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Effect of processing conditions on saponin content and antioxidant activity of Indian varieties of soybean (*Glycine max* Linn.)

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Abstract

Saponin content and antioxidant activity of three major Indian varieties, *i.e.*, Punjab long, Hardee and Kalitur were analyzed. These contained 80.4 g/kg, 49.8 g/kg and 119.7 g/kg saponin in soyflour, respectively. The black variety 'Kalitur' showed a higher saponin content and antioxidant activity as compared to the other two yellow varieties. Saponins A and B were present as major saponins. Effect of different processing conditions on soysaponin content of soybean cake were evaluated. A variety of commonly prevalent processing practices including boiling, soaking, sprouting and drying were included. The saponin content reduced on boiling and defattening. Soaking and sprouting under 50^{o}C temperature increased the saponin content. Saponins exhibited significant antioxidant activity which can be attributed to saponins A and B.

Key words: Soybeans; Soysaponins; HPLC; Processing conditions; Antioxidant activity.

Introduction

Saponins are a class of oleanane triterpenoids which commonly occur in different plants (Hostettman and Marston, 1995). These are secondary metabolites, having important biological activity like anticarcinogenic, hypocholesterolemic, haemolytic (Francis *et al.*, 2002), immunostimulatory, antiviral and hepatoprotective agent (Wang *et al.*, 2007). The saponin content in soybean is 6.500 g kg^{-1}, which is the highest among legumes (Sagratini *et al*., 2009).

The saponins present in soybean are collectively termed soysaponins. The saponins are divided into two major categories-A and B, based on their aglycone structures. Soysaponin class-A includes bidesmosidic saponins and consists of two glycosylation sites with one aglycone moiety (soysapogenol-A) (Heng *et al.*, 2006). The soysaponin class-B is monodesmosidic, having only one glycosylation site and two different aglycon moieties (soyasapogenol-B and E) (Shiraiwa *et al.*, 1991). Soysaponin class-E is only a transitory form which quickly converts into either of the other two classes. The type of saponin and its content largely depends on the soya variety, agroclimatic factors and the conditions of growth. Zou and Chang (2011) have reported the difference in saponin content of the commonly grown soybean varieties.

Soybean having a black seed coat, commonly called 'black soybean', has widespread consumption in Asia. It is rich in protein and also contains important bioactive components like isoflavones, anthocyanins, saponins and vitamin E (Messina, 1999). Recent studies have shown enormous health benefits of black soybean. It helps in preventing cardiovascular diseases, breast cancer and it also acts as an immunostimulatory agent (Xu and Chang, 2008).

Since, the soybean is consumed in the form of various food products according to regional preferences, it is important to understand the effects of various processing methods on the availability of the bioactive component in it. The present work was done to mainly address the effect of processing on the saponin contents of soybean. Soybean is generally

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cooked before consumption and processed by different methods. The commonly used processing methods are boiling, germination, soaking, sprouting and drying (Della *et al*., 1994). These are known to improve the nutritional status and taste. But the individual effect of temperature, crushing, incubation *etc*., may cause an alteration in the saponin level and their bioavailability (Chau *et al*., 1997). The saponin content and its antioxidant activity are likely to diminish during harsh processing conditions. No systematic study has been done in this aspect, especially with reference to black soybean. The studies on the impact of soaking and cooking on the saponin contents are limited and often contradictory. This study aimed to compare the effects of the different processing conditions and varietal differences on the saponin content and the antioxidant activity.

The level of saponin in Kalitur variety is being reported first time. The effect of processing condition, *viz*. soaking, sprouting, drying, roasting and boiling *etc*., on saponin and antioxidant activity of soybean meal of Punjab long (commonly used in India) is also presented.

Materials and Methods

Materials

Three cultivars of soybean (*Glycine max* Linn.) Punjab long, Hardee and Kalitur were obtained from 'National Research Centre for Soybean, Indore, India'.

