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A New Method of Isolation of Isoflavones from *Glycine max* (Soya Beans) by Complexation Technique

[1]Priya R. Rao, [1]K. Girish Kumar and [1]Manoj C. Narayanan
[1]Pelican Biotech. and Chemical Labs, Vayalar P.O., Cherthala,
Alappuzha District, Kerala-688 536, South India
[2]Department of Applied Chemistry, Cochin University of Science and Technology,
Kochi, Kerala-682 022, South India

Abstract: The technique of complexation was used to prepare isoflavones-enriched soya extract and to isolate genistein, from soya bean extract. The finding shows that isoflavones, in the extracts, form stable complexes with metals. This can be used as a novel method for isolation of flavonoids from plant sources.

Key words: Soya extract, genistein, isoflavones, glycine max, complexation

INTRODUCTION

Flavonoids are known to be highly potent anti-oxidants as well as very effective bioactive compounds. Use of flavonoids as therapeutic drugs are increasing and the technology for isolating them are of high importance to keep the cost of these drugs low. Conventional methods of isolation include extraction using a suitable solvent, fractionating into alkaline soluble fraction, chromatography and crystallization. Earlier reports explain the effective extraction procedures and concentration of isoflavones by single phase extraction (Levytskyi and Bohatov, 2002; Rostagno *et al*., 2005). There are reports of the methods for extraction and analysis of soybean isoflavones by RP-HPLC, LC-MS and fast column HPLC (Qu *et al*., 2005; Griffith and Collison, 2001; Klejdus *et al*., 2005). These processes are multi-step, uses sophisticated technology and the cost of solvents and consumables are high. For the present study, we have taken up the isolation of the flavonoids present in *Glycine max*, commonly known as Soya beans. Soya beans have been consumed by humans for years owing to their high protein and nutritional quality. Soya bean extracts are mainly comprised of Soya saponins and chemoprotective isoflavones. The major isoflavones are genistein and daidzein (Franke *et al*., 1994; Liggins *et al*., 1998). These isoflavones have been reported to show excellent estrogenic, antifungal, anti-tumor and antimutagenic properties (Rishi, 2002; George and Sheehan, 2002; Lori Coward *et al*., 1993; Miyazawa, 1999). Soy isoflavones have been shown to play a role in estrogen-dependant breast cancer (Barnes *et al*., 1990).

There have been reports on the interaction of metals with flavonoids and studies of the anti-oxidant activity of flavonoids by complexation of transition metals (Shizuo Toda and Yoshiaki Shirataki, 2001; Afranas'ev *et al*., 1989; de Souza and de Giovani, 2005; Torreggiani *et al*., 2005). This technique was utilized to study the use of metal complexation as a technological tool to isolate isoflavones from herbal sources. There are no reports on the isolation of flavonoids from plant sources, which uses the interaction of metals with flavonoids. In this study a new technology of isolating isoflavones is discussed, which is expected to be more cost effective and simpler.

Corresponding Author: Priya R. Rao, Pelican Biotech. and Chemical Labs, Vayalar P.O., Cherthala, Alappuzha District, Kerala-688 536, South India

Table 1: Percentage yields of isoflavones (genistein+daidzein) from soya beans, by complexation method

Parameters	Copper chloride	Copper sulphate	Copper nitrate	Copper acetate	Aluminium chloride	Ferric chloride	Zinc chloride
Room temp.							
MeOH extract	0.0233	0.0135	0.0143	0.0210	0.0233	0.0180	0.0240
Fresh soya beans	0.0750	0.0420	0.1050	0.1150	0.0650	0.0550	0.1600
40°C							
MeOH extract	0.0233	0.0180	0.0160	0.0240	0.0150	0.0160	0.0233
Fresh soya beans	0.0550	0.1550	0.0650	0.1400	0.1600	0.0850	0.1000

MATERIALS AND METHODS

A part of the present study was carried out at the Analytical Chemistry Lab at the Department of Applied Chemistry, Cochin University, Kochi, Kerala, India and a part of the work at the R and D Labs of Pelican Biotech. and Chemical Labs, Cherthala, Kerala during the period June 2006 to February 2007.

