Ethanol blended with water proved to be the best extraction agent. The optimum concentration was tested as well as the duration of extraction and temperature of the bath. The composition of the extraction agent varied between 50% and 80% ethyl hydroxide EtOH in water (v/v). The duration of extraction was not definite either but varied between 30 min and 120 minutes. Nevertheless, the optimum temperature appeared to be 100°C in all cases. In all cases, the analysis was preceded by centrifugation and filtration through a microfilter. The mobile phases varied again. In one of the cases, demineralised water was used while in another one it was acetonitrile and water, 75% and 25% (v/v), respectively (Vaňha & Kvasnička 2005; Ekvall *et al*. 2007).

High Performance Anion Exchange Chromatography HPAEC

Pulsed amperometric detection (PAD) – the modification of the sample was identical to the ones described above, however, the sample was evaporated to dryness and subsequently diluted with water prior to the microfiltration. The supernatant was recovered and evaporated at reduced pressure. The extract was finally dissolved in 1 ml water, filtered, and analysed. The mobile phase was created using 50% NaOH. Analysis duration could be reduced substantially by using NaOH gradiant elution (Vaňha & Kvasnička 2005; Bansleben *et al*. 2008).

Mass spectrometry detection (MS) – the process of sample preparation was the same as in the case of PAD detection. Carbohydrates were detected using KOH gradient elution. A desalter with a constant current 65 mA and post-column addition of LiCl had to be used. MS data were acquired on quadrupole spectrometer with an electrospray interface (ESI) (Guignard *et al*. 2005).

High Performance Capillary Electrophoresis

HPCE represents another fast, easy and reproducible method of determination of the raffinose family of oligosaccharides (RFO) or α-galactosides. The identification was carried out using indirect UV detection at moderate alkaline pH 9.2 and using pyridine-2.6-dicarboxylic acid as the background electrolyte in a sodium tetraborite buffer with added cetyltrimethylammonium bromide. In the following instance, the extraction took place in quite a different manner – twice with a hot mixture of ethanol and water. The solution of manitol was used as the internal standard (www.noack.cz).

Enzyme analysis

One of the fast and precise ways of identifying raffinose is based on its hydrolysis at pH 4.5 to D-galactose and saccharose in the presence of α-galaktosidase.

$$\text{raffinose} + H_2O \xrightarrow{\alpha\text{-galaktosidase}} \text{D-galactose} + \text{sucrose}$$

At the 2nd stage, nicotinamide adenine dinucleotide (NAD^+) is oxidised by D-galactose to D-galactonic acid in the presence of β-galactose dehydrogenase (Gal-DH)

$$\text{D-galaktose} + NAD^+ \xrightarrow{\text{GAL-DH}} \text{D-galactonic acid} + NADH + H^+$$

The amount of NADH formed in the reaction is stoichiometric to the amounts of lactose and D-galactose, respectively. The increase in NADH is measured by its light absorbance at 334, 340, or 365 nm (Vaňha & Kvasnička 2005; www.noack.cz).

Phytic acid

Phytic acid is the ester of myo-inositol and phosphoric acid (myo-inositolhexakisposphate, IP6). It represents around 50–80% of phosphorus found in the seeds of cereals, oil plants, and legumes. The content of phytic acid in plants depends on the environmental conditions. It is important to say that even though the products of phytic acid hydrolysis are decomposed and absorbed within the digestive system of different types of livestock, they are never present in their meat.

Gas chromatography

GC-MS determination of phytic acid is based on anion-exchange chromatography, enzymatic hydrolysis of phytic acid to myo-inositol, and derivation trimethylsilyl derivate, with scyllo-inostiol as

an internal standard. Gas chromatograph uses a fused-silica capillary column SPB-20, and He as the carrier gas. The column temperature was programmed from 120°C to 250°C at 8°C per minutes. The column pressure was increased from 47.8 kPa to 95.4 kPa at 2.9 kPa·min in order to keep the gas flow at 0.8 ml/minutes. The injector port and the detector interface were kept at 250°C (March *et al.* 2001).

GC-FID detection – The sample was treated twice with chloroform-methanol (2:1, v:v). The defatted samples were extracted with 2.4M HCl except for the GC analysis, for which they were extracted with 0.5M HCl. The extract was centrifuged at 3500 rpm for 10 minutes. The detection was carried out at 260°C (Park *et al.* 2006).