Preparation of defatted soybean meal and other processing treatments

The soybean flour was prepared by milling the beans at 'Centre for Rural Development and Technology' facility. The flour was subjected to different processing treatments. (i) It was defatted for 48 h with hexane, (ii) boiling of flour in an open vessel without pressure for 5 min. at 100 ÚC, (iii) boiling of the flour in a closed cooker with pressure (1.4 psi) for 5 min, (iv) soaking of seeds for 6 h at 30^{o}C followed by sprouting for 72 h at 30^{o}C, (v) soaking of seeds for 6 h at 30^{o}C, (vi) ultraviolet irradiation of the flour for 20 min., (vii) microwave based processing of the flour for 5 min., and (viii) roasting of the flour for 5 min.

Distilled water, pH 6.9 was used for all processing. The wet samples were air-dried at room temperature inside a laminar hood for 48 h so that any type of impurities could be avoided. These dried samples were grounded properly into powder and sieved through a sieve 8-mesh size and then powdered samples were stored in dark brown, air-tight bottles at -80 ÚC for further extraction process.

Extraction of saponins

Ten grams of soybean flour was mixed with 50 ml methanol in a capped and sealed flask and stirred for 48 h at 120 rpm in a shaker. The samples were then centrifuged at 8000 rpm for 10 minutes at room temperature. The supernatant was collected and dried in a flash evaporator. The residue was dissolved in 1 ml butanol-water mixture (1:1 ratio). This was kept overnight and was dried again. The dried mixture was dissolved in 2 ml methanol and was analysed by HPLC.

HPLC *analysis of extracted saponins*

The extracted saponins were analyzed by HPLC (Agilent Technologies, 1200 series, USA) equipped with UV detector. A C-18 reverse phase column was used. Conditions were followed as per the procedure of Zhang *et al*. (2009). Trifluoroacetic acid (0.05%) in water (solvent A) and acetonitrile (solvent B) were used as solvents. The flow rate was kept at 1ml/min with elution under gradient condition. The solvent A started with 60% and decreased up to 52% over 60 min. The peaks were analyzed by UV-VIS detector.

ESI- MS *of saponins*

Individual peaks of soysaponins were collected by HPLC and analyzed by ESI-MS on a Bruker Compass MicroTOF-QII.

Antioxidant activity of saponins

The cupric ion reducing antioxidant capacity of soybean was determined according to the method of Apak *et al.* (2008). Briefly, 0.1 ml of sample was mixed with 1ml each of copper chloride solution (1.0×10^{-2} mol/l), neocuproine alcoholic solution (7.5×10^{-3} mol/l), and ammonium acetate buffer (1 mol/l, pH 7.0) solution and water. The mixture was incubated for 30 min. and absorbance was recorded at 450 nm against the reagent blank. Trolox was used as the standard. The results were expressed as µmol Trolox /g, using molar absorptivity of Trolox as $1.67\text{x}10^{4}$ l mol^{-1} cm^{-1}.

Results and Discussion

Soysaponins in different variety of soybeans

Saponin contents from full fat soybean cake were extracted by methanol and analysed by HPLC. The typical HPLC chromatogram of saponin extracted from the full fat soy flour (FFSF) of all the three varieties are shown in Figure1. The results show presence of four major saponins and five minor derivatives in each of the Indian soybean varieties, Punjab long, Hardee and Kalitur.

Soybean is reported to have nine types of saponin or its derivatives (Zhang and Popovich, 2008). In the present study, four major and five minor peaks by FFSF are shown. The first major peak from HPLC chromatogram appeared between 13.3-13.9 min. The second and the third major peaks appeared between 16.9-17.7 and 17.6-19.0 min. The fourth major peak appeared between 25.4-26.8 min. Zhang and Popovich (2008) have previously reported four major peaks and identified them as Group I-B non DDMP soysaponin, Group III-B non DDMP soysaponin, soysapogenol-A and B Group-B DDMP soysaponin, respectively. The five minor peaks were also in agreement with their reports. Individual peaks and their identification are summarized in Table 1.