The commercially available Soya extract was taken to study the feasibility of complex formation with metal ions (Fe^{3+}, Al^{3+}, Cu^{2+} and Zn^{2+}) under different conditions. The conditions were optimized and then used to study the isolation of isoflavones from fresh soya beans. The different salts taken up for the present study were ferric chloride, aluminium chloride, copper chloride, copper nitrate, copper sulphate, copper acetate and zinc chloride.

Initial study using nitrates, oxides, sulphates and chlorides of Fe^{3+}, Al^{3+} and Zn^{2+} showed that chlorides easily formed complexes with the isoflavones and so were taken up for the study.

Experiment 1

Commercially available Soya extract (40% free isoflavones) was treated with various salts under various concentrations in methanol at Room Temperature (RT) for 72 h and at 40°C, for 4 h. The residue was filtered out washed with methanol and dried under vacuum to give a solid residue R1. The filtrate was concentrated to dryness and washed with acetone (to remove unreacted soy extract) and dried to give a solid residue R2. Both R1 and R2 were analyzed by IR spectroscopy to ascertain any complexation. Both residues were hydrolyzed using dilute acid, to release free isoflavones which was extracted using ethyl acetate. The ethyl acetate layer was analyzed for the presence of isoflavones by TLC on comparison with standard genistein and daidzein.

Experiment 2

Soya beans procured commercially was powdered, extracted in methanol (for 6×6 h) and filtered. The extract was treated with various salts and studied (a) at 40°C, for 4 h and (b) at RT for 72 h and worked up as in Exp. 1 (Table 1).

Experiment 3

Soya beans procured commercially was powdered and was directly treated with various salt solutions (a) at 40°C, for 4 h and (b) at RT for 72 h and worked up as in Exp. 1 (Table 1).

RESULTS AND DISCUSSION

Previous studies have shown that pure flavonoids from stable complexes with metal ions (Toda and Shirataki, 2001; Afranas'ev *et al*., 1989; de Souza and de Giovani, 2005; Armida *et al*., 2005). The present study shows how flavonoids in the plants can from stable complexes and how this can be utilized as a tool for the isolation of the same from plants. It was seen that the isoflavones, genistein and daidzein were able to from stable complexes with the metal ions. The complexes, after hydrolysis using dilute acid, yielded a mixture of daidzein and genistein, which was studied and analyzed using TLC.

Table 2: Yield of genistein from soya extract (reaction with copper acetate)

Wt. of extract taken (g)	Wt. of copper acetate (g)	Yield of genistein (%)
1.00	1.00	13.57
1.00	0.50	4.78
5.00	5.00	14.04
10.00	10.00	13.56

The formation of residues R1 and R2 insoluble in organic solvents and its solubility in dilute acid shows that the isoflavones, in plant extracts form complexes with selected salts under suitable conditions of time and temperature. In the IR spectra, the shifting of Carbonyl stretching by 50 cm^{-1} shows the involvement of carbonyl group in the complex formation.

It was observed that R1 derived from soy extract (as in 1) with Fe^{3+}, Al^{3+}, Cu^{2+} and Zn^{2+} salts did not result in the isolation of any flavonoids, whereas residue R2 of all the salts resulted in the isolation of both the isoflavones genistein and daidzein as a mixture. Though the experiments were initiated using the stoichiometric ratio of the isoflavones and salts, the maximum yields were obtained when the ratio of extract to salt is at least 1:1 by weight.

When complexation was carried out using soya extract (40%) and copper acetate, the residue R2 yielded only genistein as a pure isolate, the purity of which was confirmed by TLC and HPLC. The results are shown in Table 2.

In the case of methanol extract from fresh Soya beans (Exp. 2) and direct treatment of fresh Soya beans with salts in the presence of methanol (Exp. 3), the residues R1 did not yield any isoflavones, whereas residues R2 showed the presence of a mixture of genistein and daidzein.

The table shows that when 1:1 salt: extract is used; the yield was around 13.5%. The yield was reduced when the amount of salts was reduced to 0.5: 1 extract by weight. The yield of 13.5% of isoflavone from 20% extract is a 67.5% recovery which is notable in natural products isolation. Daidzein could not be isolated as a pure compound in this method.

When the results from the experiments given in Table 2 were extrapolated to soy beans as shown in Table 1, it was found that the two isoflavones genistein and daidzein can be isolated as a mixture in a single cost effective step.

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