KAUNAS UNIVERSITY OF TECHNOLOGY

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# PHYTOESTROGENS AND THEIR METABOLITES IN BERRIES, VEGETABLES AND CEREAL PRODUCTS AND THE RELATIONSHIP WITH COMPONENTS OF DIETARY FIBRE

Summary of Doctoral Dissertation

Physical sciences, Chemistry (03P)

KAUNAS, 2005

The research was accomplished at Kaunas University of Technology, Department of Food Technology (Lithuania); Vienna University, Institute of Analytical Chemistry and Food Chemistry (Austria), VTT Biotechnology (Finland) and Martin-Luther University, Institute of Nutrition Sciences (Germany) during 2000-2004.
It was supported by Lithuanian State Science and Studies Foundation.

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The summary of the disertation has been sent 11-04-2005.
The Dissertation is available at the library of Kaunas University of Technology.

**KAUNO TECHNOLOGIJOS UNIVERSITETAS**

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# UOGŲ, DARŽOVIŲ IR GRŪDŲ PRODUKTŲ FITOESTROGENAI, JŲ METABOLITAI IR RYŠYS SU SKAIDULINIŲ MEDŽIAGŲ KOMPONENTAIS

**Daktaro disertacijos santrauka**

**Fiziniai mokslai, chemija (03P)**

**KAUNAS, 2005**

Disertacija rengta 2000-2004 metais Kauno technologijos universiteto Cheminės technologijos fakultete, Maisto produktų technologijos katedroje (Lietuva); Vienos universitete, Analizinės chemijos ir maisto chemijos institute (Austrija); VTT Biotechnologijos centras (Suomija) ir Martino Liuterio universitete, Mitybos mokslų institute (Vokietija).
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Disertacija bus ginama viešame Chemijos mokslo krypties disertacijos gynimo tarybos posėdyje, kuris įvyks 2005 m. gegužės 11 d. 11 val. Kauno technologijos universitete, Centrinių rūmų Disertacijų gynimo salėje (K. Donelaičio g. 73 - 403, Kaunas).

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Daktaro disertacijos santrauka išsiųsta 2005-04-11.
Su disertacija galima susipažinti Kauno technologijos universiteto bibliotekoje.

**Relevance of the topic.** The International Agency on Research on Cancer (IARC) and the International Union of Nutritional Sciences (IUNS) noted that dietary fibre plays a very important role in human nutrition. Nutrition studies reveal that in some EU countries the intake of dietary fibre is insufficient. The most richest sources of dietary fibre are whole grain foods, legumes, vegetables, fruits and berries which differ from each other in specific chemical composition.

From a nutritional point of view the most studied biologically active components of dietary fibre are polysaccharides. It was reported that non-starch polysaccarides improve digestion and decrease the level of glucose and cholesterol in blood and also decrease the risk of diabetes, obesity and stomach cancer.

Recently special attention has been paid to dietary fibre associated phytochemicals such as phytoestrogens. Epidemiological- and *in vitro* fermentation- studies and experiments with animals have suggested that the biologically activity of these compounds are associated with the prevention of different diseases. Till now phytoestrogens (lignans and isoflavones) in cereals, legumes and oilseeds, especilally in rye, soy beans and flaxseed have been generally studied. According to researchers, a high daily intake of plant lignans is probably important for the intestinal conversion of plant lignans to the mammalian lignans enterodiol and enterolactone. Enterodiol and enterolactone decrease the risk of hormone-dependent diseases, such as breast and prostate cancer, because of hormone level changes are also important for the prevention of coronary heart disease.

There are different opinions about the influence of soy isoflavones and their metabolites on human health. One group of researchers explain that the influence of soy isoflavones on humans has an anti-cancer effect while the other group advocates an opposite view. In the human organism soy isoflavones seem to work like estrogens and seem to stimulate hormone-dependent diseases.

Therefore, dietary fibre and with them the associated phytoestrogens and their metabolites are actual the object of investigation. Special attention should also be paid to less investigated plant products which could be rich sources of phytoestrogens. In this area a lot of research has to be carried out to understand the phytoestrogens role in the disease prevention mechanism and to determine the recomended daily intake. Further solutions of the problem are linked with the development of new methods of investigation.

**Aim of the work.** To investigate phytoestrogens and products of their bioconversion, and dietary fibre components in most of the common plant products and also to determine the possible relationships between each other.

**Goals to be achieved:**

- To develop a simple and rapid high performance liquid chromtography (HPLC) method with coulometric dual electrode detection for the investigation of matairesinol, daidzein, genistein and formononetin and apply this method for the analysis of these phytoestrogens in soy beans.

- To evaluate the influence of some factors: genotype and thermal treatment on the quantity of phytoestrogens in soy beans.
- To investigate the metabolism of plant lignans to mammalian lignans enterodiol and enterolactone by using *in vitro* fermentation with human fecal microflora.
- To compare the contents of enterodiol and enterolactone produced from various plant foods: berries, vegetables, cereal products and flaxseed.
- To investigate dietary fibre and its components in different berries, cereal products, soy beans and flaxseed as well as to collect more information for **a** dietary fibre database.
- To measure possible quantitative relationships between the non-starch polysaccharides, their constituent sugars and with dietary fibre associated phytoestrogens and their metabolites.

**Scientific novelity.** A simple and rapid method was developed to extract the isoflavones daidzein and genistein from plant products and to analyse them quantitatively by HPLC with coulometric dual electrode detector.

This method was applied to determine the influence of several factors such as genotype and thermal treatment on the quantities of these compounds in soy beans.

For the investigation of the bioconversion of plant lignans the technique of *in vitro* fermentation was used and the quantitative analysis of their metabolites enterodiol and enterolactone was performed by HPLC with coulometric elektrode array detector. For the first time *in vitro* fermentation of different berries was carried out. The contents of enterodiol and enterolactone produced from different berries, vegetables, cereal products and flaxseed were determined and compared. The dietary fibre as non-starch polysaccharides of some berries, vegetables, cereal products, soy beans and flaxseed were measured by a modified enzymatic-chemical method using gas liquid chromatography (GLC) for determination of constituent sugars. The quantitative relationship between non-starch polysaccharide components and their associated phytoestrogens and their metabolites were determined.

**The practical value.** The results of the determination of phytoestrogens and their metabolites gives more information of bioactive compounds for databases and their possible plant sources. The data obtained may be used to estimate lignan production from a given diet or to formulate diet with a given lignan producing potential. Berries are an important source of phytoestrogens and a high producer of mammalian lignans. Traditional Lithuanian dietary fibre sources – cereal products and especially rye are very important from a nutritional point of view. Quantitative information about mammalian lignans production from cereals bran, cranberries, cloudberry, raspberries and strawberries shows that these plant products can be recommended for the creation of food supplements and functional foods. The method for the determination of daidzein and genistein can be used for the qualitative and quantitative analysis of these compounds in soy beans. Material

presented in this dissertation is useful for nutritionists, phytotherapists, agriculturalists and food scientists.

## RESEARCH OBJECTS AND METHODS

**Research objects.** Because of scarce comprehensive data in the literature of dietary fibre (DF) and their associated bioactive compounds are available, the various plant foods most common used in the human diet in Lithuania such as (1) berries; (2) vegetables and (3) cereal and cereal products were chosen for the experiments.

Additional flaxseed and soy beans were investigated which according too literature, contains the highest quantity of phytoestrogens.

*Soy beans.* For the determination of daidzein and genistein, soy beans grown at the experimental farm of the Lithuanian Agriculture University were used. For evaluation of the influence of the different genotype**s** on the quantities of phytoestrogens in soy beans, three genotypes were chosen for analysis: one – new, at this time in approval stage (sample 1) and another two – developed in Poland (sample 2) and in Latvia (sample 3). For evaluation of the influence of thermal heating on the quantities of phytoestrogens, soy beans before milling have been heated for 30 minutes at 100$^{o}$C and for 5 minutes at 280$^{o}$C.

*Plant products as sources of lignans.* For the investigation of enterodiol (END) and enterolactone (ENL) production from their precursors have been chosen: (1) berries – blackcurrant, blueberry, buckthorn, chokeberry, cloudberry, cranberry, crowberry, lingonberry, strawberry; (2) vegetables – broccoli, carrot, garlic, onion, potato, pumpkin, rape, red cabbage, red paprika, zucchini; (3) cereal products – barley bran, barley whole flour, oat bran, oat whole flour, rye bran, rye whole flour, wheat bran, wheat whole flour, wheat flour; (4) flaxseed.

For analysis foods with higher water content (berries and vegetables) have been freeze-dried and milled, and those with low water content (flaxseed and cereal products) were milled as such.

*Cereal grains.* Components of DF: cellulose, hemicelluloses, lignin, cutin and β-glucan have been analyzed in cereals of different varieties grown in Lithuania. Traditional and also new varieties have been analyzed: (1) rye – Duoniai, Rūkai, Tolovskaja, Kustro, SW 870493, Hibridas 346, Hibridas 341, Hibridas 347, Hibridas 345, Hibridas 339, Hibridas 343; (2) wheat – Širvinta, Alba, Kosack, Moskovskaja niskostebelnaja, LŽI 2828-47, LŽI 2804-8, LŽI 2905-1, LŽI 2828-47, LŽI 2901-26, LŽI 2804-24, LŽI 2804-33, LŽI 3182; (3) barley - Aidas, Ula, Rolandas, Auksiniai-3; (4) oat – Javor, Dragon, German, Radius, Jaugila, Celsia.

After representative sampling, the grains were milled.

*Plant products for the analysis of non-starch polysaccharides.* The quantities of the constituent sugars of non-starch polysaccharides (NSP), such as arabinose, xylose, mannose, glucose and galactose, were analyzed in: (1) berries –

blackcurrant, blueberry, buckthorn, chokeberry, cloudberry, cranberry, crowberry, lingonberry, strawberry; (2) vegetables – broccoli, garlic, onion, potato, red paprika, zucchini; (3) cereal products – barley whole flour, oat whole flour, rye bran and wheat flour, (4) flaxseed; (5) soy beans.

For analysis the samples were freeze-dried and milled.

