INVESTIGATION OF SOY ISOFLAVONES DISTRIBUTION DURING THE SOY BEEN PROCESSING

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Abstract: *Soy bean contain soy isoflavones which have an effect on different metabolic disorders in human. In this study we have analysed isomeric composition of isoflavones in soy bean and investigated distribution of this substances between different product under soy bean processing. Using mass-spectroscopy and NMR analysis we have detected two 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 of them were 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 the scheme of soy bean processing with isoflavone concentrate producing.

Keywords: *soy, soy isoflavones, protein concentrate, protein isolate.*

Introduction

Isoflavones are the phenolic substances that are not so widely spreadible in plants but are detected only in some plant family. The highest content of isoflavones have been found in soy bean. There were 12 isomers of isoflavones. It was shown that these substances had are weakly estrogenic and anticarcinogenic effect on human [1-3], they could be used for prevention cardiovascular and another metabolic disorders [4-7]. In particular, epidemiological and clinical studies suggest potential chemopreventive effects for the soy isoflavone genistein against breast cancer in womens.
In the same time soy isoflavones have also antioxidant properties as it was shown Georgetti SR et al. [8] by the Chemiluminescence Method.
Thus soy isoflavones could be used for prevention of different human desease. Commonly soy been processed for vegetable oil and different protein products and the retention of isoflavones during processing was not taking into account to nowadays. Only some studies were publicated devoted to the problem of soy isoflavones in connection with protein production. Jun Lin et al. have shown effect of precipitating and washing temperatures on retention of isoflavones and saponins during processing of soy protein isolate [9]. Authors have shown lowering of isoflavone lost in the protein isolate with reducing of processing temperature.

The purpose of our study was to investigate the isomeric composition of soy isoflavones and their distribution between different by-products of soy processing.

Materials and methods

*Soy been defatting.*Crushed soy bean were defatted in apparatus of Soks-lett to contain less 1 % fat. Soy cake was dryed to 9-10 % fluidity.

Isoflavones extraction. Isoflavones were extracted from defatted soy meal using ethyl alcohol solution in the same extractor during some 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. Insoluble residue on the filter was dried in vacuum and it was a mixture of isoflavones. This mixture was analysed on the isoflavones composition.

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 to reveal the molecular mass and the identification 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.

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 withdrawn and isoflavone content was analysed. 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 some exposition protein pellet was separated by centrifugation (3 000 x g). Protein pellet was collected and dried to 6-8 % fluidity. Protein and isoflavone content was analysed in protein product and in supernatant (whey water).

Results and discussion

Our procedure of extraction of isoflavones from soy bean have given output 0.39 % isoflavone concentrate. Obtained mixture of isoflavones from defatted soy seed was analysed on the isoflavone composition. The three individual substances were detected on chromatogram in a ratio 1:1:0,03 (Fig.1). 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. We could not determine the third component due to its very insignificant content.

For identification of detected isomers we have analysed NMR spectra of isoflavone mixture. Aromatic part of spectrum was taken into account for this purpose (Fig.2).

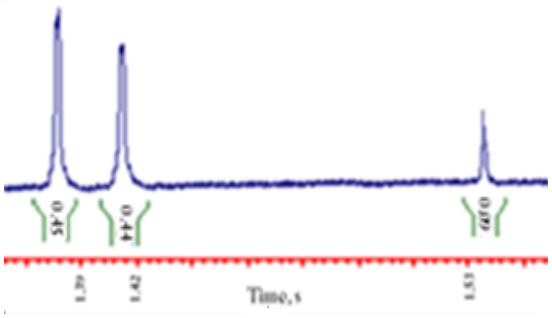

Fig. 1. Chromatogram of isoflavone extract

And it was confirmed the presence of daidzin and genistin in isoflavone extract.
We used defatted soy meal for processing of protein concentrate. We had proposed that isoflavone substances could extract from defatted soy meal simultaneously with other soluble substances to ethyl alcohol solution.

