

## Germination as a processing technique for soybeans in small-scale farming

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### Abstract

Heat processing is an effective way of reducing antinutritional factors (ANFs) in legumes, but requires expensive facilities and equipment. Accurate control of temperature is critical to avoid under- or overheating. Therefore, heat treatment of soybeans is not a viable option for the average small-scale farmer in remote areas. Germination and other traditional methods, namely soaking and dehulling, were evaluated as alternative processing methods for soybeans. The effect of the processing treatment on the level of different ANFs, nutritional composition and *in vitro* protein digestibility (IVPD) of soybean seeds was determined. Soybeans were soaked for 24 hours and allowed to germinate for one to six days. Soaked soybeans were manually dehulled. Changes within seeds were noted for nutritional and ANFs during germination. Crude protein and fat content increased with increasing number of days germinated, but germination caused a decrease in starch content. Dehulling also increased the crude protein and fat content of the seeds. All treatments caused a significant decrease in condensed tannins of the soybeans. Trypsin inhibitor levels were numerically lower after germinating, soaking and dehulling of seeds, but not significantly so. There were no changes in IVPD after treatment of the soybeans. It was concluded that germination for a period of three days effectively improved the nutritional value of soybeans and can be considered an alternative treatment of soybeans for the small-scale farmer where heat treatment is impractical or impossible.

**Keywords:** Soybeans seed, *Glycine max*, TIA, anti-nutritional factors, traditional methods

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### Introduction

Legume seeds are important sources of energy and protein in many parts of the world, both in animal and human nutrition (Kaushik *et al.*, 2010). However, their nutritional value may be compromised in part by the presence of undesirable components, known as antinutritional factors (ANFs) (Donangelo *et al.*, 1995).

Processing legumes with heat is a quick technique for decreasing or destroying ANFs, but strict guidelines must be applied for the treatment to be effective. An optimal processing temperature must be applied, as overheating can cause protein and amino acid damage. On the other hand, underprocessing does not completely destroy ANFs (Kabeya & Kiatoko, 2004). Expensive facilities and equipment are required for the proper heat treatment of legumes, putting this technique out of reach of the average small-scale farmer in remote areas.

Globally, soybean (*Glycine max*) seed is one of the largest sources of vegetable seed oil and protein in the feed and food industry, and contains about 40% crude protein and 20% oil. It is also a source of calcium, iron, carotene and ascorbic acid. Soybean oilcake meal has become the principal protein supplement for livestock in many countries (Ghaly & Sutherland, 1982) and is one of the legumes that is most frequently used in the poultry industry. However, soybeans contain undesirable components such as lipoxygenase and trypsin inhibitors that limit their utilisation. Trypsin inhibitor is an ANF that affects protein digestibility (Kumar *et al.*, 2006). A high level of trypsin inhibitors in a diet stimulates pancreatic juice secretion and

causes pancreatic hypertrophy and poor growth in animals (Liener & Kakade, 1980, cited by Huisman & Tolman, 2001).

Traditional processing methods of soybeans such as germinating, soaking and dehulling are sometimes used to reduce or eliminate the ANFs that affect protein utilisation. The trial reported here was designed to evaluate the efficacy of germination and other traditional treatments as alternative processing methods in reducing the levels of ANFs in soybean seed.

## Materials and Methods

One ton of soybean seeds (variety RR5409 Monsanto) was procured locally (Brits, North West Province, South Africa). The seeds were kept raw or subjected to one of four processing methods: roasted, soaked for one or two days, dehulled or germinated from one to six days. The methods of processing were as follows:

- As a heat-treated control group, a representative sample of seed was roasted in an oven for 30 min at 130 °C. After cooling, the seeds were milled and stored in plastic bottles at room temperature for further analysis.
- For the soaking treatment, 500 g representative soybean samples were weighed into 5000 mL plastic containers, which were then filled with tap water. The seeds were soaked at room temperature for 24 or 48 h. The soaked seeds were spread on hessian sacks for 48 to 72 h under sunlight until dry. After drying, the seeds were milled through a 1 mm sieve and kept in plastic containers at room temperature for further analysis.
- The soybean seeds were dehulled after being soaked for 24 h in tap water (1 : 10, w/v). The hulls were removed manually by rubbing the seeds between the palms of the hands (Sangronis, 2007). After dehulling, the hulls were removed by flotation, and the cotyledons were dried under sunlight. The dehulled seeds were milled through a 1 mm sieve, and then stored at room temperature, pending further analysis.
- Germination was achieved by soaking the seeds for 24 h. Afterwards they were spread indoors on hessian sacks on the floor, and covered with aluminium foil to exclude light. Six groups of seeds were kept at room temperature (22 - 26 °C) and allowed to germinate for one to six days, respectively. Water was applied once daily to provide moisture during sprouting. Thereafter, the germinated beans were dried for 48 to 72 h under sunlight, ground through a 1 mm sieve, and then kept at room temperature pending further analysis.

The sampling of seeds was carefully conducted to ensure homogenous and representative samples for analysis. Large samples were collected from various locations from the original batch of beans, and were properly mixed, divided and subdivided until the required sample size was obtained.

All treatments were replicated four times, independent of one another. Representative samples from each treatment from all four batches were analysed in duplicate for moisture (AOAC, 2000), crude protein (AOAC, 2000), starch (Davie, 1986), condensed tannins (Porter *et al.*, 1986), total polyphenols (Makkar, 1993), trypsin inhibitor activity (TIA) (Kakade *et al.*, 1974), crude fat (AOAC, 2000) and *in vitro* protein digestibility. Analyses were done at the UP-Nutrilab (Department of Animal and Wildlife Sciences, University of Pretoria, South Africa).

*In vitro* protein digestibility (IVPD) of the seed was determined by pepsin or papain enzyme digestion. For the pepsin method, the soybeans (300 g) were weighed into a series of test tubes. A solution of 5 mL of 0.075 N HCl and 0.5 mL of pepsin solution (2.0 mg/mL) in 0.075 N HCl was added to each tube. The tubes were incubated at 37 °C and enzyme action was stopped after 24 h by adding 5 mL 10% (w/v) trichloroacetic acid (TCA). Digestion was performed in duplicate. The digest was filtered through Whatman No. 2 filter paper, and the residue was washed with warm water on the filter. Nitrogen in the residue was estimated by the micro-Kjeldahl method. The IVPD was obtained by calculating the difference between the amount of total nitrogen in the sample before and after *in vitro* digestion with pepsin (AOAC, 1965). Kjeldahl nitrogen was multiplied by the factor 6.25 to obtain crude protein.

*In vitro* protein digestibility with papain was conducted according to the method used by Buchanan & Byers (1969) and modified by Nanda *et al.* (1977). The activator solution of 0.5 M thioglycolic acid was prepared by adding 4.6 g (3.5 mL) laboratory-grade thioglycolic acid to distilled water and making it up to 100 mL, after having adjusted the pH to 6.6 with a 0.45 M NaOH solution. A phosphate-citrate buffer

solution of pH 6.6 was made by mixing 7.7 parts 0.2 M Na<sub>2</sub>HPO<sub>4</sub> with 2.3 parts 0.1 M citric acid. Quantities of substrate representing about 10 mg of protein N were weighed accurately in 75 mL centrifuge tubes. Then 0.4 mL papain solution was pipetted into each digestion tube, together with 0.5 mL 0.5 M thioglycolic acid and 9.1 mL citrate-phosphate buffer. Two drops of toluene were added as preservative, the tubes were stoppered, and the contents were mixed gently. Care was taken to ensure that all particles of the protein sample remained immersed in the digestion liquid. The samples were incubated in duplicate at 37 °C for 24 h. The control tubes contained only enzyme, activator and buffer. Immediately after incubation, the unhydrolyzed protein fraction was precipitated by adding 10 mL trichloroacetic acid (TCA) solution (100 g/L) to each tube. Thereafter the procedure was followed as described for pepsin.

An analysis of variance with the GLM model (SAS, 2010) was used to determine the significance between treatments. Mean and standard deviations were calculated. Treatment means were separated using Fisher's protected least significant difference (LSD) at the 5% level of significance.

## Results & Discussions

Soybeans were germinated for various time periods, from 1 to 6 days, in order to find the optimal germination period. Raw and roasted soybeans were used as negative and positive controls, respectively. Soaked and dehulled soybeans were also assessed for their nutritional value, levels of ANFs and *in vitro* protein digestibility.