**The test methods**

***Phytoestrogens analysis in soy beans by HPLC with coulometric dual elektrode detector.*** The HPLC system consisted of a pump model 5200A (ESA, Chelmsford, USA) and a Rheodyne 7125 injector (Cotati, C.A., USA) adapted with a 5µl sample loop. A Lichrospher 60 RP Select B (Merck, Darmstadt, Germany) column (250×4 mm, 5 µm) in combination with a precolumn (10×4 mm, same material) and for detection a Coulochem II (ESA, Chelmsford, USA) equipped with a dual electrode cell, model 5010 (ESA, Chelmsford, USA) were used. Data acquisition and evaluation were carried out with an IBM PC/AT compatible computer provided with Bischoff (Leonberg, Germany) Mc Dacq software.

*Sample preparation of soy beans.* Two types of sample preparation for phytoestrogens analysis were performed: 1) simultaneously extraction and acid hydrolysis, and 2) only extraction.

From 10 g of milled soy beans a 50 mg sample was taken. To each sample 35 ml ethanol, 5 ml of 10 M HCl and 1 ml internal standard (125 mg estriol) were added. The prepared mixture in the first case was refluxed for two hours and cooled afterwards to room temperature, and in the second case the mixture was just kept by room temerature for two hours. The pH was adjusted to 3 with 6 M NaOH and the solution was filled up to 50 ml with ethanol. After that 1 ml of the solution was diluted with mobile phase **[**ethanol/THF/buffer solution (sodium acetate), 396/9/595, v/v/v, pH 2.6 was adjusted by adding glacial acetic acid**]** in a flask to 10 ml.

*Qualitative and quantitative analysis of daidzein and genistein.* 5 µl of the diluted and filtered sample extracts or standard solutions (daidzein: 6.5 to 262 µg/l, genistein: 7.1 to 284 µg/l, the concentration of estriol was held constant at 250 µg/l) were injected; the substances were separated (flow rate: 0.8 ml/min) on the reversed phase column and were detected at +350 mV (channel 1) and +500 mV (channel 2).

Daidzein and genistein were identified by measuring the retention time (RT) and the hydrodynamic voltammograms of the substances in samples and standard solutions and were quantified using the calibration curves. The detection limits for daidzein and genistein were determined using diluted standard solutions. Triplicate samples were analyzed and the standard deviation was calculated. The recovery was obtained by adding estriol as internal standard.

***Investigation of metabolism of plant lignans by using in vitro fermentation with human fecal inoculum and by HPLC with coulometric elektrode array detector.*** *In vitro* fermentation was performed according to a modified incubation method of Karppinen *et.al*. A carbonate-phosphate buffer solution with trace elements was held in an anaerobic chamber for 2 days prior to fermentation. Feces were collected from three healthy human volunteers, who suffered no digestive disease, and had not received antibiotics for at least 3 month. Freshly passed feces were immediately taken in an anaerobic chamber, pooled, and homogenized with an equal weight of culture medium using a Waring blender. The slurry was diluted to 16.7% (v/v) with the culture medium, filtered through a 1 mm sieve, and used immediately as inoculums.

0.1 g of a analyzed food sample was weighed into 50 ml glass vials, and 10 ml of the inoculum was added and stored in a 30°C anaerobic chamber. The vials were sealed with rubber stoppers and shaken in a 37°C water bath for 24 h. The fermentation was stopped by plunging the vials into iced water, after which the vial contents were freeze-dried. Dublicate incubations were carried out for each sample. Also, dublicate blanks, containing only culture medium and inoculum, were incubated for 0 and 24 h.

*Quantitative analysis of END and ENL by HPLC with coulometric elektrode array detector*. The HPLC system consisted of a pump model 580 (ESA, Chelmsford, USA) and an automatic injector model 540 (ESA, Chelmsford, USA). An intersil ODS-3 (GL Science Inc., Japan) column (3.0×150 mm, 3.3 μm, 9LI 500 10) in combination with precolumn Quick Releate $C_{18}$ (Upchurch Scientific Inc., WA) and for detection Coulochem Electrode Array Detector (ESA, Chelmsford, USA) equipped with a eight electrode cell were used.

The freeze-dried incubated samples were weighed (approximately 20 mg) and 500 μl of water and 10 μl of 6 M HCl was added. The samples were extracted twice with 5 ml of diethyl ether. The extracts were combined and evaporated to dryness under $N_2$ flow. The samples were dissolved in 500 μl MeOH and subsequent diluted with the mobile phase.

The mobile phase consisted of 20% solution B (50 mM NaAc, pH 5/MeOH/ACN, 40/40/20, v/v/v) and 80% solution A (50 mM NaAc, pH 5/MeOH, 80/20, v/v).

Prepared sample extracts or standard solutions (END: 7.0 to 350 μg/l, ENL: 10.834 to 541.7 μg/l) were injected, the compounds were separated (flow rate of 1.2 ml/min) on the reversed phase column and were detected at +180 mV (channel 1) till +720 mV (channel 8).

The END and ENL were quantified using calibration curves and by evaluation of their quantities determined in a blank sample. The precision of the method was determined using END and ENL standard solutions. Duplicate samples were analyzed for each incubation sample and the standard deviation was calculated.

The quantities of END and ENL produced in 24 h fecal blanks were subtracted from the results obtained from the incubations carried out with the food samples.

***The methods of investigation of dietary fibre.*** The analysis of the dietary fibre components: NDF (neutral detergent fibre), ADF (acid detergent fibre) and ADL (acid detergent lignin) was carried out by the ***Van Soest and Wine detergent method.*** The NDF fraction contains hemicelluloses, cellulose, cutin and lignin, the ADF – cellulose, cutin and lignin, and the ADL – cutin and lignin.

The hemicelluloses and cellulose contents were calculated as difference between NDF and ADF fractions, and ADF and ADL fractions, respectively. Using this method lignin could be determined together with cutin by means of the quantity of the ADL fraction.

Before extraction of the dietary fibre components, starch was removed enzymatically with α-amylase and amyloglucosidase. For NDF analysis, starch-free material was extracted with a neutral detergent, and the residue was filtered and weighed after washing, drying and ashing. By sample extraction with acid detergent hemicelluloses were removed and the ADF fraction was precipitated. The quantities of lignin and cutin were determined by extraction of the ADF fraction with sulphuric acid.

*β-glucan* was determined enzymatically according to the ***McCleary and Cood method,*** using a Megazyme kit (BBG 3/96, Ireland). Samples were suspended in sodium phosphate buffer solution (pH 6.5) and digested sequentially with lichenase and β-glucosidase enzymes. The β-glucan was hydrolyzed to β-glucan-oligosaccharides by lichenase and oligosaccharides were hydrolyzed to glucose by β-glucosidase. By adding glucose-oxidaze-peroxidase reagent chinoidic colors are formed. Light absorption of the colored solution is measured by a spectrophotometer at 510 nm, which values correlate with the β-glucan quantity.

The constituent sugars of NSP were measured according to the modified ***H. N. Englyst*** **and** ***J.H. Cummings method*** by GLC**.**

In the modified method, starch including resistant starch that is resistant to gelatinization in boiling water, was dispersed with dimethyl sulphoxide and removed from the sample matrixes enzymatically (with α-amylase and amyloglucosidase). NSP was precipitated with ethanol and then hydrolyzed with sulfuric acid for 2 h to monosaccharides. The constituent sugars of NSP: arabinose, xylose, mannose, glucose and galactose were determined by GLC. Alditol acetates prepared by using *N*-methylimidazole to catalyze the acetylation were used for GLC determination of released monosaccharides. The total NSP were expressed as sum of constituent sugars. GLC was performed by using a gas chromatograph of *Shimadzu – GC – 17 AAF FID* with a flame ionization detector (FID) and an analytical column *RTX 50* (30 m×0.25 mm, 0.25 μm) and an automatic sample injector *AOC-17.* The column was maintained at 230 ºC and the injector and detector were kept at 300 ºC. The carrier gas (helium) flow rate was 1.67 ml/min

(30 $cm^3$/min). The contents of monosaccharides were determined by using a compatible computer provided with CLASSGC10 Chemstation software.

## RESULTS AND DISCUSSION

**The use of HPLC with coulometric detector for the determination of phytoestrogens in soy beans.** Two methods for the analysis of phytoestrogens by HPLC with coulometric dual electrode detection have been developed: first – for the determination of daidzein and genistein and second – for a wider spectrum of phytoestrogens, such as daidzein, genistein, matairesinol, and formononetin.

***Method for the determination of daidzein and genistein.*** To determine daidzein and genistein, optimal chromatographic conditions, such as a mobile phase, internal standard and potentials of working electrodes of the coulometric detector, have been chosen.

*Selection of the mobile phase.* Ten different eluents (E1 - E10) were tested as mobile phases (Table 1). For their preparation 4 different organic substances: ethanol, methanol, tetrahydrofuran and acetonitril were used. The optimal retention time (RT) of daidzein and genistein in the column and the optimal chromatograms obtained from standard solutions were determined by using the mobile phase E10 (pH 2.6). Also, using this mobile phase, the isoflavones were eluted isocratically from the column within 11 minutes and a minimum flow rate (0.8 ml/min) was noticed, which contributes to the efficiency of the analysis (less chemicals are needed).

T**able 1.** Different eluents which were tested as mobile phases

| Eluents | EtOH | 5 mmol NaAc | $H_2O$ | MeOH | THF | ACN | pH | Flow rate, ml/min |
|---|---|---|---|---|---|---|---|---|
| | ml | | | | | | | |
| E1 | – | 6.8 | 500 | 500 | – | – | 4.8 | 1.2 |
| E2 | – | 3.6 | 450 | 550 | – | – | 4.8 | 1.2 |
| E3 | – | 6.8 | 500 | – | 88.24 | – | 4.5 | 1.2 |
| E4 | – | 6.8 | 500 | – | 17.65 | – | 4.5 | 1.2 |
| E5 | – | 6.8 | 500 | – | 10.00 | – | 4.5 | 1.2 |
| E6 | – | 6.8 | 500 | – | – | 167 | 4.5 | 1.2 |
| E7 | – | 6.8 | 500 | – | – | 300 | 4.5 | 1.2 |
| E8 | – | 1.8 | 500 | – | – | 321 | 4.5 | 1.2 |
| E9 | 333.3 | 1.8 | 500 | – | 8.00 | – | 3.0 | 0.8 |
| E10 | 333.3 | – | 500 | – | 8.00 | – | 2.6 | 0.8 |
| E11 | 333.3 | – | 500 | – | 7.8 | – | 3.0 | 0.8 |

*Selection of the internal standard.* Various phenolic compounds, such as β-estradiol, bisphenol A, estriol, vanilin, ferulic methylacetate ester, ferulic acetate and vanilin acetate, were tested as internal standards. The retention time (RT) of these substances are presented in Table 2.