That is why we had used obtained ethyl alcohol extract 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 soy bean) output of isoflavone concentrate, that means that whole isoflavone fraction is extracted to ethyl alcohol extract in processing of protein concentrate.
Mass-spectroscopy and NMR analysis have confirmed the presence of two isomers of isoflavones - daidzin and genistin and their hydrolysed derivatives (Table 1).
We have studied also isoflavone content in different product during protein isolate processing. First of all we have analysed

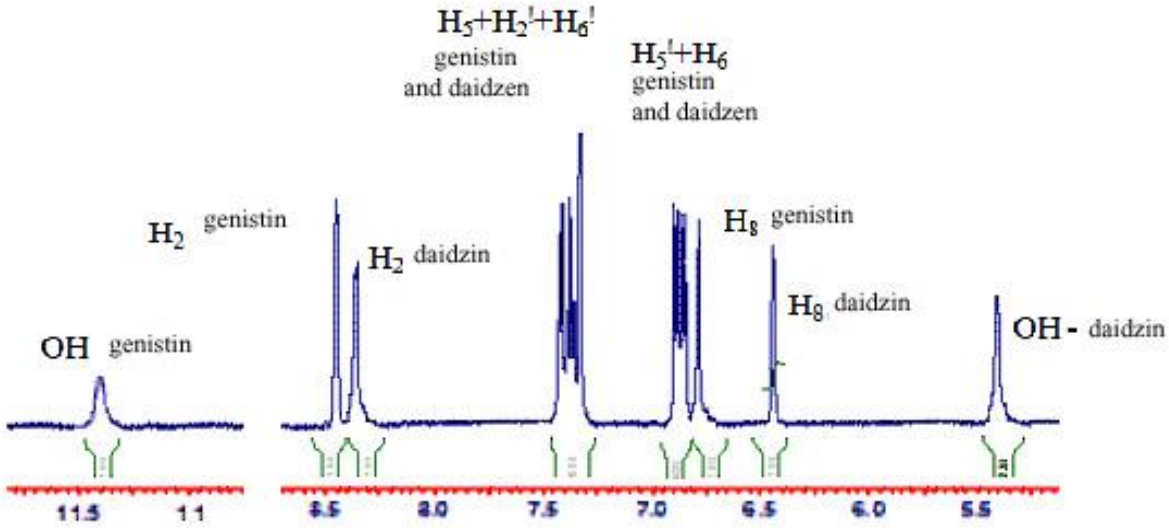

Fig. 2. ^{1}H Nuclear magnetic resonnce (NMR) spectrum of isoflavone extract

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	
	255,3	daidzein	-	
	271,4	genistein	-	
Protein concentrate	Not detected			
Insoluble residue after protein extraction	417,0	daidzin	45	0.01
	433,6	genistin	43	
	503.2	malonildaidzin	9	
	255,3	daidzein	-	
	271,4	genistein	-	
Whey water after protein precipitation	417,0	daidzin	39	0.26
	433,6	genistin	43	
	503.2	malonildaidzin	14	
	255,3	daidzein	-	
	271,4	genistein	-	
Protein isolate	417,0	daidzin	46	0.05
	433,6	genistin	43	
	503.2	malonildaidzin	7	
	255,3	daidzein	-	
	271,4	genistein	-	