Punjab long variety contain highest amount of I-Group-B Non-DDMP Soy Saponin, Soy Sapogenol B, Ba and Bg – GroupB-DDMP Soy Saponin. Kalitur variety contain highest amount of III GroupB-non-DDMP Soy Saponin, Soy Sapogenol A, Ba group B-DDMP Soy Saponins and Ya Group B-DDMP Soy saponins.

Punjab long and Kalitur varieties have about equal amount of Soy sapogenol (A and B), which are the major and well characterized saponins in soybean.

The total saponin content in FFSF of each soybean variety is summarized in Figure 2. It is evident that Kalitur, the black variety of soybean showed significantly higher values of saponin content in comparison to Punjab long and Hardee. There are no previous reports of the quantification of saponin content in these varieties. Due to the lack of purification standards and an effective quantification method of saponins, the research on the effect of processing conditions on saponin content has received less attention than isoflavones (Rickert *et al*., 2004).

The antioxidant activity of the saponins

Antioxidant activity of saponins extracted from each variety is shown in Figure 3. The Punjab long and Kalitur have almost same total antioxidant activity, though Kalitur has much higher saponin content (Figure 2). However, they have same amount of soy sapogenol content which might be contributing to the antioxidant property. Lee *et al*. (2002). also found varietal difference and processing conditions, affect the antioxidant activity.

Effect of processing method on the saponin contents of soybean flour

Processing methods are known to affect the availability of nutrients and bioactive constituents of the seed cakes (Chau *et al*., 1997). The impact of processing on saponins are rarely available in literature (Rickert *et al*., 2004). In the present study, the soybean flour of Punjab long was subjected to commonly employed processing conditions. The residual content was monitored by HPLC. The changes in total saponins and individual saponins content are also shown in Tables 2 and 3, respectively. It is evident that soaking, boiling and defatting caused a substantial reduction in the saponin content of soybean flour. The saponin content was reduced by about 18% on defatting. The removal of saponins may be due to their dissolution in n-hexane that had been used for defatting the soybean flour. The free aglycons may be extracted by hexane during defatting (Booth *et al.*, 1960). Boiling in an open vessel caused a 28.6 % reduction in the saponin content. This may be due to the fact that some saponins are heat labile. The level of saponin loss is consistent with previous reports by Xu and Chang (2009) and Wang *et al.* (2009), where 21.9 and 15.9% reductions are observed after cooking (boiling).

Boiling under pressure (1.4 psi) caused more reduction (53.11%) as compared to open vessel cooking. Elhardallow and Walker (1994) also found significant reduction (60%) in saponin content after closed pressure cooking of the lentils. Pressure and ordinary cooking of soaked legumes also reduce the saponin content by 28-38% (Duhan *et al*., 2001). Simple soaking of seeds led to a 32.2% increase in saponin content. This is in contrast with earlier reports, wherein saponin content was found to decrease due to leaching out of saponin in water (Kataria *et al*.,1988). However, short duration (6 h) soaking of navy beans did not complete the hydration of beans so the water soluble saponin content remains as such in the sample so more or less similar amount of saponin was found in soaked samples as compared to unprocessed sample (Shi *et al*., 2009).

The exposure of the seeds to microwave radiation, prior to grinding to prepare flour results in increase in the content of saponin by 39.2%. An increase in the isoflavone content under similar processing conditions was reported by Aguiar *et al*. (2010). They found that their processing methods decreased the malonyl form while aglycon and glycoside forms increased. The saponin content has aglycone and glycoside moieties in it, so perhaps because of this reason our experiment also showed an increase in saponin values. Microwave treatment has been reported to reduce the antinutritional factors in some legumes (El-Beltagy, 1996). However, the present study has displayed the opposite result.

Roasting of soy flour also caused 23.38% increase in saponin content. The effect of roasting has been previously reported in green gram where a reduction in saponin content was observed, (Kataria *et al*., 1988). However, some researchers reported that saponins are relatively heat stable components (Oenning *et al*., 1994).