Finally estriol was chosen for the internal standards due to its electrochemical behaviour similar to that of daidzein and genistein and its RT in between the analysed isoflavones (8.8 minutes)**.** Additionally estriol is an endogenic mammalian hormone and does not occur naturally in plant products.

**Table 2.** The retention times of internal standards and analyzed substances

| Internal standards | RT, min | Analyzed substances | RT, min |
|---|---|---|---|
| β-estradiol | 35.3 | Daidzein | 7.1 |
| Bisphenol A | 23.4 | Genistein | 10.4 |
| Estriol | 8.8 | | |
| Vanilin | 6.8 | | |
| Ferulic methylacetate ester | 6.4 | | |
| Ferulic acetate | 5.9 | | |
| Vanilin acetate | 5.5 | | |

*Selection of the potentials of the working electrodes.* In oder to get the voltammograms, the working elektrode of the first cell was set at +200 mV and the potential of the second elektrode was changed in increments of 50 mV from +150 mV till +500 mV.

The hydrodynamic voltammograms were established by plotting the signals of daidzein, genistein and estriol agains the potential applied on the second working elektrode (Figure 1).

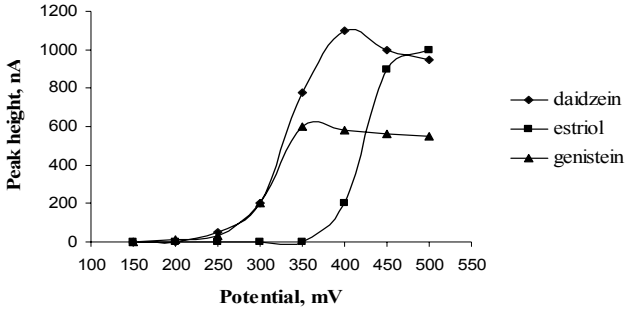


**Figure 1.** Hydrodynamic voltammograms of daidzein, genistein and estriol

At potentials lower than +200 mV against electrodes neither the internal standard nor the isoflavones were oxidized. By increasing the potentials of the second electrode, daidzein and genistein started to oxidize and at the potential +350 mV also estriol started to oxidize. The biggest peak areas of all three

compounds in the chromatogram were noticed when potentials ranged from +400 mV till +500 mV.

Considering the hydrodynamic voltammograms, potentials of +350 mV (channel 1) and +500 mV (channel 2) were chosen for the detection of daidzein, genistein and estriol.

*Determination of calibration curves and detection limits.* Under chosen chromatographic conditions a linear correlation between the signals in channel 2 and the concentration of daidzein and genistein in the working standard solutions was found. The calibration curves for analyzed compounds were calculated with the linear regression equation and the correlation coefficients (R), which represent the precission of the coulometric detector to analyse the substances, were determined. In all cases R was higher than 0.99 (when daidzein concentration is in the range from 6.5 to 262 μg/l, R = 0.9985 and when genistein from 7.1 to 284 μg/l, R = 0.9973). The detection limits were 0.5 pg daidzein and 1.0 pg genistein.

This shows that a coulometric dual elektrode detector can be used for the separation of daidzein and genistein and the determination of their quantities.

***Method for the determination of the phytoestrogens: matairesinol, daidzein, genistein and formononetin.*** The application of the above method for the determination of daidzein, genistein was extended for other phytoestrogens, such as matairesinol and formononetin, determination. For this, different internal standards and mobile phases have been analyzed.

*Selection of mobile phase.* By using the mobile phases E1 - E10, it was not possible to obtain good isocratically separation of analyzed substances. Therefore a new mobile phase E11, in which in compare with E10 the concentration of tetrahydrofuran was decreased and the pH was increased till 3.0 (Table 1), was tested. These changes in E11 allowed to extend the RT of analysed phytoestrogens and separate without interference these compounds in one chromatogram.

*The selection of the internal standard.* In addition to the phenolic compounds described above, cumestrol and biochanin were used in the experiment. The determined RT of internal standards and analyzed substances are presented in Table 3.

Estriol was again chosen as internal standard due to its eluation behavior between the analyzed compounds in the „empty“ part of the chromatogram.

*The determination of the calibration curves.* A linear correlation was determined between the peak areas and the concentration of matairesinol (14.2 - 142 μg/l), daidzein (14.6 - 146 μg/l), genistein (13.6 - 136 μg/l) and formononetin (24 - 240 μg/l) in the working standard solutions. For the analyzed substances a linear regression equation of calibration curves and correlation coefficients were calculated: for matairesinol – R=0.9827; daidzein – R=0.9994; genistein – R=0.9721 and formononetin – R=0.9386.

In all cases R was higher than 0.93, so the coulometric dual elektrode detector could be used for the separation of matairesinol, daidzein, genistein and formononetin and as well for the determination of their quantities.

**Table 3.** The retention times of internal standards and analyzed substances

| Internal standards | RT, min | Analyzed substances | RT, min |
|---|---|---|---|
| β-estradiol | 36.4 | Matairesinol | 9.5 |
| Cumestrol | 35.0 | Daidzein | 11.5 |
| Bisphenol A | 25.1 | Genistein | 15.1 |
| Estriol | 13.5 | Formononetin | 20.6 |
| Vanilin | 9.4 | | |
| Vanilin acetate | 8.5 | | |
| Ferulic acetate | 8.2 | | |
| Ferulic methyl acetate ester | 7.9 | | |
| Biochanin | 7.4 | | |

**Daidzein and genistein in soy beans.** To apply the developed HPLC method for the qualitative and quantitative analysis of analyzed phytoestrogens in soy beans, two methods of sample preparation described in literature were tested.

The result of the investigations (Figure 2) showed that the levels of phytoestrogens were lower by using only sample extraction in compare with the combination of sample extraction and acid hydrolysis: 7.3 - 10.6% less daidzein and 11.9 - 37.9% less genistein. Therefore the method for sample preparation by using simultaneously extraction and acid hydrolysis was chosen for phytoestrogens analysis.

During these procedures phytoestrogen conjugates are converted into their respective aglycons. The results showed that using the proposed method, relative high concentrations of phytoestrogen aglycons were formed. Therefore low sample amounts can be used for analysis, which cause a decreased disturbance in the chromatograms of the sample matrix.

Additionally, by using this method of sample preparation the quantities of phytoestrogens can be determined in soy beans without defatting and a cleaning up procedure.

The analysis of phytoestrogens showed that the quantities of maitairesinol and formononetin in tested soy beans were less then the detection limits for these substances, and under chosen chromatographic conditions the peaks of maiteresinol and formononetein could not be seen in the chromatograms. Therefore by using the developed method, matairesinol, daidzein, genistein and formononetin can not be quantified in one chromatogram.

It was shown that the recovery of daidzein and genistein determined with standard solutions and the standard addition method both depends on the matrix of the food and that isoflavones behave very similar to the internal standard. By analyzing three different soy bean samples (each three times) a recovery of 81.9% ± 7.4%. for estriol (internal standard) was found. This value was adopted as

recovery for daidzein and genistein. It could be mentioned that the recovery for these substances determined by using the developed method in compare with other HPLC methods is reasonably high.

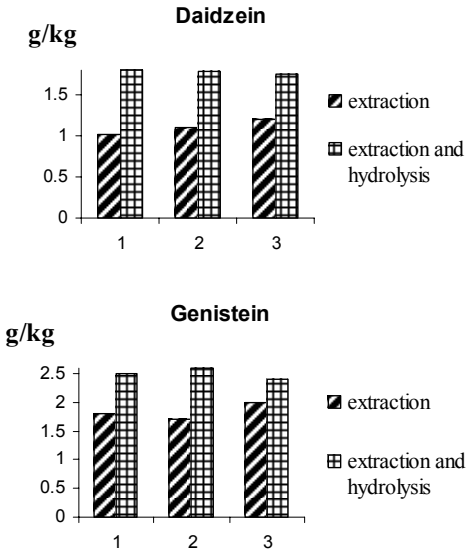


**Figure 2**. The quantities of daidzein and genistein determined by using different methods of sample preparation

The results on the precision of HPLC analysis are given in Table 4.

The repeatability was calculated from the three independent single test results, obtained on the same test material using the same equipment. It was observed that in the concentration range from 1.54 to 1.94 g daidzein/kg, and 2.04 to 2.40 g genistein/kg, the coefficients of variation of repeatability are less than 12% and 11%, respectively.

Therefore the HPLC method with coulometric dual electrode detector is enough precise and could be used for the simple and rapid determination of daidzein and genistein in soy beans.

The following advantages of the developed method should be mentioned:

- Relative low quantities of chemicals used, because the flow rate of the mobile phase [ethanol/tetrahydrofuran/buffer solution (sodium acetate/ trichloroacetic acid), 396/9/595, v/v/v, pH 2.6] for substances separation on the column is low, only 0.8 ml/min.
- Daidzein and genistein were isocritically eluted from the column within 11 minutes with the rapid chromatographic analysis.

- Simple identification and quantification of daidzein and genistein, because using chosen potentials of working electrodes: +350 mV (channel 1) and +500 mV (channel 2) only analyzed substances are oxidized at the second working electrode.
- The method is sensitive and precise for the qualitative and quantitative determination of daidzein and genistein in one chromatogram. The recovery for these substances is 81.9% ± 7.4%, and the coefficient of variation of repeatability is less than 12%.