High Performance Liquid Chromatography

HPLC method on reverse phase with UV-Vis detection was based on the metal replacement reaction of phytic acid from its coloured complex (iron (III)-thiocyanate), separation and monitoring of the decrease in the concentration of the coloured complex with UV detection. The content of phytic acid in the samples was calculated using the calibration curve obtained from the standard solution of phytic acid and iron (III)-thiocyanate solutions containing 100 µg/ml iron (III) ion. The spectrophotometric determination was based on the metal replacement reaction of phytic acid from its coloured complex with iron (III)-5-sulfosalicylic acid followed by reading the absorbance at 500 nm. The method used here is a modification by some researchers who used iron (III)-5-sulfosalicylic acid to detect the absorbance changes (by a UV-vis detection) in iron (III)-5-sulfosalicylic acid after the metal replacement reaction with phytic acid, followed by a chromatographic separation (Latta & Eskin 1980; Plaami 2001; Dost & Tokul 2006).

Another possibility of using HPLC techniques is RI detection. The samples were extracted with 2.4M HCl except for the HPLC analysis, for which they were extracted with 0.5M HCl. The extract was centrifuged at 3500 rpm for 10 min and analysed on C_{18} column with the mobile phase of 0.025M KH_2PO_4 (pH 6.0) solution and at ambient temperature (Lee & Abendroth 1983; Haraland *et al.* 1986; Park *et al.* 2006)

In HPAEC-PAD pulsed amperometric detection, the sample preparation consists of its disintegration with 10M HCl. The mixture of the acid and sample is extracted in water bath and refluxed. The refluxed sample is then centrifuged and the supernatant is filtered through micro filter 0.45 µm. For the purpose of phytic acid detection, gradient elution is used; the time used for the analysis was 60 minutes (Vaňha & Kvasnička 2005).

HPAEC-CD conductivity detector, unlike many other methods, does not imply $FeCl_3$ coagulation and its reverse conversion to phytate content when determining phytic acid. This method can be reliably applied in the cases of the products with a higher lipid content, which is removed during the sample preparation (Talamond *et al.* 2000). This method can also be combined with UV detection, but in this case phytic acid can only be detected by following the previous post-column reaction with $FeCl_3$ (Chen & Li 2003).

Capillary Isotachophoresis (CI). Phytic acid was extracted using 0.95M HCl and separated from the interfering compounds by iron precipitation with $FeCl_3$. The following boiling and centrifugation determination of Fe^{3+} residual from supernatant was carried out at 565 nm. The iron concentration in the supernatant was used for the calculation of Fe present in the precipitate. The precipitate was dissolved in 1.5M NaOH, shaken and centrifuged, pH was adjusted with HCl and the solution was analysed by isotachphoresis (Blatny *et al.* 1995; Dušková *et al.* 2001; Feil 2001).

STARCH

Anhydroglucose residue, known under its empirical formula $C_6H_{10}O_5$, forms the basis of starch. This is a compound of two homopolysaccharides (amylose and amylopectin). Individual types of plants are characterised via the content, size, and shape of starch grains.

The above were therefore used in microscopic methods for the identification of starch and soy protein and determination of the amounts of these additives using model samples. The model samples contained ground muscle tissue and additives of plant origin – potato starch, wheat starch (flour), and soy protein. The additives proportions were 0.5%, 1.0%, 2.5%, 5.0%, and 10%. The samples were processed using classical histological technique of paraffin cuts. The evaluation was then done using the following methods: approximate determination of the additive amount, semi-quantitative determina-

Table 1. Characteristic of methods of isoflavone analysis

Methods	LOD	LOQ	Reproducibility (%)
GC–MS[1]	12.3 ng/ml	N/A	95.5–105.5
HPLC–DAD UV[2]	N/A	N/A	96–102
HPLC–MS[3]	0.8 ng/ml	2.8 ng/ml	103
CE–ED[4]	0.02 ng/ml	0.09 ng/ml	N/A

[1]Prasain (2004); [2]Klejdus *et al.* (2005), Rostagno *et al.* (2007); [3]Klejdus (2004); [4]Peng and Ye (2006)

tion of the additive amount by means of ocular net, quantitative determination of the additive amount using picture analysis (Renčova 2007).