The germinated soybeans contained less ( $P < 0.05$ ) starch compared with all other treatments, including the raw beans (Table 1). Mubarak (2005) reported a decrease in starch content in mung bean seeds after three days of germination. Mwikya *et al.* (2000) also found a gradual decrease in the starch content of finger millet during the first 36 h of germination. This reduction was attributed to the hydrolysis of starch that supplies the developing seedling with energy during germination (Madden *et al.*, 1985).

Crude protein was found to increase as the number of days of germination of soybeans increased (Table 1) and was higher ( $P < 0.05$ ) than the crude protein content of beans from the raw control group and the roasted and soaked treatments. Kaushik *et al.* (2010) also reported an increase in crude protein with germination of soybeans. Similar increases in protein have been reported for other legumes such as lablab beans (Osman, 2007), mung beans (Mubarak, 2005), faba beans and kidney beans (Alonso *et al.*, 1998). The apparent increase in protein can be attributed to the utilization of carbohydrates as an energy source for developing sprouts (Donangelo *et al.*, 1995). However, Bamdad *et al.* (2009) reported a notable decrease in protein content after lentils germinated for 1, 3 and 5 days. Echendu *et al.* (2009) found a release of free amino acids after enzymatic hydrolysis for the synthesis of new protein.

**Table 1** Effect of soaking, dehulling and germination of soybean seeds on crude protein, fat and starch content

| Treatment         | Crude protein             | Crude fat                 | Starch                    |
|-------------------|---------------------------|---------------------------|---------------------------|
| Raw               | 39.1 <sup>a</sup> ± 0.37  | 15.8 <sup>a</sup> ± 1.34  | 1.24 <sup>ab</sup> ± 0.11 |
| Roasted           | 40.3 <sup>b</sup> ± 0.57  | 19.6 <sup>b</sup> ± 1.03  | 1.38 <sup>a</sup> ± 0.20  |
| Soaking – 1 day   | 40.2 <sup>b</sup> ± 0.10  | 17.7 <sup>b</sup> ± 0.82  | 1.17 <sup>b</sup> ± 0.11  |
| Soaking – 2 day   | 42.1 <sup>c</sup> ± 0.25  | 19.0 <sup>c</sup> ± 0.40  | 0.96 <sup>c</sup> ± 0.03  |
| Dehulling         | 45.1 <sup>d</sup> ± 0.35  | 21.0 <sup>d</sup> ± 0.54  | 1.11 <sup>bc</sup> ± 0.12 |
| Germination 1 day | 41.3 <sup>e</sup> ± 0.19  | 18.1 <sup>d</sup> ± 0.89  | 0.99 <sup>d</sup> ± 0.89  |
| Germination 2 day | 42.2 <sup>f</sup> ± 0.54  | 19.1 <sup>e</sup> ± 1.37  | 0.84 <sup>d</sup> ± 0.12  |
| Germination 3 day | 43.4 <sup>g</sup> ± 0.20  | 19.2 <sup>ef</sup> ± 0.99 | 0.75 <sup>d</sup> ± 0.10  |
| Germination 4 day | 43.8 <sup>g</sup> ± 0.52  | 19.2 <sup>e</sup> ± 1.05  | 0.71 <sup>d</sup> ± 0.13  |
| Germination 5 day | 44.1 <sup>gh</sup> ± 0.70 | 18.5 <sup>e</sup> ± 0.97  | 0.94 <sup>d</sup> ± 0.20  |
| Germination 6 day | 45.1 <sup>di</sup> ± 0.30 | 20.6 <sup>f</sup> ± 0.60  | 0.86 <sup>d</sup> ± 0.24  |

Mean (± standard deviation) values within the same column with different superscripts differ significantly at  $P < 0.05$ .

Germination of the soybeans increased the fat contents significantly ( $P < 0.05$ ) (Table 1). This agrees with Echendu *et al.*'s (2009) report on ground beans. However, Ghavidel & Prakash (2007) found a significant decrease of fat content after germination of some legume seeds. Osman (2007) and Mubarak (2005) reported a significant decrease of fat content when mung bean seeds were allowed to germinate for three days. This could be owing to the use of fat as energy during the sprouting process. The other processing methods that were included in this study, that is, soaking, dehulling and roasting, led to significant increases of crude protein and fat content. These increases are owing to the removal of the hull portion and therefore the concentration of endosperm in the legume product (Ghavidel & Prakash, 2007) as protein and fat are characteristically present in the cotyledon fraction of seeds (Alonso *et al.*, 1998).