**Table 4.** Statistical results

| Parameters | Daidzein | | | Genistein | | |
|---|---|---|---|---|---|---|
| | Samples | | | | | |
| | 1 | 2 | 3 | 1 | 2 | 3 |
| Average mean, g/kg | 1.79 | 1.54 | 1.94 | 2.38 | 2.04 | 2.40 |
| Repeatability standard deviation $s_r$, g/kg | 0.10 | 0.17 | 0.12 | 0.11 | 0.22 | 0.18 |
| Coefficient of variation of repeatability, % | 5.59 | 11.04 | 6.19 | 4.62 | 10.78 | 7.50 |

**The influence of technological parameters on the content of phytoestrogens in soy beans.** The results of the investigation of daidzein and genistein content in different genotypes of soy beans, untreated and heated to 100°C and 280°C, are summarized in Table 5.

Results showed that the content of daidzein in soy beans ranged from 1.36 g/kg to 2.10 g/kg. The content of genistein were slightly higher – from 1.83 g/kg till 2.63 g/kg. It should be mentioned that the content of tested phytoestrogens in soy beans of various genotypes grown in Lithuania are slightly higher than those mentioned in literature. According to the investigation it can be assumed that the genotype did not influence the content of daidzein and genistein in soy beans.

The investigation of the thermostability of daidzein and genistein is also important because heat treatment of soy beans is performed to eliminate enzyme inhibitors and various soy products are therefore processed at higher temperatures. Therefore three sorts of soy beans, untreated, heated at 100°C for 30 minutes and at 280°C for 5 minutes were analyzed to investigate the influence of temperature on the content of phytoestrogens. Results showed that heat treatment did not alter the daidzein and genistein content in soy beans. The deviations between the values at different temperatures could be due to the inhomogeneity of the powdered soy beans caused by milling. Therefore, these compounds are not decomposed in food prepared by higher temperatures. These results also correspond to literature mentioned that isoflavones are thermostabile phytoestrogens.

**Table 5.** The influence of temperature on the contents of daidzein and genistein in soy beans

| Samples of soy beans | Time and temperature of heat treatment | Daidzein, g/kg | Genistein, g/kg |
|---|---|---|---|
| Sample 1 | Untreated (25°C) | 1.79±0.10 | 2.38±0.11 |
| | 100°C, 30 min | 1.93±0.19 | 2.63±0.34 |
| | 280°C, 5 min | 1.72±0.10 | 2.46±0.14 |
| | Range | 1.72-1.93 | 2.38-2.63 |
| Sample 2 | Untreated (25°C) | 1.54±0.17 | 2.04±0.22 |
| | 100°C, 30 min | 1.36±0.10 | 1.83±0.05 |
| | 280°C, 5 min | 1.42±0.25 | 1.97±0.37 |
| | Range | 1.36-1.54 | 1.83-2.04 |
| Sample 3 | Untreated (25°C) | 1.94±0.12 | 2.40±0.18 |
| | 100°C, 30 min | 1.80±0.13 | 1.90±0.08 |
| | 280°C, 5 min | 2.10±0.16 | 2.40±0.19 |
| | Range | 1.80-2.10 | 1.90-2.40 |

**The mammalian lignan production of enterodiol and enterolactone from various plant foods using *in vitro* fermentation.** The quantative results of enterodiol (END) and enterolactone (ENL) produced during the 24 h fecal incubation of lignans of berries, vegetable and cereal products are summarized in Tables 6, 7 and 8.

*Berries.* The mammalian lignan production from various berries ranged from 7.8 to 382.8 nmol/g, for END – from 0 to 327.2 nmol/g and for ENL – from 7.8 to 55.6 nmol/g (Table 6).

By comparing different kind of berries on a wet (as-is) basis, the cloudberry (382.8 nmol/g), raspberry (227.9 nmol/g), and strawberry (150.8 nmol/g) were the highest lignans producers. The lignan production from other berries, such as lingonberry, blueberry and cranberry was 12 to 18 times lower than that from the cloudberry. The buckthorn and crowberry did not appear to be good producers, because the total lignan quantity was lower than 10 nmol/g.

It has been noticed that of the higher quantities of total lignans (in cloudberry, raspberry, strawberry), more END was produced than ENL and the ratios of END to ENL ranged from 6:1 to 5:1. In berries in which lower quantities of total lignans were found, the levels of ENL were higher than END. The reason for it could be the particularities of mammalian lignan precursors in different berries and END oxidation to the more stable ENL. It can be affirmed that by longer fermentation (48 h or 72 h) this ratio of END to ENL could be different, so the total lignan amount could better characterize the mammalian lignans production.

By knowing that matairesinol can be transformed by the bacterial enzymes directly to ENL through reactions involving hydrolysis, dehydroxylation and demethylation, the higher levels of ENL rather than END in a majority of the berries suggest that matairesinol may be more abundant present.

**Table 6.** Mammalian lignan production from different berries

| Analyzed berries | Enterodiol, nmol/g | | Enterolactone, nmol/g | | Total lignans, nmol/g | |
|---|---|---|---|---|---|---|
| | Wet basis | Dry basis | Wet basis | Dry basis | Wet basis | Dry basis |
| Cloudberry | 327.2 | 1817.5±112 | 55.6 | 308.7 ±36 | 382.8 | 2126.2 |
| Raspberry | 191.9 | 1066.0 ±42 | 36.0 | 199.6 ±17 | 227.9 | 1265.6 |
| Strawberry | 124.5 | 803.3 ±4.6 | 26.3 | 169.4 ±0.6 | 150.8 | 972.7 |
| Cranberry | 3.2 | 23.2 ±2.2 | 30.4 | 217.2 ±3 | 33.6 | 240.4 |
| Blueberry | 6.3 | 34.9 ±1.8 | 26.8 | 148.7 ±3.5 | 33.1 | 183.6 |
| Lingonberry | 0 | 0 | 21.6 | 154.5 ±0.5 | 21.6 | 154.5 |
| Chokeberry | 1.8 | 12.6 ±2.2 | 14.2 | 101.7 ±4.1 | 16.0 | 114.3 |
| Blackcurrant | 5.0 | 33.3 ±1.3 | 8.9 | 59.6 ±1.3 | 13.9 | 92.9 |
| Crowberry | 0 | 0 | 8.6 | 47.8 ±0.4 | 8.6 | 47.8 |
| Buckthorn | 0 | 0 | 7.8 | 45.9 ±1.8 | 7.8 | 45.9 |
| ***Minimum*** | ***0*** | ***0*** | ***7.8*** | ***45.9*** | ***7.8*** | ***45.9*** |
| ***Average*** | ***66.0***±113.9 | ***379.1***±636.9 | ***23.6***±15.0 | ***145.3***±84.4 | ***89.6***±126.6 | ***524.4***±725.1 |
| ***Maximum*** | ***327.2*** | ***1817.5*** | ***55.6*** | ***308.7*** | ***382.8*** | ***2126.2*** |

*Vegetables.* The quantities of mammalian lignans produced from different vegetables ranged from 10.5 to 91.2 nmol/g, END – from 5.8 to 45.7 nmol/g and ENL – from 4.7 to 45.5 nmol/g (Table 7).

**Table 7.** Mammalian lignan production from different vegetables

| Analyzed vegetables | Enterodiol, nmol/g | | Enterolactone, nmol/g | | Total lignans, nmol/g | |
|---|---|---|---|---|---|---|
| | Wet basis | Dry basis | Wet basis | Dry basis | Wet basis | Dry basis |
| Potato | 45.7 | 190.5±13.9 | 45.5 | 189.7 ±13.5 | 91.2 | 380.2 |
| Garlic | 42.9 | 214.4 ±2.2 | 23.3 | 116.5 ±1.5 | 66.2 | 330.9 |
| Zucchini | 33.6 | 239.9 ±32.9 | 26.8 | 191.5 ±35.4 | 60.4 | 431.4 |
| Broccoli | 24.9 | 177.6 ±1.1 | 17.4 | 124.0 ±0.3 | 42.3 | 301.6 |
| Red paprika | 15.3 | 153.4 ±3 | 20.0 | 199.8 ±4.1 | 35.3 | 353.2 |
| Red cabbage | 10.1 | 111.6 ±2.3 | 22.1 | 245.4 ±1.2 | 32.2 | 357.0 |
| Pumpkin | 16.0 | 114.4 ±0.2 | 16.0 | 114.6 ±0.2 | 32.0 | 229.0 |
| Onion | 19.2 | 137.3 ±0.3 | 10.2 | 72.9 ±0.2 | 29.4 | 210.2 |
| Carrot | 12.7 | 106.1 ±0.8 | 13.8 | 115.0 ±0.4 | 26.5 | 221.1 |
| Rape | 5.8 | 55.1 ±4.9 | 4.7 | 44.4 ±2.7 | 10.5 | 99.5 |
| ***Minimum.*** | ***5.8*** | ***55.1*** | ***4.7*** | ***44.4*** | ***10.5*** | ***99.5*** |
| ***Average*** | ***22.6***±14.2 | ***150.0***±56.3 | ***20.0***±11.1 | ***141.4***±62.8 | ***42.6***±23.5 | ***291.4***±99.7 |
| ***Maximum*** | ***45.7*** | ***239.9*** | ***45.5*** | ***245.4*** | ***91.2*** | ***380.2*** |

Considering individual vegetables on a wet basis, potatoes produced the highest quantity of total lignans (91.2 nmol/g). Garlic (66.2 nmol/g), zucchini (60.4 nmol/g) and broccoli (42.3 nmol/g) were the other good producers of lignans in this food product group. Rape in compare with other vegetables was the lowest lignan producer (10.5 nmol/g).

In most of the vegetables (although the END to ENL ratio was close to 1) slightly more END was produced than ENL with the exception of red cabbage where the level of ENL was higher than the level of END.

Considering this, the larger quantities of END rather than ENL produced from vegetables may be a reflection of their higher concentration of the other lignan precursor – secoisolariciresinol coupled with a low rate of conversion of END to ENL.

*Cereal products.* From cereal products the production of mammalian lignans ranged from 78.3 to 321.9 nmol/g depending on the product type, END – from 8.7 to 149.3 nmol/g and ENL – from 64.4 to 278.3 nmol/g (Table 9).

Cereal brans were the highest producers of mammalian lignans (294.1 - 321.9 nmol/g). The lignan production from bran was 2.2 - 2.3 times higher than that from whole flour of the same kind of cereals. It is known that dietary fibre of cereals with their associated substances (as phytoestrogens) are distributed in the outer layer of grains. The higher quantities of dietary fibre were found in the aleurone and pericarp layers of grains, and during milling end up in the bran fraction.

