Kinetic study of saponins B stability in navy beans under different processing conditions

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Abstract

Saponins are rich in the legumes which are known to provide many health benefits for human beings. Saponin B is the main component in the saponins group present in navy beans. The stability of saponin B during food processing is a key issue in evaluating the quality and nutrition of food products. The effects of different soaking and cooking methods and conditions on the stability of saponin B were investigated. The effects of the soaking process on saponin reduction followed a first order kinetic model. The soaking time and the seed-to-water ratio significantly affected the stability of saponin B during the soaking process. Short time soaking and lower seed-to-water ratio would keep more saponin B in the soaked beans. The cooking medium and methods greatly influenced saponin B degradation during cooking. Water–oil mixed cooking media enhanced saponins stability in the seeds during the cooking process, as compared to a water-only cooking medium. Combined soaking and ordinary cooking induced more saponin degradation in ordinary cooked seed samples. An autoclave cooking method eliminated most of the saponin B from the autoclaved beans.

1. Introduction

There is a long-standing controversy about saponins’ functions in foods because saponins have generally been considered as undesirable antinutrient components in legumes (Gestetner et al., 1968; Khalil and El-Adawy, 1994). However, recent extensive studies of their biological activity in vivo have shown that saponins’ stronger bioactivities are associated with several health benefits. The results suggested that saponins in the diet have a wide spectrum of activity as antifungal and antibacterial agents, in lowering of blood cholesterol, and inhibition of cancer cell growth (Rao and Sung, 1995; Jeon and Sung, 2000; Berhow et al., 2000; Oh and Sung, 2001; Matsuura, 2001; Lee et al., 2005; Wang et al., 2007). Legumes are one of the richest and least expensive natural sources of saponin in the human diet.

Saponins possess both water-soluble and lipid-soluble components. They consist of a lipoid-soluble nucleus, having either a steroid or a triterpenoid aglycone structure, with one or more side chains of water-soluble carbohydrates. Based on their aglycone structure, saponins are generally categorized into three main groups as groups A, B, and E (Heng et al., 2006). The main saponin components in legumes are the group B saponins. The group B saponins have the aglycone, sapogenol B, as sapogenin and which make up a majority of the saponins in legumes (Gurfinkel and Rao, 2002; Hubert et al., 2005; Shiraiwa et al., 1991). However, legumes are generally subjected to a cooking process before they are consumed. Soaking and cooking are common processing methods for bean products in the food industry. Processing methods and operating conditions tend to modify the composition and availability of nutrients in the finished products (Van der Poel, 1990; Della et al., 1994). The stability of saponins in the beans undergoing processing directly influences the quality and nutritional value of the end products. With advancements in qualitative and quantitative analysis techniques, the precise analyses of saponin contents in processed legume products is becoming more and more significant to the food industry. More systemic and detailed reports on this aspect are needed. The processing methods and operating conditions could affect the stability of saponins in the final bean products. Our knowledge about the extent and nature of saponins’ kinetic degradation is incomplete. The objective of the present study therefore was to determine the degradation kinetics for saponins in beans under various processing procedures that are commonly used in the food industry, i.e. different soaking conditions and cooking methods, and the effects of various thermal process methods and conditions on the characteristics of the saponins during different...
process stages using navy beans as a model material. This research focused on the major saponins B in navy bean seeds.

2. Materials and methods

2.1. Plant materials

Dry raw navy bean (Phaseolus vulgaris L.) seeds were obtained from the H.J. Heinz Company Canada Ltd. The dry beans were first screened to get a uniform size free from broken or spoiled seeds. The selected seeds were stored in a refrigerator at 4 °C in airtight containers to prevent moisture absorption before applying various treatments.

2.2. Processing methods

The processing methods used to study the kinetic degradation of saponins were soaking, ordinary cooking, autoclaving, and their combinations. In the soaking process, navy beans were soaked in distilled water at different seed-to-water ratios with different soaking times. In the ordinary cooking processes, effects of different cooking media on the changes in saponins in the cooked unsoaked and soaked beans were investigated. Comparisons of different cooking methods on the degradation of saponins during the thermal processes were determined. The experiments were prepared and tested in triplicate in each step. The initial saponin B content in the unprocessed dried navy beans was used as a control to compare with the treated navy bean samples.

