ISOFLAVONES DISTRIBUTION IN THE PROCESS OF SOY PROTEIN RECOVERY

***Nosenko Tamara T., Mank Valeriy V., Maksimova Olena S.**

National University of Food Technologies – Kyiv, Ukraine

***Nosenko Tamara, tnosenko@yahoo.com**

Abstract

Soy bean contain soy isoflavones which have an effect on different metabolic disorders in human. In this study we have analysed distribution of soy isoflavone between different product under recovery of soy protein. Using mass-spectroscopy and NMR analysis we have detected main isomers in soy bean daidzin and genistin. It was shown that isoflavone fraction is extracted to alcohol solution in processing of protein concentrate.

Having analysed products of protein isolate processing we detected that isoflavones were extracted to alkaline solution during protein extraction. Only about 17 % of isoflavones have been remaining in protein isolate. Main part of them was detected in whey water after protein precipitation. And it is possible to elicit them according to standart procedure using extraction by ethylacetate and precipitation by chloroform. Taking into account our results we are proposing to use the by-products of soy protein processing for isoflavone concentrate producing.

Keywords: soy isoflavones, protein concentrate, protein isolate

Introduction

Isoflavones are the phenolic substances that are detected only in some plant family. The highest content of isoflavones have been found in soy bean. The content of these substances differs in different soy lines, depends on weather conditions, maturity of seed and so on. (Britz, Schomburg & Kenworthy, 2011). It was shown that these substances had weakly estrogenic and anticarcinogenic effect on human (Lu, Anderson, Grady & Nagamani, 1996), they could be used for prevention cardiovascular and another metabolic disorders (Wagner et al, 1997, Nestel et al, 1997).

A number of studies have been done to investigate isoflavone content in different soy products and soy protein supplements. Having done research on isoflavone concentration in soybean samples on a C18 reverse-phase column using a two-step gradient solvent system, Wu & Muir (2008) showed that producing soybean hydrolysate led to a nearly 40% loss of isoflavones compared with the original soybean flour. Barnes, Kirk & Coward (1994) showed that high temperature treatment (higher than 80 °C) resulted in changes in isoflavone isomers content and toasted flour and protein isolate contained only moderate amounts of each isomer. Yerramsetty, Mathias, Bunzel & Ismail (2011) detected interconversions of malonylgenistin and its isomer in buffered and soymilk systems. Significant interconversions of isoflavone isomers was also observed in thermally treated soymilk at pH 7 and pH 9 (Nufer, Ismail & Hayes, 2009).

Genovese & Lajolo (2010) reported that production of soy isolates leads to an increase in the percentage of free aglycones. Coward, Barnes, Setchell & Barnes (1993) have shown decreased isoflavone content in such soy protein products from American and Asian diets as soy sauce, alcogol-extracted protein concentrates and protein isolates. Low isoflavone content in alcogol-extracted protein concentrates and protein isolates had

demonstrated also by Wang & Murphy (1994). Lin, Krishnan & Chunyang (2006) have shown effect of precipitating and washing temperatures on retention of isoflavones and saponins during processing of soy protein isolate. The purpose of our study was to investigate the soy isoflavones distribution between different by-products and final products during soy protein processing.

Materials and methods

Isoflavones extraction. Isoflavones were extracted from defatted soy meal using ethyl alcohol solution (70 %, v/v) in the same extractor during some 4 hour. Than solvent was evaporated on the rotor evaporator and residue was treated by hot water. Received solution was filtrated through paper filter to remove insoluble substances. Filtrate was evaporated again and residues were extracted three time by ethylacetate. Obtained extracts were combined and dried by sodium sulfate. Isoflavones were precipitated by chloroform and filtrated.

Isoflavones analysis. Isoflavones composition was analysed by column chromatography on silicagele with system solvents ethyl alcohol:water. Eluate was analysed photometrically. Mass-spectroscopy was used for identification molecular mass of isoflavone isomers.

Isoflavones identification. ^{1}H nuclear magnetic resonance (NMR) spectra were measured on a Bruker Avance DRX300 spectrometer and were used for isoflavones identification. Chemical shifts obtained NMR spectra were compared with standarts (Pouchert, 1983) .

Obtaining of protein concentrates. Defatted soy seed was used for protein concentrate obtaining according to next procedure. Defatted soy seed was mixed with ethyl alcohol solution (70 %, v/v) in relation 1:10 and exposed during 40-50 min with stirring under 45-50 °C. After this insoluble residue was precipitated by centrifugation. The supernatant (extract of soluble substances) was analysed on isoflavone isomer content. Pellet was dried to 8-10 % fluidity and protein and isoflavone content was analysed.