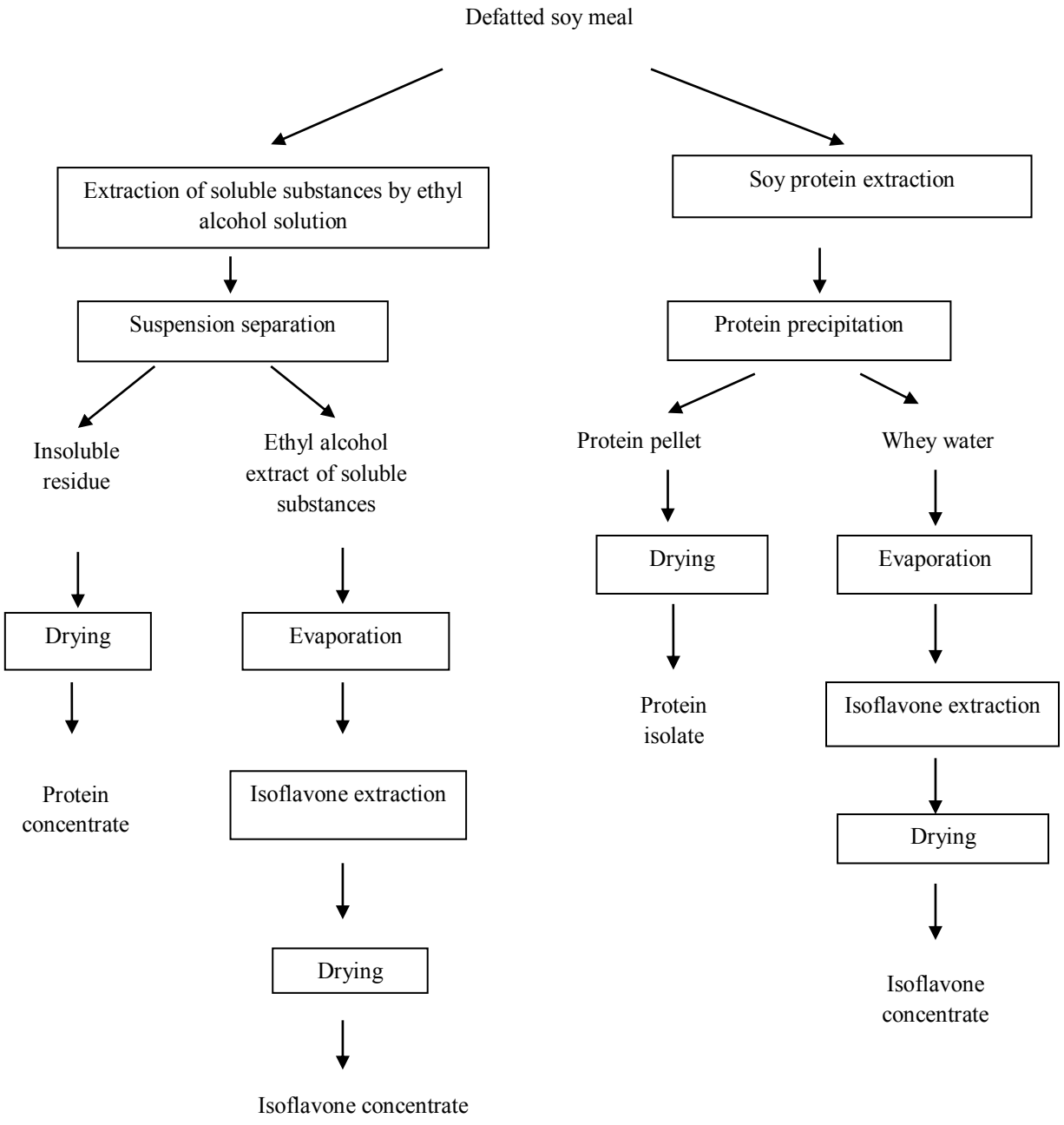

Fig.3 The scheme of soy bean processing with soy isoflavone producing.

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. They contain all detectable isomers. In the same time malonildaidzin content in this fraction was higher.

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Analysis of insoluble residue after protein extraction indicated only trace contain of isoflavone in this by-product (Table 1). Thus almost whole isoflavone fraction is extracted to alkaline solution together with protein

Our results demonstrated insignificant isoflavone content in protein isolate. It was about 17 % of total their content on soy bean. Although authors of work [9] have shown that isoflavone content in protein isolate was significantly higher including whole range of temperature during processing.

In agreement with our results we can propose the scheme of soy bean processing with isoflavone concentrate producing (Fig.3).

Conclusion

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 water and alcohol 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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