Table 2 shows the effect of the different treatments on the levels of various ANFs. The time of germination and the duration of soaking were important factors affecting the ANFs measured in the study. All treatments numerically decreased the activity of trypsin inhibitor measured in the soybeans. These differences did not prove to be statistically significant, probably because of large variations found among replicates. However, germination may be useful in removing or reducing the activity of trypsin inhibitors in soybeans.

Germination significantly reduced the levels of condensed tannins in the soybeans. Condensed tannins were significantly decreased by 46%, 55% and 54%, respectively, after 2, 3 and 4 days of germination. This reduction in tannin content in soybeans is attributed to the tannin activity during germination (Mubarak, 2005) that leads to the formation of hydrophobic association of tannins with seed proteins and enzymes. Some loss of condensed tannins during germination may be because of the leaching of tannins into water. Reddy *et al.* (1985) found that overnight soaking of grains followed by germination for two days significantly reduced the tannin content by 50% in a wide variety of legumes. The decrease in condensed tannins after soaking, as observed in this study, is in agreement with results reported by Alonso *et al.* (2000).

Dehulling decreased the levels of condensed tannins, similarly to the findings of Alonso *et al.* (1998). Tannins are located mainly in the seed coats, which could explain their reduction after dehulling (Matthäus & Angelini, 2003).

**Table 2** Effect of soaking, dehulling and germination of soybeans on trypsin inhibitor activity (mg/g), total polyphenols (%) and condensed tannins (%)

| Treatment         | Trypsin inhibitor activity | Total polyphenols         | Condensed tannins         |
|-------------------|----------------------------|---------------------------|---------------------------|
| Raw               | 83.1 ± 8.71                | 10.8 <sup>a</sup> ± 0.42  | 1.24 <sup>a</sup> ± 0.34  |
| Roasted           | 52.3 ± 14.55               | 12.4 <sup>b</sup> ± 1.07  | 1.50 <sup>a</sup> ± 0.46  |
| Soaking – 1 day   | 77.3 ± 9.67                | 12.4 <sup>ab</sup> ± 0.20 | 1.04 <sup>ab</sup> ± 0.62 |
| Soaking – 2 day   | 66.2 ± 5.31                | 12.4 <sup>ab</sup> ± 1.56 | 0.71 <sup>c</sup> ± 0.21  |
| Dehulling         | 68.7 ± 9.20                | 12.8 <sup>ab</sup> ± 1.35 | 0.64 <sup>c</sup> ± 0.18  |
| Germination 1 day | 64.0 ± 24.97               | 11.8 <sup>ab</sup> ± 0.24 | 0.82 <sup>c</sup> ± 0.29  |
| Germination 2 day | 67.9 ± 21.54               | 11.2 <sup>ab</sup> ± 0.77 | 0.67 <sup>c</sup> ± 0.14  |
| Germination 3 day | 64.4 ± 18.86               | 10.6 <sup>ab</sup> ± 0.69 | 0.56 <sup>c</sup> ± 0.22  |
| Germination 4 day | 69.0 ± 8.35                | 10.4 <sup>ab</sup> ± 0.70 | 0.57 <sup>c</sup> ± 0.28  |
| Germination 5 day | 63.9 ± 6.67                | 12.4 <sup>bc</sup> ± 1.41 | 0.96 <sup>c</sup> ± 0.39  |
| Germination 6 day | 67.3 ± 10.77               | 12.8 <sup>ac</sup> ± 0.69 | 0.87 <sup>c</sup> ± 0.26  |

Mean (± standard deviation) values within the same column with different superscripts differ significantly at  $P < 0.05$ .

In this study, the germination of soybean seeds did not have a significant effect on the level of total polyphenols in the beans. The increase of total polyphenols observed in the soybeans that were germinated for a period of five to six days could be attributed to the presence of fungi at this time (Wu *et al.*, 2011), as

fungal growth on the seeds was evident from the fifth day of germination. Further research is needed before definite conclusions can be made.