Obtaining of protein isolate. Protein was extracted from defatted soy seed by alkaline solution (pH 8.5-9.5) under constant stirring and temperature 50-55 °C during 40-50 min, relation cake:solution was 1:10. After this insoluble residue was precipitated by centrifugation. The supernatant (protein extract) was used for isoelectric protein precipitation at pH 3.8-4.5. After protein coagulation pellet was separated by centrifugation (3 000 x g). Protein pellet was collected and dried to 6-8 % fluidity. Protein and isoflavone isomer content was analysed in protein product and in supernatant (whey water).

Results and discussion

After extraction of isoflavones from soy bean we have detected that total content of isoflavones was 0.39 %, calculated on the dry substances of soy meal. Obtained mixture of isoflavones from defatted soy seed was studied on the isoflavone composition. The three individual substances were detected on chromatogram in a ratio 1:1:0,03. The holding time of these components was very close – 1.38, 1.41 and 1.53 min.

Mass-spectroscopy analysis gave possibility to estimate molecular mass of detected isomers. They were 417,4 and 433,4. Such molecular masses correspond to daidzin and genistin respectively. Daidzin is a glukoside of isoflavone daidzein and genistin

correspondingly - of genisteine. We could not determine the third component due to its very insignificant content.

For identification of detected isomers we have analysed ^{1}H NMR spectra of isoflavone mixture from soy seed. We have analysed aromatic part of spectrum for this purpose (Fig.1). And it was confirmed the presence of daidzin and genistin in isoflavone extract, their ratio was about 1:1.

We used defatted soy meal for processing of protein concentrate. We had proposed that isoflavone substances could be extracted from soy meal simultaneously with other soluble substances to ethyl alcohol solution.That is why we had used ethyl alcohol extract obtained under protein concentrate producing to recover isoflavones. In this case we followed the same procedure as for isoflavone extraction from defatted soy meal. We have obtained 0.39 % (from the initial mass of dry substances meal) output of isoflavone concentrate, that means that almost whole isoflavone fraction is extracted to ethyl alcohol extract in processing of protein concentrate. Mass-spectroscopy and ^{1}H NMR analysis have confirmed the presence of two isomers of isoflavones - daidzin and genistin in ratio about 1:1 (Fig.2).

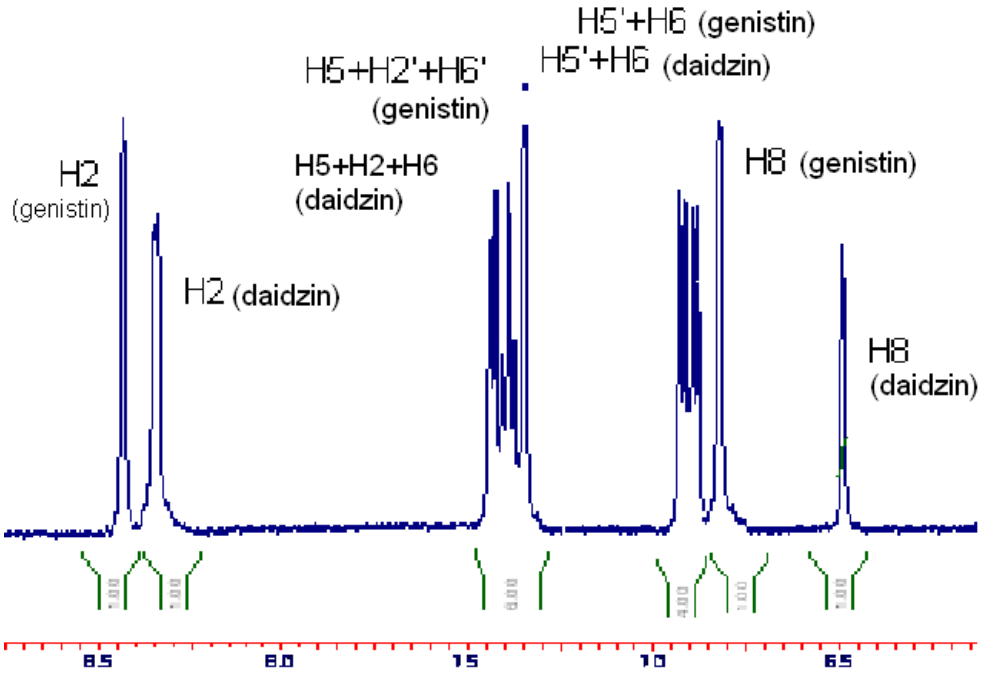

Fig. 1. ^{1}H Nuclear magnetic resonance (NMR) spectrum of isoflavone extract from soy bean.