From the results, it can be concluded that there was a substantial reduction in the saponin content of soyabean flour when it was processed through defatting and boiling (in a closed vessel) but increased in soaking, sprouting, roasting, exposure to ultra violet radiation and exposure to microwave irradiation.

ESI-MS of soysaponin samples

The molecular mass of the different peaks of soysaponin separated by HPLC were analyzed by ESI-MS. The peak numbers 6, 7 and 2 (Table.1) gave mass values corresponding to 1035.86, 1225.07 and 568.29. These were identified as for the peaks-soysaponin βa, soysaponin βg, and soysaponin III. The values for soysapogenol A and soysapogenol B correspond to the values reported in literature *viz.*, 473.3 and

457.4 (Zhang *et al.*, 2008). The results confirm the presence of major types of saponins in Indian soybean varieties Punjab long, Hardee and Kalitur.

Conclusions

The black variety 'Kalitur was found superior in the saponin content. The processing conditions especially boiling reduced the saponin significantly whereas soaking and sprouting increased the saponin content. The study has the following important implications:

1. 'Kalitur' which is a non-edible variety of soybean can be very useful for obtaining saponin at high concentration, which can be marketed as cholesterol-lowering and anticarcinogenic agents.
2. While producing soy foods it should be kept in mind that excessive boiling might leads to a loss of saponins.
3. The processing methods such as microwave treatment, soaking or sprouting are better techniques to improve the saponin content.
4. Among individual saponins, soy sapogenols A and B are important for antioxidant activity.

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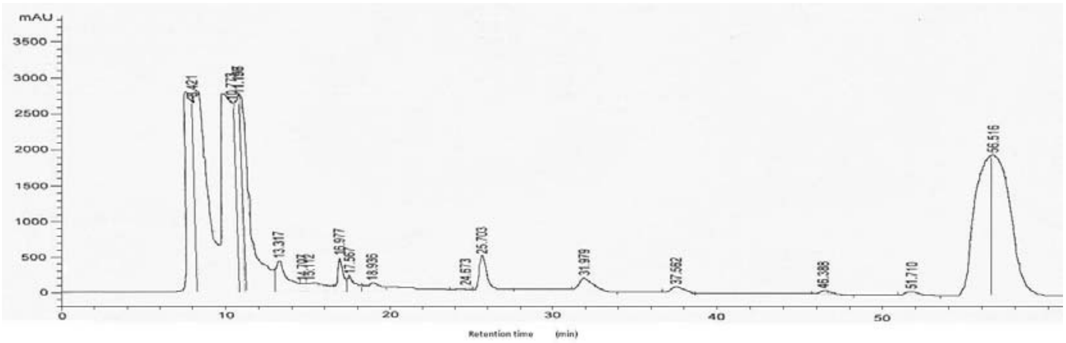
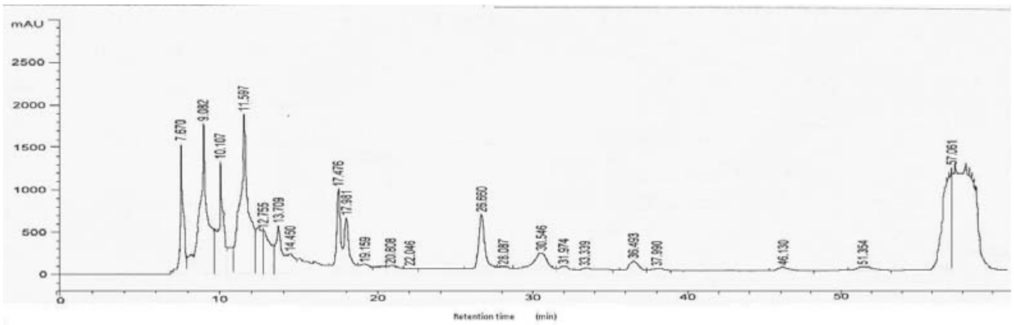


(a)