**Table 8.** Mammalian lignan production from different cereal products

| Analyzed cereal products | Enterodiol, nmol/g | | Enterolactone, nmol/g | | Total lignans, nmol/g | |
|---|---|---|---|---|---|---|
| | Wet basis | Dry basis | Wet basis | Dry basis | Wet basis | Dry basis |
| Rye bran | 34.6 | 40.2 ±0.2 | 278.3 | 323.6 ±1.1 | 312.9 | 363.8 |
| Wheat bran | 141.3 | 164.3 ±2.6 | 180.6 | 210.0 ±0.1 | 321.9 | 374.3 |
| Oat bran | 149.3 | 173.7 ±3.3 | 171.4 | 198.9 ±0.8 | 320.7 | 372.6 |
| Barley bran | 121.5 | 141.2 ±6.7 | 172.6 | 200.7 ±4.8 | 294.1 | 341.9 |
| Whole wheat flour | 119.3 | 138.7 ±5.7 | 111.3 | 129.4 ±1.5 | 230.6 | 268.1 |
| Whole rye flour | 66.1 | 76.8 ±5.1 | 76.8 | 89.2 ±4.9 | 142.9 | 166.0 |
| Oat whole flour | 65.9 | 76.6 ±3.5 | 71.8 | 83.5 ±1.2 | 137.7 | 160.1 |
| Barley whole flour | 62.5 | 72.7 ±4.2 | 64.4 | 74.9 ±3.6 | 126.9 | 147.6 |
| Wheat flour | 8.7 | 10.1 ±0.2 | 69.6 | 80.9 ±1.1 | 78.3 | 91.0 |
| ***Minimum*** | ***8.7*** | ***10.1*** | ***64.4*** | ***74.9*** | ***78.3*** | ***91.0*** |
| ***Average*** | ***85.47***±49.3 | ***99.37***±57.3 | ***132.98***±72.9 | ***154.57***±84.8 | ***218.4***±97.6 | ***253.93***±113.5 |
| ***Maximum*** | ***149.3*** | ***164.3*** | ***278.3*** | ***323.6*** | ***321.9*** | ***374.3*** |
| **Flaxseed** | **4350.8** | **5059.1 ±41** | **6082.4** | **7072.6 ±41** | **10433.2** | **12131.7** |

From most of analyzed cereal products slightly more ENL was produced than END, and with rye bran and wheat flour the ratios of ENL to END were even 8:1. It seems that in this food product group matairesinol is a quantitatively more important precursor than secoisolariciresinol.

***Comparison of enterodiol and enterolactone production from different products.*** The results of investigation showed that the amount of mammalian lignans produced from flaxseed (10433.2 nmol/g) was of another magnitude than in other food product groups (Table 8), where vegetables (42.6 nmol/g) and berries

(89.6 nmol/g) produced less than cereal products (321.9 nmol/g). A larger amount of END (5059.1 nmol/g) rather than ENL (7072.6 nmol/g) produced from flaxseed was found. Similar results of mammalian lignans production from flaxseed were noticed in literature.

On a wet basis, the total lignan production from flaxseed was 27 times higher than that from the berries, 32 times higher than that from the cereal products and 114 times higher than from the vegetables. Cloudberry, raspberry, strawberry, wheat bran, oat bran, rye bran, barley bran, whole wheat flour, rye and whole rye flour were the other good producers of lignans, but still there magnitudes were lower than flaxseed.

Considering the ratio of END to ENL, it can be suggested that in most of the cereal products, and berries (except cloudberries, raspberries, and strawberries) matairesinol is one of the most important mammalian lignan precursor. Vice versa in vegetables, other lignan precursors such as secoisolariciresinol may be more abundant present.

It can be noticed that berries and vegetables did not appear to be good producers because of their high moisture content (up to 96%) in compare with cereal products (about 14%). However, when the data are expressed on a dry matter basis, eliminating the moisture effect, in most of the berries and vegetables the highest amount of these substances could be determined. On a dry matter basis, the total amount of lignans produced from such berries as cloudberry (2126.2 nmol/g), raspberry (1265.6 nmol/g), strawberry (972.7 nmol/g) and such vegetables as zucchini (431.4 nmol/g), potato (380.2 nmol/g), red cabbage (357.0 nmol/g) and red paprika (353.2 n mol/g) were higher or similar than that from cereal brans (341.9 – 374.3 nmol/g).

How much the different foods contribute to the total lignan production in humans depends on their level of use in the diet. Flaxseed produced the highest quantity of mammalians lignans (that shows also this investigation), but currently its dietary contribution is low because it is not widely used for food since it contains a high concentration of toxic cyanoglycosides. In the Western type of diet whole grain foods, fruits, berries and vegetables due to their higher level of intake are the main sources of lignans.

In Lithuania cereal products are the most important sources of mammalian lignan precursors. It needs to pay attention to increase in the diet the intake of whole grain foods, especialy rye products. Among grains, rye has a relatively high content of important dietary fiber components combined with other bioactive compounds, such us plant lignans, which are termostable and are not lost during baking.

Although fresh berries and vegetables, on the average, produce lower quantities of mammalian lignans, among them there are some with high mammalian lignan producing capability, such as cloudberry, raspberry and

strawberry, potato and zucchini, which could contribute to reduce the risk of cancer.

No data were found in literature as of the ability of berries to produce mammalian lignans. Although the data on lignan production of cereals and vegetables differ slightly with the data available from literature tendencies seem to be the same with this findings. The reason could be that intestinal bioconversion of plant lignans to mammalian lignans is subject to large interindividual variation, probably due to differences in bacteria microflora and also the use of antibiotics.

Concerning the latter antibiotics we recall that our human volunteers in the experiment did not received antibiotics for at least 3 month. It is probably that the diet-host interactions are largely determined by genetic variation between individuals, and also by dietary differences with respect to the content and type of non-digestible food components which provide substrates for the gut microflora. Therefore, much research is still needed to understand plant lignan metabolism and absorbtion in humans, to verify their role and mechanisms in desease prevention and to create recommendations for their intake.

**Complex analysis of dietary fibre of cereals grown in Lithuania.** The quantities of hemicelluloses, cellulose, lignin + cutin also β-glucan determined in Lithuanian wheat, rye, barley and oat are presented in figure 3.

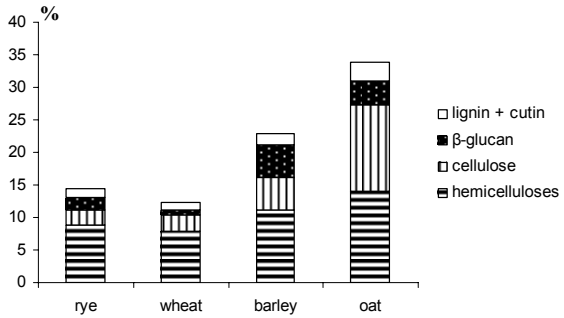


**Figure 3.** Dietary fibre fractions of cereal

The highest quantities of total dietary fibre and most of its fractions were found in oats. In this type of cereals the hemicellulose and cellulose content was, respectively, 1.3 and 2.7 times higher than in barley, 1.6 and 5.5 times higher than in rye and 1.8 and 5.1 higher than in wheat. The lignin and cutin content together in oats was from 1.8 to 2.4 times higher in compare with other cereals.

Among the tested cereals, barley was specific for the highest content of β-glucan (5.1 ± 0.4 % dm), which was 1.4 times higher than in oats, 2.8 times higher than in rye and 6.4 times higher than in wheat. In compare with rye and wheat the

content of hemicellulose (11.2 ± 0.7 % dm) and cellulose (4.9 ± 0.8 % dm) in barley were also about 1.4 and 2 times higher, respectively.

In rye only the content of β-glucan (1.8 ± 0.3 % dm) was higher than in wheat (0.8 ± 0.3 % dm). The quantities of hemicellulose, cellulose and lignin in rye and wheat were equivalent.

By comparing different genotypes of cereals it was noticed that the variation in composition of dietary fibre fractions depends not only on the kind of cereals but also on the genotype.

No significant relationships have been determined between the amounts of mammalian lignans produced from cereal products and the amounts of total dietary fibre and their fractions. Obviously, the lignan production more depends on dietary fibre specific composition and structure, which varies between different kind of cereals, than on the amount of dietary fibre.

**Non-starch polysaccharides and their constituent sugars in plant products.** The results of the investigation of non-starch polysaccharides (NSP) of different berries, vegetables and cereal products, flaxseed and soy beans are presented in Figures 4, 5, 6.

*Berries.* For berries, the total NSP content ranged from 1.08 to 2.88 g/100 g. The main constituent sugar was glucose, which content varied from 0.67 to 1.99 g/100 g. The contents of other constituent sugars were substantially lower: xylose (0.11 - 0.63 g/100 g), arabinose (0.08 - 0.30 g/100 g), galactose (0.11 - 0.24 g/100 g) and mannose (0.04 - 0.14 g/100 g). The following proportion of NSP components in berries was found: 49.1 - 69.1 % glucose, 7.3 - 37.3 % xylose, 4.7 - 16.2 % arabinose, 5.9 - 11.1 % galactose and 2.4 - 5.6 % mannose (Figure 4).

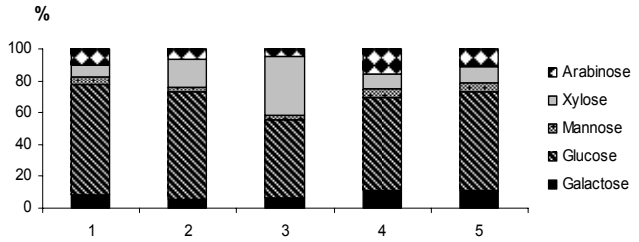


**Figure 4.** The proportion of NSP constituent sugars in berries (1- crowberry, 2- raspberry, 3- blueberry, 4- lingonberry, 5- strawberry)

The comparison of different berries showed, that the highest total NSP content was in crowberry (2.88 g/100 g) and raspberry (2.71 g/100 g). The highest glucose content was in crowberry and raspberry, arabinose – in lingonberry and crowberry, mannose and galactose – also in crowberry, xylose – in blueberry and raspberry.