2.2.1. Soaking

Navy bean seeds were soaked in distilled water (pH 6.2) for 3, 6, 9, or 12 h at room temperature, respectively. To avoid the possible problem that the soaking water may cause germination in the seeds, the dry seed to soaking water ratio must be selected such that the beans are completely covered by water. Therefore, the ratios of dry beans to soaking water (called seed-to-water ratio), 1:3 and 1:7 (w/w), were chosen. Any incompletely soaked seeds were discarded. The soaked bean seeds were rinsed in distilled water, and then air-dried in a fume hood for 24 h at room temperature. The dried beans were immediately milled into flour (8-mesh size) and stored in dark brown polyethylene bottles at −30 °C until further extraction and analysis.

2.2.2. Ordinary cooking

Two cooking media, distilled water and water–oil mixture (1:1, v/v), were selected to study the effects of different cooking media on the kinetic degradation of the saponins B group under different cooking times. The ratio of seed-to-cooking media was fixed at the 1:4 ratio (cooking media is four times the weight of soaked seeds) that is normally used in the traditional bean cooking process. After soaking 50 g dry navy beans in distilled water at the seed-to-water ratio of 1:3 for 6 or 12 h at room temperature, the soaked bean seeds were rinsed with distilled water and dried with a paper towel, then put in round mouth tall beakers fitted with condensers. After adding 200 g distilled water or water–oil mixture, the samples were cooked on a hot plate for 15, 25, or 35 min at 100 °C. The cooking times chosen for this study are commonly used in the food industry for bean processing. Cooking of unsoaked control seeds was also performed by the same treatment method as described above. After cooking, the medium was discarded and the beans were air-dried overnight at room temperature. The dried cooked bean seeds were ground into flour (8-mesh) while being cooled with liquid nitrogen to avoid thermally induced degradation generated during milling. The bean flours were stored in dark brown polyethylene bottles at −30 °C, pending further extraction and analyses.

2.2.3. Autoclaving (pressure) cooking

The unsoaked and soaked (soaking for 6 h at a seed-to-water ratio of 1:3) seeds were autoclaved (MRH Biotechnical Ltd, Boston, MA, USA) in distilled water at 1.41 kg/cm² pressure at a temperature of 121 °C for 15, 25, or 35 min at the seed-to-cooking media ratio of 1:4 (w/w). The cooking times and ratios of seed-to-cooking media used in the autoclave were the same as those used for ordinary cooking. After pressure cooking, the media were discarded, and the seeds were air-dried at room temperature, and then subsequently milled into flour (8-mesh size) with liquid nitrogen to avoid mechanically induced thermal degradation during milling.

2.3. Extraction of saponins B

The saponins B in different processed navy bean seeds and control unprocessed seeds were extracted according to the method of Khalil and Ei-Adawy (1994) with some modifications. Five grams samples of bean flour from each of the different processes were extracted twice by refluxing for 4 h with 50 mL of 70% (v/v) aqueous ethanol in a water bath at 60 °C. The extracts were centrifuged at 3000 rpm for 15 min. The supernatants were collected and dried in a rotary evaporator at 45 °C for further hydrolysis. The dried supernatants were dissolved in 8 mL of 1.5 N hydrochloric acid–methanol solutions and hydrolyzed for 2.5 h at 75 °C. The hydrolysates were cooled, and then purified and concentrated by solid phase extraction (SPE). Seven milliliters of hydrolyzed samples were passed through an SPE extraction cartridge (Strata C18-E; 55 μm, 70 Å; 500 mg/6 mL; Phenomenex, USA), preconditioned with methanol (5 mL) and distilled water (5 mL), and subsequently rinsed with 5 mL of distilled water to remove unbound material. The fraction containing sapogenol B was eluted with 5 mL methanol. The fraction was then filtered through a 0.45 μm syringe filter for HPLC analysis.