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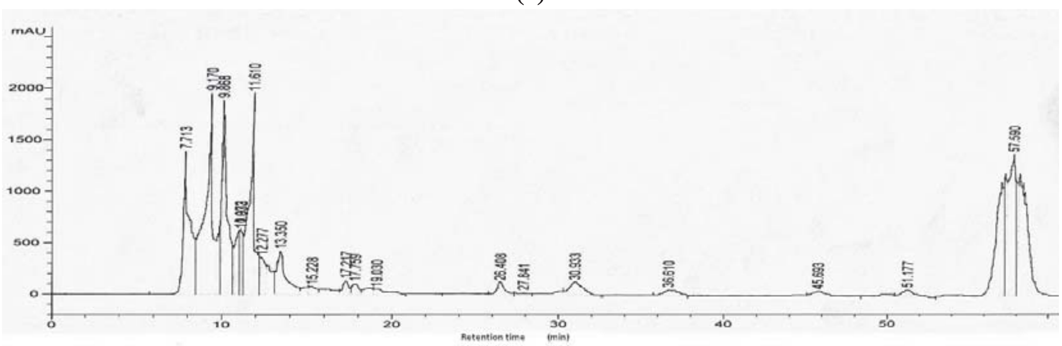

(c)

Figure 1. HPLC chromatogram of saponin extracted from the full fat soyflour of three soybean varieties (a) Punjab long (b) Hardee (c) Kalitur

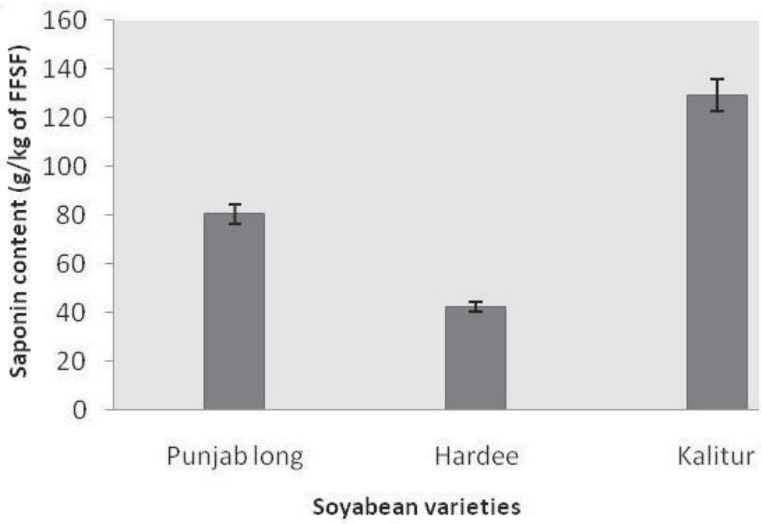

Figure 2. Total saponin content in full fat soyflour of Indian soybean varieties (a) Punjab long (b) Hardee (c) Kalitur

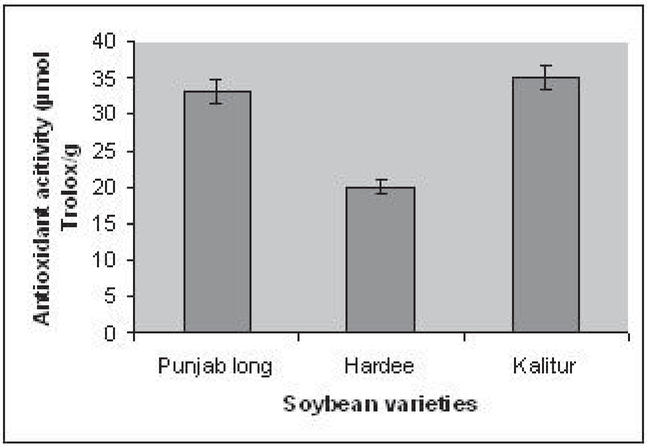

Figure 3. Antioxidant activity of saponins extracted from Indian soybean varieties (a) Punjab long (b) Hardee (c) Kalitur

Table 1. Saponin types in full fat soy flour (FFSF) of different soybean varieties