*Vegetables.* For the analyzed vegetables, the total NSP content varied from 0.29 to 3.39 g/100 g. As in berries, the dominant constituent sugar was glucose (0.11 - 1.87 g/100 g), the lowest level was of xylose (0.03 - 0.20 g/100 g). The proportion of NSP components in vegetables was the following: 23.9 - 55.2 % glucose, 13.3 - 53.5 % galactose, 4.0 - 24.8 % mannose, 4.1 - 27.4 % arabinose and 2.7 - 19.6 % xylose (Figure 5).

The highest NSP content was determined in garlic (3.40 g/100 g), and the lowest – in red paprika (0.28 g/100 g). Among the analyzed vegetables, garlic had the highest amounts of glucose (1.87 g/100 g) and mannose (0.84 g/100 g), broccoli – the highest amount of xylose (1.41 g/100 g) and arabinose (2.30 g/100 g), and onion – the highest amount of galactose (3.78 g/100 g).

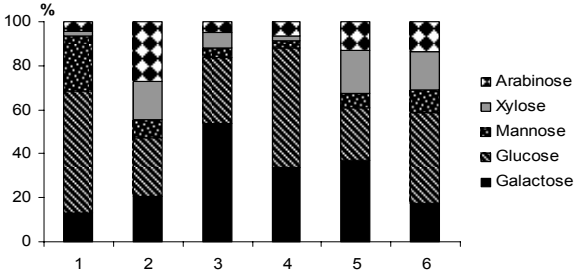


**Figure 5.** The proportion of NSP constituent sugars in vegetables (1- garlic, 2- broccoli, 3- onion, 4- potato, 5 – zucchini, 6 - red paprika )

*Cereal products.* The NSP content of cereal products ranged from 3.10 to 15.38 g/100 g. The proportion of NSP components in cereal products was the following: 29.1 - 68.3 % glucose, 12.1 - 43.1 % xylose, 12.4 - 22.4 % arabinose, 1.9 - 20.3 % galactose and 3.6 - 7.1 % mannose (Figure 6).

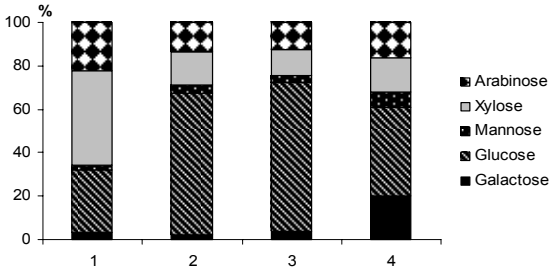


**Figure 6.** The proportion of NSP constituent sugars in cereal products (1- rye bran, 2- whole barley flour, 3- whole oat flour, 4- wheat flour)

The highest total NSP content was in rye bran (15.38 g/100 g), also – the highest arabinose and xylose content (3.45 g/100 g and 6.63 g/100 g, respectively). The reason could be that during cereal processing the outer layers of the grains, containing the highest amount of dietary fibre, are passed to the rye bran fraction.

Therefore, the lower amounts of NSP are passed to the flour, especially with low ash content. For example, wheat flour contained the lowest amount of total NSP (3.10 g/100 g) and specific proportion of constituent sugars: the highest amount of galactose and the lowest amounts of other monosaccharides. Compared to wheat flour, whole grain flour (whole barley and oat flour) produced using simple milling schemes contained higher amounts of NSP, also xylose, glucose and arabinose.

**Comparison of NSP composition of various plant foods.** For this, additional investigation of flaxseed and soy beans which according to literature have the highest amount of phytoestrogens, was carried out.

Compared with other analyzed plant foods, flaxseed and soy beans next to rye bran contain the highest amount of NSP. Also, galactose content was the highest in these products, especially in soy beans. The amount of this monosaccharide in soy beans and flaxseed was, respectively, 19.3 and 7.8 times higher - than in berries, 8.8 and 3.5 – than in vegetables and 7.4 and 3.0 – than in cereal products. Mannose content in flaxseed (0.15 g/100 g) was lower than in cereal products and garlic. Vice versa, soy beans as garlic contained the most of this monosaccharide (0.68 g/100 g).

Among analyzed plant food groups, the total NSP contain in cereal products was about 6.4 times higher than in vegetables and 4.1 times higher than in berries, however by comparing these results on a dry matter basis the differences are insignificant.

Considering the NSP composition of different plant foods groups, it can be suggested that the main NSP constituent sugars in berries are glucose and xylose, in vegetables – glucose, galactose and mannose, and in cereal products – glucose, xylose, and arabinose. This confirms that in berries higher quantities of cellulose are present, in vegetables – cellulose, galactans and mannans, and in cereal products – cellulose, β-glucan and arabinoxylans. The analysis of NSP constituent sugars of flaxseed and soy beans shows that cellulose, β-glucan, arabinoxylans and galactans are main NSP components in flaxseed, and cellulose, galactans and arabinans – in soy beans.

Analysis of different types of sugars also showed that there are differences between investigated plant food groups. In soy beans, berries and vegetables the amount of hexoses were respectively 2.9; 3.0 and 5.8 times higher than that of pentoses, therefore in cereal products and flaxseed the amounts of hexoses and pentoses were almost similar (content of hexoses compared to pentoses was only

1.2 - 1.3 times higher). These variations could have influence on the amount of produced mammalian lignans and their precursors in different plant products.

**Correlation between NSP constituent sugars of plants and plant lignan metabolites.** By analyzing the NSP composition of selected plant foods and mammalian lignans produced during their fermentation it was noticed that some correlations existed between quantities of total NSP, their constituent sugars and mammalian lignans (Table 9).

**Table 9.** Squared correlation coefficient ($R^2$) between total NSP, their constituent sugars and mammalian lignans

| Mamma-lian lignans | NSP constituent sugars | | | | | | | |
|---|---|---|---|---|---|---|---|---|
| | ara | xyl | man | glu | gal | pen | hex | Total |
| *berries* | | | | | | | | |
| END | 0.0451 | 0.1296 | 0.0009 | 0.2247 | 0.0106 | 0.1762 | 0.1713 | 0.2435 |
| ENL | 0.0037 | 0.1691 | 0.1126 | **0.6339** | 0.0558 | 0.3510 | **0.5535** | **0.7264** |
| Total | 0.0422 | 0.1347 | 0.0024 | 0.2488 | 0.0070 | 0.1891 | 0.1922 | 0.2711 |
| *vegetables* | | | | | | | | |
| END | 0.0025 | 0.0350 | 0.0053 | **0.4294** | 0.1729 | 0.0010 | 0.2602 | **0.3615** |
| ENL | 0.0285 | 0.1963 | 0.0018 | 0.0318 | 0.0009 | 0.0743 | 0.0110 | 0.0036 |
| Total | 0.0037 | 0.1101 | 0.0586 | 0.2009 | 0.0583 | 0.0259 | 0.0670 | 0.1278 |
| *cereal products* | | | | | | | | |
| END | 0.0052 | 0.0193 | 0.0143 | 0.7425 | **0.9382** | 0.0139 | **0.6444** | 0.0191 |
| ENL | **0.9710** | **0.9887** | **0.6797** | 0.0836 | 0.0850 | **0.9841** | 0.1537 | **0.8687** |
| Total | **0.9772** | **0.9610** | **0.7640** | 0.2709 | 0.0021 | **0.9671** | 0.3724 | **0.9780** |
| ara – arabinose, xyl – xylose, man – mannose, glu – glucose, gal – galactose, hex – hexose, pen - pentose | | | | | | | | |

For berries, an intermediate correlation was found between the total NSP and ENL values, and a loose correlation – between total NSP and the total amount of mammalian lignans. The studies showed that among the NSP components, the biggest influence on the amount of lignans has hexoses, especially glucose. An intermediate correlation was found between the glucose and ENL values and a loose correlation – between the glucose and total amount of mammalian lignans. On the contrary to ENL, no correlations have been found between NSP, their constituent sugars and the less stable END.

For vegetables, higher correlations between NSP and END were found. The intermediate correlations were found between the total NSP, glucose and END values. It can be suggested that hexoses, especially glucose are more important for lignan structure and content in vegetables and their bioconversion to mammalian lignans.

For cereal products, close correlations were found between the total NSP and the total amount of mammalian lignans, and also ENL, between pentoses and the

total amount of mammalian lignans, and also ENL, between arabinose, xylose and ENL, between galactose and END values.

The results showed that there is a correlation between the particularities of fermented food matrixes and the production of mammalian lignans. Considering the correlations between glucose and ENL found in berries and between glucose and END found in vegetables, it can be assumed that in these plant materials lignans are present in a conjugated form as glucosides. While in cereal products, in which close correlations were found between hexoses and END as well as between pentoses and ENL, lignans could occur as different glycosides e.g. not only conjugated with glucose but also with other NSP constituent sugars as xylose, arabinose. This indicates that pentoses are closely related to the quantities of plant lignans in cereal products.

## CONCLUSIONS

1. A rapid and simple HPLC method with coulometric dual electrode detector for the investigation of daidzein and genistein in soy beans was developed: as internal standard was chosen estriol; as a mobile phase – a mixture of ethanol, tetrahydrofuran, buffer solution (sodium acetate/trichloroacetic acid) [396/9/595, v/v/v, pH 2.6]; potentials of working electrodes +350 and +500 mV (R = 0.9985 for daidzein, and R = 0.9973 for genistein; recovery 81.9% ± 7.4%; coefficient of variation of repeatability by analyzing soy beans less than 12% )
2. The genotype of soy beans has no influence on the quantities of daidzein (1.36 - 2.10 g/kg) and genistein (1.83 - 2.63 g/kg) and these substances are termostable for heating at 100°C/30 minutes and at 280°C/5 minutes.
3. The quantities of mammalian lignans enterodiol and enterolactone produced during 24 h *in vitro* fermentation of berries, vegetables and cereal products were determined:
   a) the mammalian lignans production from berries ranged from 7.8 till 382.8 nmol/g, for enterodiol – from 0 till 327.2 nmol/g and for enterolactone – from 7.8 till 55.6 nmol/g;
   b) the mammalian lignans production from vegetables ranged from 10.5 till 91.2 nmol/g, for enterodiol – from 5.8 till 45.7 nmol/g and for enterolactone – from 4.7 till 45.5 nmol/g;
   c) the mammalian lignans production from cereal products ranged from 78.3 till 321.9 nmol/g, for enterodiol – from 8.7 till 149.3 nmol/g and for enterolactone – from 64.4 till 278.3 nmol/g;

   The mammalian lignan production from berries (except cloudberry) and vegetables was substantial lower than from flaxseeds and cereal brans. It was noticed, that from beries with higher mammalian lignan producing capability more enterodiol was produced than enterolactone, and from berries with low

producing capability – more enterolactone. In vegetables big differences between the quantities of enterodiol and enterolactone were not noticed. In cereal products the levels of enterolactone were higher than enterodiol.