Starch content in foodstuffs may also be determined polarographically. Starch is converted into its soluble form using HCl. This method utilises its specific optical rotation, which ranges from $(\alpha)_D$ +90° to +202°. Even small amounts of starch can be thus detected (CSN EN ISO 10520).

In the case that just sheer presence of starch is to be determined, its colour reaction with iodine solution can be utilised. A very specific colour reaction ensues due to the reaction with iodine mixed with potassium iodide (Vaňha 2007).

Starch can also be detected using enzymatic sets. In such case, starch is determined colorimetrically (www.noack.cz).

DISCUSSION

The evaluation of the methods used to determine the individual markers of plant raw materials in meat products is discussed in terms of their time consumption, duration of analysis, limits of detection, and suitability for the analysis of the meat products.

Isoflavones

The analysis of isoflavones using gas chromatography requires a lengthy preparation of the sample and up to now has only been successfully tested on liquid biological samples. It is further disadvantaged by the long period of time needed for the derivation, of the samples and high costs of energy required for the analysis. Sensitivity is comparable to that with liquid chromatography.

The methods of liquid chromatography used to identify isoflavones have already been described on many occasions in the past. DAD and MS detection of isoflavones is a prevalent method of liquid chromatography. The preparation of the samples is again time consuming, however, the subsequent analysis is very rapid and has already been tested on isoflavones in meat products.

Capillary electrophoresis technique is also very suitable for this type of products. Time needed to prepare and analyse the samples is comparable to those given above. In comparison with GC and HPLC methods, capillary electrophoresis is characteristic for its higher sensitivity. Table 1 shows the characteristics of isoflavone analysis methods.

Galactooligosaccharides

Gas chromatography method for determining galactooligosaccharides is applicable to products containing higher amounts of these saccharides. No quality and reproducible results could be obtained, when products with the content of 4% and lower of plant additives were analysed.

Table 2. Characteristic of methods of galakto-oligosaccharides analysis

Methods	LOD	LOQ	Reproducibility (%)
GC–MS[1]	2.5 mg/l	8.3 mg/l	96.6–105.5
HPLC–RID[2]	0.7 mg/l	2.3 mg/l	98–101
HPLC–PAD[3]	2.4 µg/l	8 µg/l	97–99
Enzyme[4]	3 mg/l	5 mg/l	97

[1]Sanz *et al.* (2004); [2]Vaňha and Kvasnička (2005); [3]Guignard *et al.* (2005); [4] www.noack.cz

Table 3. Characteristic of methods of phytic acid analysis

Methods	LOD	LOQ	Reproducibility (%)
GC – FID[1]	N/A	N/A	72
GC – MS[2]	19 µg/l	N/A	98
HPLC – UV Vis[3]	0.5 µg/ml	1.6 µg/ml	96
HPLC – RI[1]	1 mg/ml	3.1 mg/ml	79–96
HPAEC – PAD[4]	0.34 mg/ml	1.1 mg/ml	93–97
HPAEC – CD[5]	N/A	N/A	N/A
CI[6]	2 mg/ml	N/A	93

[1]Park *et al.* (2006); [2]March *et al.* (2001); [2]Plaami (2001); [4]Vaňha and Kvasnička (2005); [5]Talamond *et al.* (2000); [6]Dušková *et al.* (2001)

HPAEC-PAD method for the detection of raffinose, stachyose, and verbascose in meat products was used in the past and proved to be highly sensitive. In terms of the detection limits and concentration of saccharides in the sample, the remaining methods such as HPAEC-MS, HPCE-DAD are also convenient. A slight disadvantage is caused by the time consuming preparation of the samples.

Enzymatic identification of raffinose can be used to analyse materials with a higher content of this saccharide, i.e. meat products in the lower price range, where a high content of plant materials is expected. Table 2 shows the characteristics of the methods of galactooligosaccharides analysis.

Phytic acid

The procedure of phytic acid detection using gas chromatography is time consuming due to the fact that it involves hydrolysis of phytic acid. It is also unsuitable for testing meat products, which contain only low levels of phytic acid.

Both methods of phytic acid detection using HPLC with UV detection are based on the absorption of Fe^{3+} ions and according to the measured values seem to be suitable for identifying phytic acid in meat products due to their high sensitivity. The detection of phytic acid using the HPAEC technique with the conductivity detector has already proved suitable even for the products with very low contents of plant additives (long life salamis). The time of analysis is twice as long when compared to gas chromatography; however, the reproducibility is considerably higher and ranged between 93–97%.