Peak No.	Retention time (min)	Saponin	Relative amount of individual saponins		
			"Punjab long"[a]	"Hardee"	"Kalitur"
1.	13.3	I-Group-B Non-DDMP Soy Saponin	100	(-)24.6	(-)21.4
2.	16.9	III-Group-B Non-DDMP Soy Saponin	100	(-)72.2	(+)37.3
3.	17.5	Soy Sapogenol-A	100	(-)61.6	(+)51.6
4.	18.9	Soy Sapogenol-B	100	(-)55.7	(-)40.3
5.	21.0	Be-Group-B -DDMP Soy Saponin	100	nd	nd
6.	24.6	Bg-Group-B -DDMP Soy Saponin	100	nd	nd
7.	25.7	Ba-Group-B -DDMP Soy Saponin	100	(-)78.8	(+)16.2
8.	31.9	Not identified	nd	(-)39.2	(+)22.2
9.	37.5	Yg-Group-B -DDMP Soy Saponin	100	(-)67.6	(-)54.8
10.	46.3	Ya-Group-B -DDMP Soy Saponin	100	(+)8.8	(+)15.2

[a]The content of Punjab long variety are taken as 100, nd- Not detected

Table 2. Effect of various processing conditions on saponin content of Punjab long variety of soybean

Processing conditions	Soy saponin content (g/kg)	Percent residual saponin respect to unprocessed (control)
Full fat soy flour without any process (control)	80.4±1.32	100
Defatting	73.67±1.15	92
Boiling in an open vessel	57.4±1.36	71
Pressure cooking	37.7±0.93	47
Soaking of seed (6 h) and than sprouting (72 h) at 30°C	87±1.52	108
Soaking of seeds for 6 h	106.3±2.77	132
Flour exposed to Ultra violet rays for 20 min	92.4±1.91	115
Microwave cooking for 5 min	111.9±2.06	139
Roasting	99.2±1.75	123

Table 3. Effect of processing conditions on individual saponin content of Full Fat Soya Flour (Punjab long variety of soybean)

Type of saponin	Unprocessed FFSF [a] (control)	Defatted Soy Flour (DSF)	Boiling of flour open vessel	Pressure cooking the flour	Soaking of seed (6 h) followed by sprouting (72 h) at 30 ˚C	Soaking of seed (6 h)	Microwave cooking 5 min	Roasting
I-Group-B Non-DDMP Soy Saponin	100	(+)15.0	(+)0.4	(-)2.2	(-)17.9	(-)55.4	(+)67.2	(-)53.1
III-Group-B Non-DDMP Soy Saponin	100	(+)93.5	(-)43.8	(+)33.7	(+)20.8	(+)40.8	(+)37.1	(+)42.5
Soy Sapogenol-A	100	(+)53.5	(-)3.5	(+)36.7	(+)39.2	(+)57.4	(+)51.7	(+)52.2
Soy Sapogenol-B	100	(-)41.8	(+)45.9	(+)45.5	(-)59.9	(+)30.4	(-)59.5	(+)35.5
Be-Group-B -DDMP Soy Saponin	100	nd	nd	nd	nd	nd	nd	nd
Bg-Group-B -DDMP Soy Saponin	100	nd	(+)66.5	(+)87.0	(+)69.1	(+)82.0	(+)86.8	(+)89.6
Ba-Group-B -DDMP Soy Saponin	100	(+)25.5	(-)78.3	(-)73.7	(-)69.7	(-)72.3	(-)14.6	(-)13.9
Un identified	nd	nd	nd	(-)69.6	(-)81.2	(-)77.1	(-)85.1	(-)77.1
Yg-Group-B -DDMP Soy Saponin	100	(+)17.11	nd	nd	nd	nd	(+)4.7	(+)25.0
Ya-Group-B -DDMP Soy Saponin	100	(+)31.5	(+)56.2	(-)32.5	nd	nd	nd	(+)4.6

[a]The value of FFSF (control) was taken as 100%, [nd] Not detected

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