The content of phytoestrogen on legume plants

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ABSTRACT : There are many plant-derived bioactive nonnutrients that can confer significant health benefits. Among these phytochemicals is the broad class of nonsteroidal estrogens called phytoestrogens. These phytoestrogens and their metabolites have many potential hormonal and nonhormonal activities that may explain some of the biological effects of diets rich in phytoestrogens. Phytoestrogens are plant (predominantly legumes) substances, that have stuctural and functional similiarity to 17β-estradiol, that influence a variety of biological processes. Phytoestrogens are widely used in the human reproductive system, but in livestock, it has not been used yet. Prior study on its role livestock, as a first step, it needs to be exploring the content of phytoestrogen on legumes plants. This study was aimed to determine the content of phytoestrogens of various legumes plants (soya bean straw, peanut straw and green bean straw) by using KLT Densitometry method with genistein standard. The research found that from several legume plants, the legume plant that contained soy phytoestrogens was only soya bean straw either dried using a drying oven or using a freeze dryer content with genistein of each was 0,498 g/100 g dry matter, and 1,748 g/100 g dry matter, uspectinely.

Key words : phytoestrogen, legume plants, soya bean straw, peanut straw, green bean straw

INTRODUCTION

Environmental estrogens are devided into two main groups : phytoestrogens and xenoestrogens. Xenoestrogens are man-made synthetic product, where as phytoestrogens are derived from plants (Woclawek *et al.*, 2005). Phytoestrogen is the group of chemistry content estrogen that can be found in different kinds of plant (Jefferson *et al.*, 2007). Within this chemical compound has isoflavone which is an organic compound belonging to a group that occurs in legumes especially beans.

The part of the plant which contains this compound group varies from its seed, flower, leaf, stem, and root. The greatest kind of phytoestrogen is found in soybean in the form of isoflavone and the kind of phytoestrogen that getting serious consideration is genistein (Jefferson *et al.*, 2007). Genistein is well-known because it is easily found in soybean seed or *Glycine soja* and also on leguminous plants (Paris, 1963), daidzein is in pigeon pea or *Cajanus cajan* (Matos, 1975) and likoisoflavone A is contained in string bean or *Phaseolus vulgaris* (Woodward, 1979), while flat nut or *Dolichos biflorus* has dolikhin A (Ingham, 1981).

The content of phytoestrogen can be found in many legumes (Adams, 1995) and its content depend on a number of factors. The most important of estrogenic component on that plants is isoflavone and the estrogenic of coumestans is approximately 1/1.000 and of isoflavones approximately 1/10.000 and relatively to the activity of estradiol-17β.

In health, phytoestrogen is often used in human reproductive system and with animals are not yet uncovered. Phytoestrogens are compounds that exert estrogenic effects on the central nervous system, induce oestrus and stimulate growth of the genital tract of female animals (Kurzer and Xu, 1997). Before having a research with animal, it is best to find out the content of the animal's feed which are made of legumes plants. The aim of this study was to determine the contents of the phytoestrogen on animal's feed which were made of legume plants.

MATERIALS AND METHODS

The needs of materials in doing this study were 100 g of peanut straw, soya bean straw, and green bean straw which were picked from those plants and afterwards the materials were washed clean, drained, aired and dried in an oven. The same process was also applied in freeze dryer. The indication of dried straw was when it was easily squeezed into powder.

5 g of each powder substance of the plants extracted into 50 ml ethanol using volatile, and the produce of filtrate was evaporated till it was solid. Then add 10 ml hot aquades which was put on a separated funnel and also put 10 ml technical hexane in. After the separating process, its base layer was taken and mixed with 10 ml acetate ethyl, next the acetate ethyl layer was steamed up till getting solid and dissolved in 2 ml of 95 % ethanol. The substance was ready to be used for the examination solution relating to analysis of isoflavone content.