2.4. HPLC analysis

The total content reductions in saponins B were evaluated by quantifying their common hydrolysate, sapogenol B. The reverse phase HPLC (Agilent 1100, Scientific Equipment Source, ON, Canada) with a UV detector was used to analyze the quantitative variations of saponins B at different conditions during soaking, ordinary cooking, and autoclave. A Nova-Pak C18 column (3.9 × 150 mm, 4 μm particle size; waters, USA) was used for separation. The mobile phase consisted of 75% acetonitrile and 25% deionized water containing 0.05% TFA. The system was run isocratically at a flow rate of 1 mL/min. The sample injection volume was 10 μL and UV absorbance was monitored at 210 nm. Chromatograms were recorded and integrated with Agilent Chemstation software. In order to quantify the saponin B, standard sapogenol B (ChromDex, CA, USA) was prepared at 1 mg/mL (1000 ppm) in methanol as a stock solution. Serial dilution of the stock solution using methanol was performed to prepare series standard working solutions with concentrations from 10 to 500 μg/mL. The representative chromatogram of sapogenol B standard and sapogenol B obtained from navy bean samples are illustrated in Fig. 1A and B. All analyses were carried out in triplicate and reported on a dry matter basis.

2.5. Kinetic modeling of degradation

In comparison with the effects of different process methods and conditions on saponins B contents in processed beans, the kinetic phenomenon of saponins B under different conditions was demonstrated by the plot of the first order degradation. The degradation rate constant (k) was calculated as the slope of the linear plot. A
first-order kinetic model was applied for characterizing saponins B stability according to the following reaction:

$$\frac{dC}{dt} = kC$$  (1)

The kinetic degradation of saponins to treatment times can be obtained by integrating Eq. (1) as:

$$\ln \left( \frac{C}{C_0} \right) = -kt$$  (2)

where $C_0$ is the initial saponins B content in unprocessed dry raw navy bean seeds and $C$ is the remaining saponins B contents in treated bean seeds after process time $t$. The $k$ is the kinetic degradation constant of saponins B (mg/g beans per min or h) and can be determined as the slope from plotting $\ln\left( \frac{C}{C_0} \right)$ vs. $t$.

The reduction in saponin B contents was determined as a percentage of the decrease over the initial saponins B content in unprocessed dry raw navy beans.

Soaking effects on saponins B reduction (%) by leaching $= \left( \frac{C_0 - C_s}{C_0} \right) \times 100$

Cooking effects on saponins B reduction (%) by degradation and leaching $= \left( \frac{C_0 - C_s - C_c}{C_0} \right) \times 100$, where $C_0$ is the initial saponins B content (mg/g) in unprocessed raw navy bean, $C_s$ is the saponins B content in the soaked seed samples, and $C_c$ is the saponins B content in the cooked seed samples.

### 2.6. Statistical analysis

All experiments were conducted using factorial experimental designs and analyzed in triplicate with a completely randomized design. All statistical analyses were done by using SAS 8.2 software from SAS Institute Inc., Cary, NC, USA. Kinetic data were analyzed by regression analysis. ANOVA was used to determine the difference and interaction effects on saponin B contents among different treatments such as the main factors and their combined effects, including the various soaking times, seed-to-water ratios, and different cooking media and cooking methods. Duncan’s multiple-range comparison tests were conducted to determine the significant effects of different processes on saponins B content of the unprocessed and variously processed seed samples. Statistical significance was accepted at $p < 0.05$.

### 3. Results and discussion

#### 3.1. Effects of soaking processes on degradation of saponins

The initial saponins B content in raw navy beans used in this study was $7.62 \pm 0.19$ mg/g as shown in **Table 1**. The reduction of saponins B amount occurs through a leaching process when the beans are soaked in water. The results of Duncan’s tests showed that 6 h or less soaking times did not significantly ($p < 0.05$) change the saponin contents in the soaked seeds when the seeds were soaked in distilled water at the seed-to-water ratio of 1:3 (w/w) compared to their initial saponins B contents. However, increasing the seed-to-water ratio to 1:7 at a soaking time of 6 h caused a significant reduction of 3.1% saponins B content in the soaked seeds.