Various researchers have reported lower levels of tannins, TIA and other ANFs in pulses as a result of domestic processing, including soaking, dehulling and germinating. Mwikya *et al.* (2000) found that the TIA decreased threefold, and reported a significant decrease in tannins to undetectable levels in finger millet during sprouting. A 20% - 38% reduction in tannins was observed during the germination of green grams, cowpeas, lentils and chickpeas (Ghavidel & Prakash, 2007). Sangronis & Machado (2007) found a significant decrease of TIA in pigeon beans, white beans and black beans after five days of germination, while Frias *et al.* (1995) found that six days of germination of lentils decreased TIA. Wang *et al.* (1997) reported that TIA decreased with shorter germination periods, but increased slightly as the length of germination increased. In this study, the level of total polyphenols increased after five days of germination, and also after roasting, soaking and dehulling soybeans (Table 2). Changes in phenolic compounds may occur, depending on the types of seed (López-Amorós *et al.*, 2006), processing conditions such as rinsing (Frias, 1995), presence of light, and germination time (Frias, 1995; Oloyo, 2004; López-Amorós *et al.*, 2006; Dueñas *et al.*, 2009). Oloyo, (2004) and Dueñas *et al.* (2009) found that polyphenols of lupin increased during germination. Similarly, Satwadhar *et al.* (1981) found that the polyphenol levels in moth beans increased from 1.0% to 1.7% at 36 h germination, while beans that germinated for 48 h had higher amounts of polyphenols than raw moth beans.

No significant differences were noted in IVPD among the treatments, regardless of the type of enzyme used (Table 3). On the contrary, Mubarak (2005) reported that dehulling, soaking and germinating mung bean seeds improved their IVPD. A significant increase was observed in the IVPD of white beans, black beans and pigeon beans after germination (Alonso *et al.*, 2000; Sangronis & Machado, 2007).

**Table 3** Effect of soaking, dehulling and germination of soybeans on *in vitro* protein digestibility (%)

| Treatment         | Pepsin digestion | Papain digestion |
|-------------------|------------------|------------------|
| Raw               | 66.5 ± 21.40     | 62.7 ± 13.01     |
| Roasted           | 44.6 ± 19.79     | 58.4 ± 2.77      |
| Soaking – 1 day   | 65.4 ± 24.44     | 55.4 ± 11.13     |
| Soaking – 2 day   | 80.0 ± 7.03      | 57.2 ± 16.58     |
| Dehulling         | 71.9 ± 24.69     | 48.0 ± 9.01      |
| Germination 1 day | 64.6 ± 14.02     | 55.4 ± 9.77      |
| Germination 2 day | 63.8 ± 15.96     | 52.6 ± 8.90      |
| Germination 3 day | 59.8 ± 19.38     | 52.5 ± 8.90      |
| Germination 4 day | 57.8 ± 15.15     | 49.5 ± 17.99     |
| Germination 5 day | 58.1 ± 14.36     | 46.8 ± 9.79      |
| Germination 6 day | 62.8 ± 11.47     | 50.7 ± 4.56      |

Mean (± SD) reported on dry basis.

## Conclusion

All traditional processing methods tested in this study significantly increased the crude protein and fat content of the soybeans, but decreased the starch content. Germination for a period of three to four days was effective in improving their nutritional value and reducing the levels of condensed tannins. Trypsin inhibitor activities were numerically lower in all treated soybeans, but not significantly. No statistical differences were noted between the soybeans that were germinated for a period of three days and those that were germinated for four days. Furthermore, longer periods of germination may result in fungal growth that may be detrimental to the quality of the soybeans. In this study, notable fungal growth after five days of germination might have contributed to an increase in the polyphenol content of the beans. It is recommended that small-

scale farmers in rural areas apply a germination period of three days for soybeans when properly controlled heat treatment is not possible. The cost of germinating soybeans is minimal as expensive equipment and specialised facilities are not necessary. Because soybeans are used mostly in poultry diets, the findings of the study are probably most applicable in the field of poultry nutrition. However, *in vivo* studies on poultry are needed to substantiate the efficacy of germination as a processing method for soya.

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