CI (capillary isotachophoresis) method was used to estimate phytic acid in feeds and faeces of pigs. Due to high sensitivity, it could probably be applicable to its determination in meat products. Table 3 shows the characteristic of the methods of phytic acid analysis.

Starch

The enzymatic method of starch identification is suitable for meat products. It is highly sensitive and provides reproducible results; it is however somewhat time and money consuming and can only be applied to small numbers of samples.

The proof of starch by using iodine solution is very fast, however, it cannot be applied to fermented meat products. The high content of fat prevents the adhesion of the solution to the surface of the product.

Microscopic and polarographic analyses of starch are both very precise, but only suitable for plant materials (seeds, flour etc.), where they provide high-quality results. Microscopic identification of plant additives in meat products is rather approximate; the values measured do not correspond precisely to the actual content of starch even in just the model samples of meat products.

CONCLUSION

The methods of electrophoretic and liquid chromatography are suitable for the analysis of meat products in relation to all markers except for starch. Both these methods are characteristic for their high detection limits, good reproducibility and

recovery. Most of the methods described above need modifications to be successfully applied onto the meat products, as they have all been tested mainly on plant materials. However, due to their high sensitivity, limits of detection and quantification, they are all deemed to be suitable for determining identical markers in the meat products. Good results in detecting starch can only be gained using enzymatic analysis.

References

Achouri A., Boye J.I., Belanger D. (2005): Soybean isoflavones: Efficacy of extraction conditions and effect of food type on extractability. Food Reserch International, **38**: 1199–1204.

Bansleben D., Schellenberg I., Wolff A.Ch. (2008): Highly automated and fast determination of raffinose family oligosaccharides in *Lupinus* seeds using pressurized liquid extraction and high-performance anion-exchance chromatography with pulsed amperometric detection. Journal of the Science of Food and Agriculture, **88**: 1949–1953.

Blatny P., Kvasnicka F., Kenndler E. (1995): Determination of phytic acid in cereal grains, legumes, and feeds by capillary isotachophoresis. Journal of Agriculture Food Chemistry, **43**: 129–133.

Chen Q.C., Li B.W. (2003): Separation of phytic acid and other related inositol phosphates by high-performance ion chromatography and its applications. Journal of Chromatography A, **1018**: 41–52.

CSN EN ISO 10520 (1999): Přírodní škrob – Stanovení obsahu škrobu – Ewersova polarimetrická metoda. .

Dost K., Tokul O. (2006): Determination of phytic acid in wheat and wheat products by reverse phase HPLC. Analytica Chimica Acta, **558**: 22–27.

Duškova D., Marounek M., Březina P. (2001): Determination of phytic acid in feeds and faeces of pigs and poultry by capillary isotachophoresis. Journal of the Science of Food and Agriculture, **81**: 36–41.

Ekvall J., Stegmark R., Nyman N. (2007): Optimization of extraction methods for determination of raffinose family oligosaccharides. Jounal of Food Composition and Analysis, **20**: 13–18.

Feil B. (2001): Phytic acid. Journal of New Seeds, **3**: 1–35.

Franke A.A., Custer L.J., Cerna C.M., Narala K.K. (1994): Quantification of phytoestrogens in legumes by HPLC. Journal of Agriculture Food Chemistry, **42**: 1905–1913.

Guignard C., Jouve L., Bogeat-Triboulot M.B., Dreyer E., Hausman J.F., Hoffmann L. (2005): Analysis of carbohydrates in plants hy HPAEC coupled with electrospray mass spectrometry. Journal of Chromatography A, **1085**: 137–142.

Haraland B.F., Oke O.L., Felix-Phipps R. (1986): Preliminary studies on the phytate content of nigerran foods. Journal of Food Composition and Analysis, **1**: 202–205.

Klejdus B., Mikelová R., Adam V., Zehnálek J., Vacek J., Kizek R., Kubáň V. (2004): Liquid chromatographic–mass spectrometric determination of genistin and daidzin in soybean food simplex after accelerated solvent extraction with modified content of extraction cell. Analytical Chimica Acta, **517**: 1–11.

Klejdus B., Mikelová R., Petrlová J., Potesil D., Adam V., Stiborová M., Hodek P., Vacek J., Kizek R., Kubán V. (2005): Determination of isoflavones in soy bits fast column high-performance liquid chromatography coupled with UV-visible diode-array detection. Journal of Chromatography A, **1084**: 71–79.