Put 16 µl on a slab of gel silica F254 (20 x 10 cm) with 1 cm distance between each dot. On the same slab, placed genistein about 2 µl as a comparison, and then aroused it (ascending) 7 cm high on a chromatography container that had been saturated using moving phase toluene-acetate ethyl-formic acid (7-3-0,1v/v; above phase). After the developing process, the next step was to detect using ultraviolet light 254, UV366, as the comparison of genistein.

Measuring used KLT Densitometry method was done in order to determine the genistein component from each extract by dropping again on the genistein contained extract quantitatively followed by genistein standard curve.

The Curve of Genistein Standard

Genistein solution was dropped about 0,5 µl; 1,0 µl; 1,5 µl; and 2,0 µl for each on the slab of KLT gel silica F254 and then was developed by using moving phase toluene –acetate ethyl-formic acid (7-3-0,1 v/v, above phase). Genistein spot which appears was measured on its content using densitometer at a long wave 270 nm.

The parameters that to be measured were:

1. The possibility of the phytoestrogen content on several legume plants
2. The phytoestrogen content from legume plants

RESULTS AND DISCUSSION

Based on the detection done using ultraviolet light 254, UV 366, it could be seen that from the sixth dropped samples (oven dried soya bean straw, freeze dried soya bean straw, oven dried peanut straw, freeze dried peanut straw, oven dried green bean straw and freeze dried green bean straw), there were just two samples which had the same dot level and color, with genistein was yellow purplish. On the other hand, the sixth other samples contained the different dot and color with genistein. The same dot and color samples with genistein were oven dried soya bean straw and freeze dried soya bean straw. From the result of evaluation it could be said that oven-dried soybean straw and freeze-dried soybean straw had genistein. To know the content of genistein from the extract, KLT Densitometry method was used for the measuring in order to drop back the extract (soya bean straw) which contain genistein quantitatively followed by genistein standard curve.

Table 1. The result of study on several legume plants

No.	The kinds of legume plants	The result of the study
1.	Soya bean straw (oven-dried)	+
2.	Soya bean straw (freeze-dried)	+
3.	Peanut straw (oven-dried)	-
4.	Peanut straw (freeze-dried)	-
5.	Green bean straw (oven-dried)	-
6.	Green bean straw (freeze-dried)	-

+ : genistein detected
- : no genistein detected

The Determining Result of Genistein Content which was Counted as Genistein on Soya Bean Straw Extract

The Calculation Result of Genistein Standard Curve. The calculation of genistein content was done using KLT Densitometry method which needs standard solution because the sample was interpolatively counted. In this study all chromatogram width area was the measuring result of densitometry and must be dividedly 55,556 × 10^3 to get the well standard curve. The calculation result can be seen on Table 2.

Table 2. The correlation between the standard genistein content and chromatogram area in making standard curve

Genistein content (µg/µl)	The width of chromatogram area (55,556 × 10^3)
0,625	0,8109
1,25	1,5626
1,875	2,2390
2,50	2,4573

Based on the data on the Table 2, a standard curve could be made as Picture 1 which shows the connection between the standard genistein content and the width of chromatogram area.

The equation of linear regression line on standard curve was Y = 0,898x + 0,363 with its correlation coefficient of R^2 = 0,952. The correlation coefficient mark is close to 1. It means that there was a correlation between the genistein content and the width of chromatogram area so that the linear equation could be used for counting the genistein content on its sample.