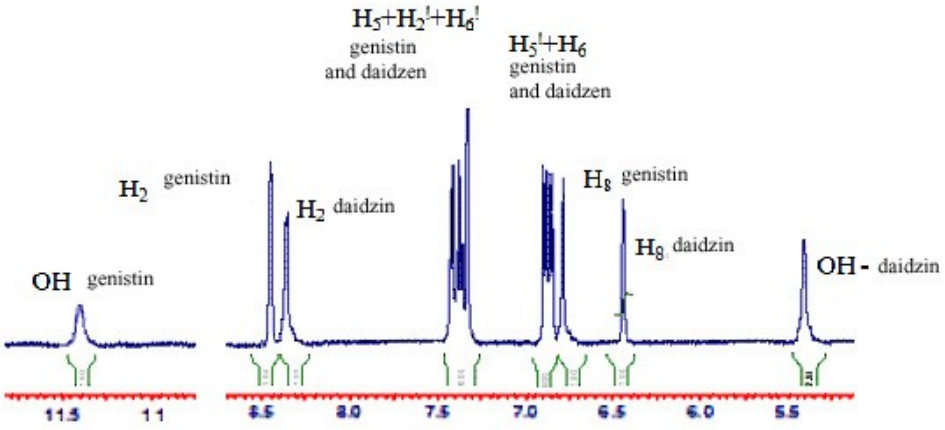

Fig. 2. [1] H Nuclear magnetic resonance (NMR) spectrum of isoflavone extracted from alcohol solution obtained in protein concentrate processing.

We have studied also isoflavone content in different by-product during protein isolate processing and in protein isolate. To obtain protein isolate protein were extracted by alkaline solution and then isoelectrically precipitated.

Firstly we have determined isoflavone content in whey water after protein precipitation. For this purpose whey water was evaporated on the rotary evaporator and residue was extracted three time by ethylacetate and obtained extracts were combined. In other respects the procedure was the same as described earlier. We have analysed the isoflavone content and composition (Table 1). About 87% of whole isoflavone fraction of soy bean is containing in whey water. We have detected three isoflavone isomers– daidzin, genistin and malonildaidzin. Their ratio was about 1:1:0.33 respectively.

Analysis of insoluble residue after protein extraction indicated only trace content of isoflavone in this by-product (Table 1). Thus almost whole isoflavone fraction is extracted to alkaline solution together with protein. These data are a little surprising because it is known that these substances are not soluble in water solution. We suppose that this result may be due to isoflavones are bound with protein in soy bean and that why these substances are extracted simultaneously.

Our results have demonstrated insignificant isoflavone content in protein isolate. It was about 17 % (on the protein isolate mass). These our results do not agree with the data obtained by Jun Lin et al. which have shown that isoflavone content in protein isolate was significantly higher including whole range of investigated temperature [Yerramsetty V.et al., 2011].

Table 1. Isoflavone isomers extracted from different products of soy bean processing. Mass-spectroscopy and NMR data.

Product	Molecular mass of isoflavone isomer	Isoflavone isomer	Ratio	Common output, % of soy bean mass
Soy bean	417,0	daidzin	1	0.39
	433,6	genistin	1	
Ethyl alcohol extract from defatted soy meal	417,0	daidzin	48	0.39
	433,6	genistin	42	
Protein concentrate	Not detected			
Insoluble residue after protein extraction	417,0	daidzin	45	0.01
	433,6	genistin	43	
	503.2	malonildaidzin	9	
Whey water after protein precipitation	417,0	daidzin	39	0.26
	433,6	genistin	43	
	503.2	malonildaidzin	14	
Protein isolate	417,0	daidzin	46	0.05
	433,6	genistin	43	
	503.2	malonildaidzin	7	

Conclusions

Our results showed the presence of three main isoflavone isomers in soy beans and in by-product of their processing – daidzin, genistin and malonildaidzin. During soy processing these substances are extracted to alcohol and water solution. Only about 17 % of initial content isoflavones is containing in protein isolate. We did not detect them in protein concentrate.To retain the main part of soy isoflavones it is possible to extract them from by-product of protein processing. It is obviously that isoflavone concentrate will have higher medicinal effect than soy protein.

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