4. The dietary fibre of cereal grains was investigated and its components were determined:
   a) the highest quantities of hemicelluloses, cellulose, lignin + cutin (respectively 14.0; 13.3 and 2.9 % d.m.) were found in oats;
   b) the highest quantity of β-glucan (5.1% d.m.) was found in barley;
   c) the quantities of hemicelluloses and cellulose in barley are higher than in wheat and rye (respectively in barley –11.2 and 4.9 % d.m., in wheat –7.8 and 2.6 % d.m., in rye –8.8 and 2.4 % d.m.).
5. The non-starch polysaccharides of various plant foods were investigated and their constituent sugars were determined. The main constituent sugars are:
   a) glucose and xylose in berries (respectively 1.23 and 1.98 g/100 g);
   b) glucose, galactose and mannose in vegetables (respectively 0.56; 0.31 and 0.18 g/100 g);
   c) glucose, xylose and arabinose in cereal products (respectively 3.74; 2.25 and 1.44 g/100 g);
   d) glucose, xylose, arabinose and galactose in flaxseed (respectively 4.5; 2.37; 2.21 and 1.87 g/100 g);
   e) galactose, glucose and arabinose in soy beans (respectively 4.64; 3.71 and 2.49 g/100 g).

   Soy beans, berries and vegetables contain higher quantities of hexoses, respectively 2.9; 3.0 and 5.8 times more than that of pentoses; in cereal products and flaxseed the quantities of hexoses and pentoses are similar, they both contain more arabinoxilans.
6. Quantitative relationships were found between non-starch polysaccharides constituent sugars and plant lignan metabolites:
   a) glucose and enterolactone ($R^2 = 0.6339$) in berries;
   b) glucose and enterodiol ($R^2 = 0.4294$) in vegetables;
   c) hexoses and enterolactone ($R^2 = 0.9841$), also between pentoses and enterodiol ($R^2 = 0.6444$) in cereal products;

   Lignans in cereal products occur not only conjugated with glucose, but also with pentoses (arabinose, xylose). The lignan production more depends on the dietary fibre specific composition and structure, which varies between the different kinds of plant foods, than on the amount of dietary fibre.
7. The results show, that cereal products and some kind of berries (raspberries, strawberries, and cloudberries) are important sources of mammalian lignan precursors. The results of the investigation adds to the information for a database for some bioactive compounds in plant foods.

## ABBREVIATIONS USED

ACN – acetonitrile
ADF – acid detergent fibre
ADL – acid detergent lignin
DF – dietary fibre
END – enterodiol
ENL – enterolactone
EtOH – ethanol
FID – flame ionization detector
GLC – gas liquid chromatography
HPLC – high performance liquid chromatography
IARC – International Agency on Research on Cancer
IUNS – International Union of Nutritional Sciences
MeOH – methanol
NaAc – natrium acetate
NDF – neutral detergent fibre
NSP – non-starch polysaccharides
R – correlation coefficient
$R^2$ – squared correlation coefficient
RT – retention time
THF – tetrahydrofuran
v/v – volume per volume

## LIST OF SCIENTIFIC PUBLICATIONS ON THE THEME OF THE THESIS

### Articles in the Scientific Journals


1. **Juodeikaitė E.**, Bašinskienė L., Juodeikienė G., Sontag G. Efektyviosios skysčių chromatografijos metodo, naudojant kulonometrinį detektorių, pritaikymas daiceinui ir genisteinui nustatyti sojos sėklose // Maisto chemija ir technologija. ISSN 1392-0227. 2003. Nr. 37 (I), 37-43.
2. **Juodeikaitė E.**, Bašinskienė L., Juodeikienė, K. Liukkonen. Žinduolių lignanų susidarymas uogų ir daržovių fermentacijos *in vitro* metu // Cheminė technologija. ISSN 1392-1231. 2004. Nr. 2 (32), 84-89.
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## Conference Papers

1. **Juodeikaitė E.**, Juodeikienė G. Fitoestrogenų tyrimai sojos sėklose didelio slėgio skystinės chromotografijos metodu // Chemija ir cheminė technologija. Studentų mokslinės konferencijos pranešimų medžiaga. Kaunas. 2000, 50-53.
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4. **Juodeikaitė E.**, Bašinskienė L., Liukkonen K. Enteradiolio ir enterolaktono susidarymo iš grūdinės žaliavos *in vitro* tyrimai // Maisto chemija ir technologija. Konferencijos pranešimų medžiaga. Kaunas. 2003, 11-12.
5. **Juodeikaitė E.**, Bašinskienė L., Juodeikienė G. and Sontag G. Use of HPLC with coulometric detection for some phytoestrogens analysis in soy beans // Book of Abstracts of the 1st International Symposium on „Recent Advances in food analysis“. Prague, Czech Republic. 2003, 265.
6. **Juodeikaitė E.**, Bašinskienė L. Juodeikienė G. The determination of mammalian lignans in an *in vitro* fermented medium of plant foods by HPLC // Book of Abstracts of the 12th International Symposium „Advances and Applications of Chromatography in Industry“. ISSN 1335-8413. Bratislava, Slovak Republik. 2004.

## CURRICULUM VITAE

Elena Juodeikaitė was born on November 26, 1975 in Kaunas. She graduated from Kaunas Secondary School No. 33 in 1993. In 1998 she finished her studies at Faculty of Chemical Technology of Kaunas Technology University with Bachelor degree in Food Technology. In 2000 she finished her studies at Faculty of Chemical Technology of Kaunas Technology University with Master degree in Food Chemistry and Technology. From 2000 till 2004 she was PhD student in the field of Chemistry at Department of Food Technology, Kaunas University of Technology.

## ACKNOWLEDGEMENTS

I would like to thank all my colleagues at Food Technology Department for sharing their particular knowledge and experience.

I am very gratefully to my supervisor assoc. prof. dr. L. BAŠINSKIENĖ for her wise supervision and knowledge, which she shared with me.

I also would like to thank prof. G. SONTAG, dr. K. LIUKKONEN, dr. H. KLÜGE for their advise and support and hosting me during my studies at their respective institutions.

## REZIUMĖ

**Temos aktualumas.** Pasaulinė vėžio tyrimo organizacija ir mitybos specialistus vienijančios asociacijos pažymi ypatingą maistinių skaidulinių medžiagų svarbą žmonių mitybos racione. Nagrinėjant gyventojų mitybą racionaliu pagrindu pastebėta, kad daugumoje ES šalių yra nepakankamas skaidulinių medžiagų suvartojimas. Svarbiausi jų šaltiniai yra grūdiniai ir ankštiniai javai, daržovės bei uogos, besiskiriantys tarpusavyje specifine, priklausančia nuo rūšies ir tipo chemine sudėtimi. Skirtingose šalyse, priklausomai nuo tradicijų, mitybos racionuose dominuoja įvairūs augaliniai produktai. Rytų Europoje ir Šiaurės šalyse daugiau suvartojama grūdų produktų, tuo tarpu Vakarų Europoje, ypač pietiniuos kraštuose – daržovės, vaisiai ir uogos gali būti pagrindiniu skaidulinių medžiagų šaltiniu.

Žmonių ir gyvūnų mitybos aspektu labiausiai ištirti biologiškai aktyvūs skaidulinių medžiagų komponentai – polisacharidai. Fiziologiniais tyrimais įrodyta, kad jie gerina žarnyno veiklą, mažina gliukozės ir cholesterolio kiekį kraujyje, o taip pat mažina susirgimų, tokių kaip cukrinis diabetas, nutukimas ir žarnyno vėžys, riziką.

Pastaruoju metu ypatingas dėmesys atkreiptas į skaidulinių medžiagų asocijuotus junginius, ypač fitoestrogenus. Epidemiologiniai stebėjimai ir laboratoriniai gyvūnų bei *in vitro* tyrimai atskleidė daugelį biologinių savybių, rodančių šių komponentų svarbą susirgimų, tarp jų ir vėžinių, profilaktikai. Iki šiol daugiausiai buvo tyrinėti grūdų ir ankštinių bei aliejingųjų sėklų, ypač linų sėmenų, sojos ir rugių, fitoestrogenai (lignanai ir izoflavonai). Mokslininkai teigia, kad lignanais praturtinta mityba gali sumažinti vėžinių susirgimų riziką. Veikiant augalų lignanus žmogaus žarnyno mikroflora, jie biokonversijos metu virsta žinduolių lignanais enterodioliu ir enterolaktonu, kurie mažina su hormonų sutrikimais susijusių vėžinių susirgimų (krūtinės ir prostatos) pavojų ir yra svarbūs koronarinės širdies ligos prevencijai.

Dėl sojos sėklų izoflavonų ir jų metabolizmo produktų poveikio literatūroje sutinkamos prieštaringos nuomonės. Vienų tyrinėtojų rezultatais jų poveikis

žmogaus organizmui yra antivėžinis, o kitų – priešingai, žmogaus organizme jie veikia kaip estrogenai, stimuliuodami šios rūšies susirgimus.

Todėl skaidulinės medžiagos ir su jomis asocijuoti fitoestrogenai bei jų pokyčiai organizme yra aktualus tyrimo objektas. Ypač reikėtų atkreipti dėmesį į mažiau ištirtus augalinius produktus. Manoma, kad šioje srityje reiktų atlikti dar daug tyrimų, norint išsiaiškinti jų vaidmenį ir ligų prevencijos mechanizmą, nustatyti vartojimo normas. Be to, tolesnis šios problemos nagrinėjimas susijęs su naujų tyrimo metodų vystymu.