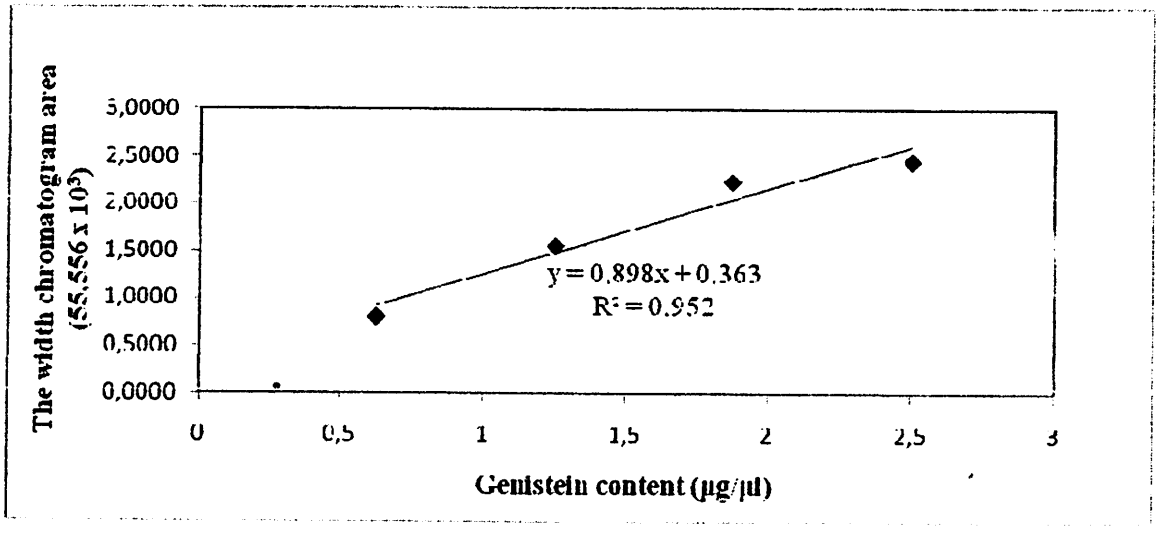

Figure 1. The normal genistein standard curve for measuring the genistein content on oven-dried soya bean straw extract and freeze-dried soya bean straw

The Determining Result of Genistein Content which is Counted as Genistein Content on Oven-Dried Soya Bean Straw Extract and Freeze-Dried Soya Bean Straw

Figure 2 is the example of dropping from various genistein standard concentrations and the sample of oven-dried soya bean straw and freeze-dried soya bean straw which used silent phase gel silica F_{254}, with moving phase of toluene mixture-acetate ethyl-formic acid (7-3-0,1, v/v, above phase) that was evaluated by ultraviolet light 356 and UV 254. The chromatogram of measuring dropped result (either

genistein standard or oven-dried soya bean straw extract or freeze-dried soya bean straw) can be seen on Figure 3.

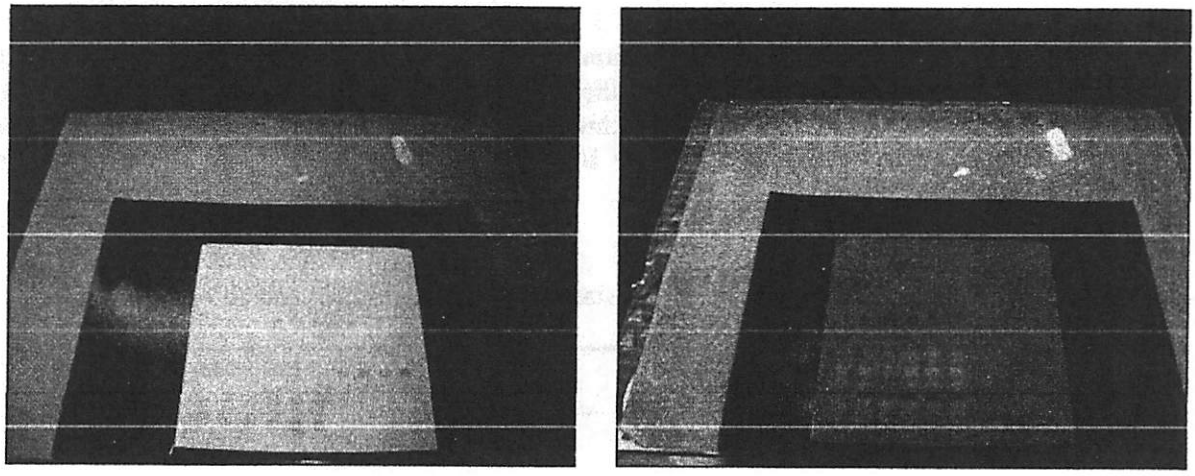

Figure 2. The example of dropping from various genistein standard concentrations and the sample of oven-dried soya bean straw and freeze-dried soya bean straw