Latta M., Eskin M. (1980): A simple and rapid colorimetric method for phytate determination. Journal of Agriculture and Food Chemistry, **28**: 1313–1315.

Lee K., Abendroth J.A. (1983): High Performance Liquid Chromatographic determination of phytis acid in foods. Journal of Food Science, **48**: 1344–1345.

Luthria D.L., Biswas R., Natarajan S. (2007): Comparison of extraction solvents and techniques used for the essay of isoflavones from soybean. Food Chemistry, **105**: 325–333.

March J.G., Simonet B.M., Grases F. (2001): Determination of phytic acid by gas chromatography – mass spectroscopy: application to biological samples. Journal of Chormatography B, **757**: 247–255.

Morton M., Arisaka O., Myiake A., Evans B. (1999): Analysis of phytoestrogens by gas chromatography – mass spectrometry. Environmental Toxicology of Pharmacology, **7**: 221–225.

Müllner C., Sontag G. (2000): HPLC with colometric electrode array detection Determination of daidzein and genistein in soy based infant food, soy milk and soy based supplements. European Food Research and Technology, **211**: 301–304.

Park H.R., Ahn H.J., Kim S.H., Lee Ch.H., Byun M.W., Lee G.W. (2006): Determination of the phytic acid levels in infant foods using different analytical methods. Food Control, **17**: 727–732.

Peng Y., Chu Q., Liu F., Ye J. (2004): Determination of isoflavones in soy products by capillary electrophoresis with electrochemical detection. Food Chemistry, **87**: 135–139.

Peng Y.Y., Ye J.N. (2006): Determination of isoflavones in red clover by capillary electrophoresis with electrochemical detection. Fitoterapia, **77**: 171–178.

Plaami S. (2001): Myoinositol phospahtes: analysis, content in foods and effects in nutrition. Lebensmittel-Wissenschaft und -Technologie, **30**: 633–647.

Prasain J.K. (2004): Mass spectrometric methods for the determination of flavonoids in biological simplex. Free Radical Biology and Medicine, **37**: 1324–1350.

Renčova E. (2007): Microscopic method and image analysis of starch in food. In: VIIIth Symposium of Zouny Research Wolker, Brno.

Rostagno M.A., Miguel P., Baroso C.G. (2007): Fast analysis of soy isoflavones by high-performance liquid chromatography with monolitics column. Analytica Chimica Acta, **582**: 243–249.

Sanz M.L., Sanz J., Castro I.M. (2004): GC-MS method for the qualitative and quantitative determination of disaccharides and trisaccharides. Journal of Chromatography A, **1059**: 143–148.

Talamond P., Doulbeau S., Gochette I., Guyot J.P., Treche S. (2000): Anion-exchange HPLC with conductivity detection for the analysis of phytic acid in food. Journal of Chromatography A, **871**: 7–12.

Vaňha J., Prokoratová V., Kvasnička F. (2002): Detekce sójových bílkovin v masných výrobcích. Maso, 13: 36–40.

Vaňha J., Kvasnička F. (2005): Detekce rostlinných surovin v masných výrobcích. Maso, **16**: 32–36.

Vaňha J. (2007): Metody pro detekci falšování masných výrobků. [Dissertation.] Vysoká škola chemicko-technologická v Praze, Praha.

Velíšek J. (1999): Chemie potravin 3, Ossis, Tábor.

Wu Q., Wang M., Simon J.E. (2004): Analytical methods to determine phytoestrogenic compounds. Journal of Chromatography B, **812**: 325–355.

Xiaoli X., Liyi Y., Shuang H., Wei L., Yi S., Hao M., Jusong Z., Xiaoxiong Z. (2008): Determination of oligosaccharide content in 19 cultivars of chickpea seeds by HPLC. Food Chemistry, **111**: 215–219.

www.noack.cz. Boehringer Mannheim/R-Biopharm, No. 0428167

Received for publication February 1, 2010
Accepted after corrections March 23, 2011

Corresponding author:

Ing. Jan Vaňha, Vysoká škola chemicko-technologická v Praze, Fakulta potravinářské a biochemické technologie, Ústav konzervace potravin a technologie masa, Technická 5, 166 28 Praha 6, Česká republika
e-mail: jan.vanha@centrum.cz