



REGULAR ARTICLE

BIOCHEMICAL ANALYSIS OF *GLYCINE MAX* SEEDS UNDER DIFFERENT GERMINATING PERIODS AND DENSITOMETRIC ANALYSIS OF GENISTEIN

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SUMMARY

Comparative analysis of soybean seeds (*Glycine max*) under different germination period has led to the discovery that germinated seeds have enhanced nutritional quality and can be used for variety of medicinal uses. The protein profiling was done by SDS-PAGE. Carbohydrate quantification showed that the total sugar content decreases during germination and found to be minimum after boiling. Additionally the starch and α -amylase activity was also checked. Finally, isoflavone profiling of these seeds with different germination periods was done by high performance thin layer chromatography. Furthermore, an important isoflavone, genistein was extracted out and identity of genistein was confirmed by similar HPTLC spectra identical with authentic genistein.

Key words: Diabetes mellitus, Secretagogues, Biguanides, Thiazolidinediones, Incretins, GLP 1 agonist, DPP 4 inhibitors, Bariatric surgery, Antidiabetic plants

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1. Introduction

Botanically, soybean belongs to the order Rosaceae, family Fabaceae, subfamily Papilionoidae, the genus *Glycine* and the botanical name *Glycine max*. Soybean has been a food in China for thousands of years. It is an abundant, economic source of protein. Soy beans contain large amounts of the Isoflavones diadzein, genistein a glycitein (1-3 mg/g) and their acetyl and malonyl conjugates (Song *et al*, 1998). Studies have shown that concentration and composition vary in different soy beans or soy protein products (Murphy, 1982) and that this variation is due to species differences (Franke *et al*, 1995), geographic and environmental conditions (Eldridge & Kwolek, 1983), and the extent of the industrial processing of soy beans (Murphy, 1982; Coward *et al*, 1998). Attention has

recently been focused on the possible role of soy beans in treatment of cancer and diabetes.

This experiment was conducted to study the effect of germination on nutritional quality of soybean seeds.

2. Materials and Methods

Soybean seeds were provided and were first kept in water overnight and then transferred to Petriplates having moist cotton at 30°C. Germinated seed samples were taken at particular interval of time. For obtaining boiled samples seeds were kept in waterbath at 90°C for 3 hours. Soybean powder was purchased from win-medicare.

Protein quantification

Fresh and germinated soy seeds were taken in a centrifuge tube and 2ml of extraction buffer (2.5ml 1.0M tris HCl pH

6.8,1 g SDS, 2.5ml beta mercaptoethanol) added. The seeds were crushed and then centrifuged at 10,000 rpm for 10 minutes at 4C. Pellet was discarded and supernatant was taken for further analysis. Above supernatant was treated with 1ml of 10% TCA, kept at 4C for 10 minutes and then centrifuged at 6000 rpm for 6 minutes at 4C. The supernatant was discarded and the pellet was washed with DDW. Finally pellet was dissolved in 0.1 N sodium hydroxide.

Protein solution containing 10 to 100 µg protein in a volume up to 0.1 ml was pipetted into 11 x 100 mm test tubes. The volume in the test tube was adjusted to 0.1 ml with appropriate buffer. Five milliliters of protein reagent was added to the test tube and the contents mixed by vortexing. The absorbance at 595 nm was measured after 5 min in 3 ml cuvettes against a reagent blank prepared from 0.1 ml of the appropriate buffer and 5 ml of protein reagent. The weight of protein was plotted against the corresponding absorbance resulting in a standard curve used to determine the protein in unknown samples. The desired amount (10ul) protein estimated in above step was taken and mixed with sample dye and beta-mercaptoethanol (30ul and 1.5ul respectively). Then it was kept at 100C for 5 minutes. 12% resolving gel and 4% stacking gel was prepared as described earlier. Gel solutions were poured in gel slab. After its polymerization, equal amount of protein of each sample was loaded in the wells.

Marker was also loaded in one of the wells of same gel. The proteins were allowed to run in the gel for 6 hours, with bromophenol blue as tracking dye. When the dye reaches the base of the gel, current was terminated. Gel was separated from the slab and poured in staining solution for 8 hours. After that gel was put into destaining solution. The destaining solution was changed after every 30 minutes till the background of the gel became clear and protein bands were visible. Molecular weights of the proteins were determined with the reference to protein molecular weight marker.

Carbohydrate quantification

Soybean seeds from different conditions were taken and dried. These dried seeds (1g) were crushed with acetone (2ml) and centrifuged at 5000 rpm for 10 minutes to remove interfering pigments. Extraction of sugars was done by using 2.5-ml aliquots of 80% ethanol. This was centrifuged at 5000 rpm for 10 minutes and then pellet was discarded. Subsequently the supernatant was filtered through whatman paper.

These samples were taken for carbohydrate quantification by anthrone method starch estimation and α-amylase activity was determined by using DNSA reagent.