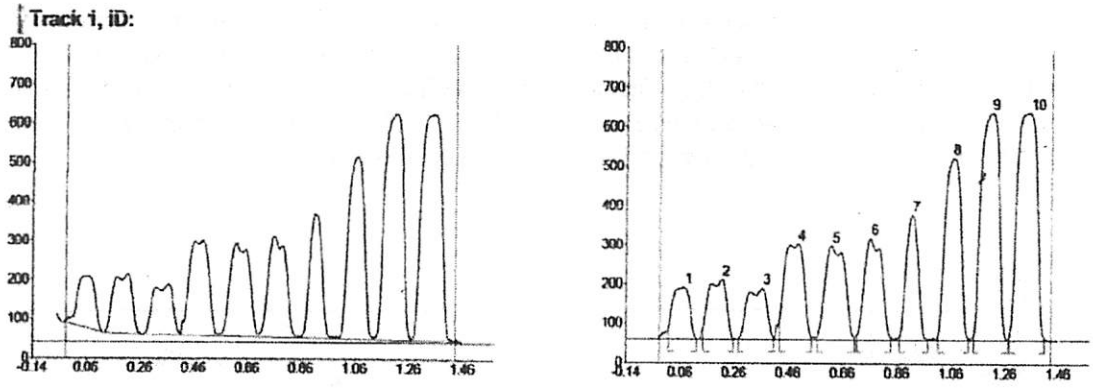

Figure 3. The chromatogram of measuring dropped result of soya bean straw sample and genistein standard using densitometer λ270 nm

Note:

1, 2, 3	: oven-dried soya bean straw sample
4, 5, 6	: freeze-dried soya bean straw sample
7, 8 ,9 ,10	: genistein standard

Genistein content was counted by inserting the width of chromatogram area sample (oven-dried soya bean straw and freeze-dried soya bean straw) which used the equation of linear regression line:

$Y = 0,898x + 0,363$

in which: Y = the width of chromatogram area sample
x = genistein content

Table 3. The width of chromatogram area sample of oven-dried and freeze-dried soya bean straw

Replication	The width of chromatogram area sample	
	Oven-dried soya bean straw	Freeze-dried soya bean straw
1	0,4833	1,0099
2	0,5992	0,993
3	0,5431	0,9697
Average	0,5418	0,9909

The accounting result showed that the genistein content of oven-dried soya bean straw and freeze-dried soya bean straw was 0,498g/100 g dry matter and 1,748g/100 g dry matter as seen on the Table 4.

Table 4. Genistein content of soya bean straw

Explanation	Genistein content, % b/b
Soya bean straw:	
- Oven-dried	0,498
- Freeze-dried	1,748

From the table above it could be seen that there was a difference between genistein content in oven-dried soya bean straw and freeze-dried soya bean straw in which the freeze-dried soya bean straw had higher genistein content than oven-dried. It was because there were a lot of substances evaporated during the drying process with an oven compared to freeze-dried. Kalela (1975), stressed that the difference of phytoestrogen content on plant caused by some factors besides dependly on the species and its age, it was also caused by plant's substance used in the form of dry or fresh. In this case the process of drying gives much impact to the phytoestrogen content of the plants.

CONCLUSIONS

From the discussion above it could be concluded that it was only the soya bean straw contained genistein (soy compound) either oven-dried or freeze-dried process. Based on the measuring result of genistein content, freeze-dried soya bean straw had higher genistein quality was 1,748 g per 100 g dry matter than oven-dried soya bean straw which was merely about 0,498 g per 100 g dry matter.

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