**Darbo tikslas:** Ištirti fitoestrogenų, jų biologinės konversijos produktų ir skaidulinių medžiagų bei jų komponentų kiekius plačiausiai vartojamuose augaliniuose produktuose ir nustatyti galimus jų tarpusavio ryšius.

**Darbo uždaviniai:**

- Parengti efektyviosios skysčių chromatografijos metodą, naudojant kulonometrinį dviejų elektrodų detektorių matairezinoliui, daiceinui, genisteinui ir formononetinui tirti ir jį pritaikyti šių fitoestrogenų analizei sojos sėklose.
- Įvertinti kai kurių technologinių veiksnių: genotipo ir terminio apdorojimo, įtaką fitoestrogenų kiekiui sojos sėklose.
- Fermentacijos *in vitro* metodu nustatyti augalų lignanų biokonversijos produktus enterodiolį ir enterolaktoną.
- Atlikti žinduolių lignanų, susidariusių fermentuojant įvairias uogas, daržoves, grūdų produktus ir linų sėmenis, palyginamąjį įvertinimą.
- Ištirti skaidulinių medžiagų ir jų komponentų kiekius įvairiose uogose, daržovėse, grūdų produktuose, sojos sėklose ir linų sėmenyse, tuo papildant duomenų bazę apie šių medžiagų kiekius augaliniuose produktuose.
- Nustatyti galimus kokybinius ryšius tarp skaidulinių medžiagų polisacharidų, jų komponentų ir su jais asocijuotų fitoestrogenų bei jų metabolitų kiekių.

**Darbo mokslinis naujumas.** Parengta paprasta ir greita metodika izoflavonams daiceinui ir genisteinui iš augalinių produktų išskirti ir jų kiekiui nustatyti efektyviosios skysčių chromatografijos metodu, pritaikant kulonometrinį dviejų elektrodų detektorių. Šiuo metodu atlikta sojos sėklų analizė, įvertinant technologinių veiksnių – genotipo ir terminio apdorojimo įtaką minėtų junginių kiekiui. Lignanų biokonversijai tirti pritaikytas fermentacijos *in vitro* metodas, o jų metabolitams enterodioliui ir enterolaktonui nustatyti – efektyviosios skysčių chromatografijos metodas su kulonometriniu aštuonių elektrodų detektoriumi. Pirmą kartą atlikti uogų fermentacijos *in vitro* tyrimai. Įvertinti ir palyginti enterodiolio ir enterolaktono kiekiai, susidarę įvairių grūdų produktų, aliejingųjų sėklų bei daržovių ir uogų fermentacijos *in vitro* metu. Atlikta detali kai kurių grūdų produktų, ankštinių ir aliejingųjų sėklų, o taip pat daržovių ir uogų skaidulinių medžiagų analizė, pritaikius jų išskyrimui fermentinį metodą, o atskirų

komponentų analizei – dujų-skysčių chromatografijos metodą. Nustatyti kokybiniai ryšiai tarp skaidulinių medžiagų polisacharidus sudarančių monomerų ir jų asocijuotų fitoestrogenų bei jų metabolitų.

**Praktinė reikšmė.** Gauti fitoestrogenų ir jų metabolitų tyrimų rezultatai papildys duomenų bazę apie šių biologiškai aktyvių medžiagų kiekius augaliniuose produktuose ir jų galimus šaltinius. Įrodyta, kad uogos taip pat yra svarbus fitoestrogenų ir žinduolių lignanų susidarymo šaltinis. Patvirtinta, kad tradiciškai Lietuvoje vartojami skaidulinių medžiagų šaltiniai – grūdų produktai ir ypač rugiai yra naudingi žmogaus mitybai. Pagal fermentacijos metu susidariusių žinduolių lignanų kiekius kai kuriuos augalinius produktus: rugių sėlenas, spanguoles, tekšes, avietes ir žemuoges galima rekomenduoti maisto papildų ir funkcinių produktų kūrimui. Sudaryta daiceino ir genisteino nustatymo metodika taikytina kokybiniam ir kiekybiniam šių junginių tyrimui sojos sėklose. Disertacijoje pateikta medžiaga naudinga mitybos, fitoterapijos, žemės ūkio ir maisto pramonės specialistams.

**Išvados:**

1. Paruoštas greitas ir paprastas ESC su kulonometriniu dviejų elektrodų detektoriumi metodas daiceinui ir genisteinui tirti sojos sėklose, vidiniu etalonu parinkus estriolį; judančiąja faze – 39,6 % (tūrio) etanolio, 0,9 % tetrahidrofurano, 59,5 % natrio acetato ir trichloracto rūgšties buferinio tirpalo mišinį (pH 2,6); elektrodų potencialo vertes +350 mV ir +500 mV (daiceinui $R^2$ = 0,9985, genisteinui $R^2$ = 0,9973, atgavimo koeficientas 81,9 % ± 7,4 %, santykinis pakartojamumo nuokrypis, analizuojant sojos sėklas, neviršija 12 %).
2. Nustatyta, kad daiceino (1,36 - 2,10 g/kg) ir genisteino (1,83 - 2,63 g/kg) kiekiai nepriklauso nuo sojos sėklų genotipo ir yra stabilūs terminio apdorojimo (100 °C temperatūroje 30 min ir 280 °C temperatūroje 5 min) poveikiui.
3. Nustatyti uogų, daržovių ir grūdų produktų fermentacijos *in vitro* metu susidarę žinduolių lignanų enterodiolio ir enterolaktono kiekiai:
   a) fermentuojant uogas susidaro nuo 7,8 iki 382,8 nmol/g žinduolių lignanų: enterodiolio – nuo 0 iki 327,2 nmol/g, enterolaktono – nuo 7,8 iki 55,6 nmol/g,
   b) fermentuojant daržoves susidaro nuo 10,5 iki 91,2 nmol/g žinduolių lignanų: enterodiolio – nuo 5,8 iki 45,7 nmol/g, enterolaktono – nuo 4,7 iki 45,5 nmol/g,
   c) fermentuojant grūdų produktus susidaro nuo 78,3 iki 321,9 nmol/g žinduolių lignanų: enterodiolio – nuo 8,7 iki 149,3 nmol/g, enterolaktono – nuo 64,4 iki 278,3 nmol/g.

   Uogų (išskyrus tekšes) ir daržovių fermentacijos metu susidarę žinduolių lignanų kiekiai yra daug mažesni nei linų sėmenų ir grūdų sėlenų. Konstatuota,

kad kai uogose žinduolių lignanų susidaro daugiau, vyraujantis komponentas yra enterodiolis, mažiau – enterolaktonas. Tirtoms daržovėms tokie dideli enterodiolio ir enterolaktono skirtumai nenustatyti. Fermentuojant grūdų produktus nustatyta daugiau enterolaktono.

4. Ištirtos varpinių javų grūdų polimeriniai skaidulinių medžiagų junginiai ir nustatyta, kad:
   a) avižose yra didžiausi hemiceliuliozių, celiuliozės, lignino (kartu su kutinu) kiekiai (atitinkamai, 14,0; 13,3 ir 2,9 % s.m.),
   b) miežiuose yra daugiausia β–gliukanų (5,1 % s.m.),
   c) celiuliozės ir hemiceliuliozių kiekiai miežiuose yra didesni negu kviečiuose ir rugiuose (atitinkamai, miežiuose – 4,9 ir 11,2 % s.m., kviečiuose – 2,6 ir 7,8 % s.m., rugiuose – 2,4 ir 8,8 % s.m.).
5. Ištirti augalinių produktų skaidulinių medžiagų polisacharidai ir nustatyta, kad jų sudėtyje vyraujantys monosacharidai yra:
   a) uogose – gliukozė ir ksilozė (atitinkamai, 1,23 ir 1,98 g/100 g),
   b) daržovėse – gliukozė, galaktozė ir manozė (atitinkamai, 0,56; 0,31 ir 0,18 g/100 g),
   c) grūdų produktuose – gliukozė, ksilozė ir arabinozė (atitinkamai, 3,74; 2,25 ir 1,44g /100g),
   d) linų sėmenyse – gliukozė, ksilozė, arabinozė ir galaktozė (atitinkamai, 4,5; 2,37; 2,21 ir 1,87 g/100 g),
   e) sojos sėklose – galaktozė, gliukozė ir arabinozė (atitinkamai, 4,64; 3,71 ir 2,49 g/100 g).

   Nustatyta, kad sojos sėklose, uogose ir daržovėse heksozių yra, atitinkamai, 2,9; 3,0 ir 5,8 karto daugiau nei pentozių, o grūdų produktuose ir linų sėmenyse heksozių ir pentozių kiekiai yra panašūs, juose daugiau arabinoksilanų.
6. Nustatytas koreliacinis ryšys:
   a) uogose – tarp skaidulinių medžiagų polisacharidus sudarančios gliukozės ir fermentacijos metu susidariusio enterolaktono ($R^2 = 0,6339$),
   b) daržovėse – tarp skaidulinių medžiagų polisacharidus sudarančios gliukozės ir fermentacijos metu susidariusio enterodiolio ($R^2 = 0,4294$),
   c) grūdų produktuose – tarp skaidulinių medžiagų polisacharidus sudarančių heksozių ir enterolaktono ($R^2 = 0,9841$), pentozių ir enterodiolio ($R^2 = 0,6444$).

   Įrodyta, kad lignanai grūdų produktuose yra kompleksuose ne tik su gliukoze, bet ir su pentozėmis (arabinoze, ksiloze). Lignanų kiekis augaliniuose produktuose daugiau priklauso ne nuo skaidulinių medžiagų kiekio, bet nuo jų kokybinės sudėties.
7. Rezultatai rodo, kad grūdų produktai ir kai kurios uogų rūšys (avietės, žemuogės ir tekšės) yra reikšmingas žinduolių lignanų susidarymo šaltinis. Gauti rezultatai papildo duomenų bazę apie biologiškai aktyvių medžiagų kiekius augaliniuose produktuose.

UDK 547.9(043)

SL 344, 2005-04-08.1. Leidyb. apsk.1. Užsakymas 166. Tiražas 70 egz..
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