3. Results and Discussion

Most of the investigators found heat to be injurious; however, in the case of legume protein, heat was found to be beneficial. However the results suggest that protein concentration decreases on boiling but this also inhibit the antinutritional compounds and also certain proteases (Nahrung 2000). In addition to this, the protein profile through SDS-PAGE shows that 6 main proteins are present in soybean seed extract. Protein profile shows little difference with germination period. Bennett et al 2005 also got the similar profile and identified these bands as subunits of 7S and I I S globulins. The results are presented in graph 2-4. The results illustrates that the total sugar content decreases during germination and found to be minimum after boiling have opined that decline in total sugars might result in limited availability of respiratory substrate for germination. (Adams et al, 1980) reported that the total sugars are rapidly utilized in germinated soybeans declining to about 20% of their initial level. The decrease in sugars might be due to their utilization in respiration the starch content in 6 day germinated seeds was found to be maximum and in non germinated seeds its content decreased. The presence of starch and the corresponding enzymes for its hydrolysis have already been well documented in soybean. The increase in starch content of seeds after germination as compared to mature seeds support the

earlier observations of number of workers that soybean produce starch during imbibition and germination which is a transient reserve material for germinating soybean cotyledons (Adams et al 1980). Lastly, activity of alpha amylase also increases with germination time. These suggest that the starch content should decrease but probably the newly formed starch is produced by gluconeogenesis using precursor from oil reserves. Soybeans are a good source of nutrients, as they contain around 40% of high quality protein, 20% oil, as well as number of minerals, vitamins and phytochemicals. Soy protein provides great commercial potential, so it is not surprising that recent advances in soy food processing have focused on developing techniques for obtaining proteins with more defined functionality. Some of this research is published in research journals, sometimes it is patented, however much of the applied research with commercial application is kept secret. Glycerin and conglycinin have different gelling, emulsification and foam properties. It is expected that over the next decade, more highly specified soy protein ingredients will provide the basis for innovative new food products and growth of the soy food market.

4. Conclusion

The protein profiling and quantification on germinated and non germinated soybean were studied. Five samples with different germinated periods were taken and checked for the variation in protein in them. Results confirmed that germination lowers the protein quantity but no much was observed in SDS profile of soybean proteins.

Fig. 1

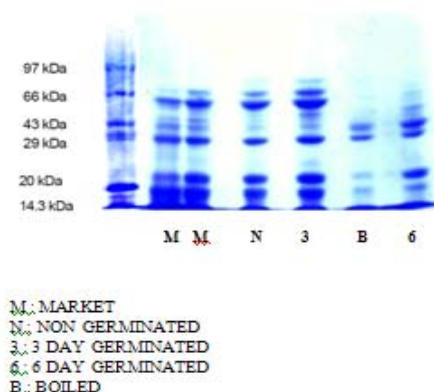


Fig. 2: Estimation of starch in *Glycine max* seeds

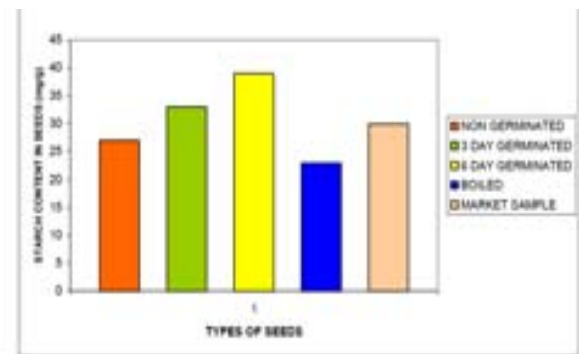


Fig. 3: Estimation of total sugar in *Glycine max* seeds

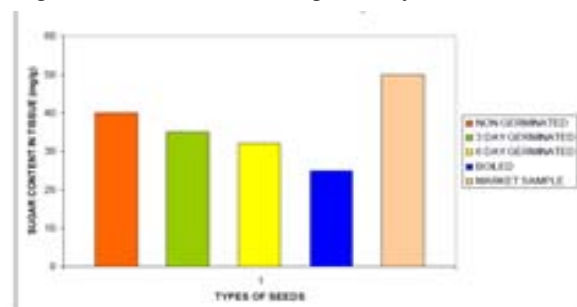
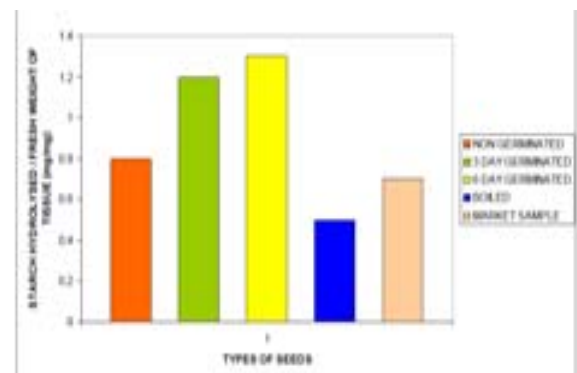


Fig. 4: Estimation of alpha amylase activity in *Glycine max* seeds



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