Moreover, when the soaking time was prolonged to 9 or 12 h, there resulted significant reductions in the amount of saponins B in the seeds soaked in distilled water at both the seed-to-water ratio of 1:3 and 1:7. The results suggested that the saponins B remained stable for short soaking times with a limited amount of soaking media. The dry beans initially absorb water to soften their hard coats. Long time soaking treatments allow the beans to continuously absorb water to breakdown the oligosaccharides and further soften the bean tissue matrix to complete hydration (Upadhyay and Garcia, 1988; Shimelis and Rakshit, 2007).

Saponins are soluble in water. The hydrated bean seeds allow more water to penetrate more deeply into their matrix to release saponins from the bean tissue matrix by simple diffusion (leaching). These phenomena could explain the reason that the short

<table>
<thead>
<tr>
<th>Table 1</th>
<th>The effects of soaking conditions on saponins B contents in navy beans.</th>
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<tbody>
<tr>
<td>Soaking time (h)</td>
<td>Seed-to-water: 1:3</td>
</tr>
<tr>
<td>Saponins B contents (mg/g)</td>
<td>Reduction (%)</td>
</tr>
<tr>
<td>0</td>
<td>$7.62 \pm 0.19^a$</td>
</tr>
<tr>
<td>3</td>
<td>$7.58 \pm 0.17^a$</td>
</tr>
<tr>
<td>6</td>
<td>$7.55 \pm 0.14^a$</td>
</tr>
<tr>
<td>9</td>
<td>$7.26 \pm 0.13^b$</td>
</tr>
<tr>
<td>12</td>
<td>$7.14 \pm 0.16^c$</td>
</tr>
</tbody>
</table>

Means with the same superscript letters are not significantly different at $p < 0.05$ levels.

“–” is not applicable.
time and less water (lower seed-to-water ratio) conditions did not complete the hydration of beans hence did not generate significant reductions of saponins contents. The highest reduction of ~10.1% saponins B content was observed in the seeds soaked for 12 h at a seed-to-water ratio of 1:7. Simultaneously, foams were observed in the soaking water when the seeds were soaked for more than 6 h, which indicated that saponin leaching occurred in the soaking media. Saponins have the ability to foam in aqueous solutions. The saponins B are more soluble in water due to their sugar chain structure composition. Therefore, water leaching resulted in a loss of saponins B (Gurfinke1 and Rao, 2003; Shi et al., 2004) in soaked beans during the soaking process. Longer soaking times and excesses water also allow a greater amount of sugar to dissolve, resulting in the increased permeability of cell membranes and seed coat to maximize the amount of saponins leached from the tissue into the soaking media. However, ANOVA analysis showed that no significant differences in saponin B contents were found in the seeds soaked for 9 and 12 h at a seed-to-water ratio of 1:7. Prolonging soaking times would not appear to influence saponin leaching after the bean tissues have been totally hydrated. The results suggest that the synergistic effects of soaking time and seed-to-water ratios have more pronounced effects on the loss of saponin B contents during short soaking treatments.

The phenomenon of saponin kinetic mass transfer (leaching) under different soaking conditions followed the first order kinetic model with a correlation coefficient ($R^2$) greater than 0.93 (Fig. 2). The results indicated that the seed-to-water ratios greatly affected the stability of saponins B in the soaked seeds. The rate of saponins B loss (in mg/g of sample) for soaked seeds at the seed-to-water ratios of 1:3 and 1:7 were 0.0117/h and 0.0073/h, respectively. This was evidence to confirm the synergistic effects of soaking time to ratio on the reduction in the saponins B contents.

From the kinetic mass transfer explanation for saponins B leaching, the large amount of soaking media increases the diffusion rate of the saponins, thus more water-soluble saponins were released from the bean tissue matrix into soaking media. Similar trends were observed by previous researchers working with kidney beans (Shimelis and Rakshit, 2007), moth beans (Khokhar and Chauhan, 1986), chickpeas (Jood et al., 1986), and black grams (Kataria et al., 1988). Kataria et al. (1989) reported that prolonging the soaking time from 12 to 18 h did not have any significant influence on the saponin contents of mung-bean seeds during soaking. However, in comparison with their range of 11–65% saponins reduction in legumes by soaking processes (Khokhar and Chauhan, 1986; Kataria et al., 1989; Curl et al., 1985; Duhan et al., 2001; Drumm et al., 1990), our experimental results showed a lower reduction in saponin contents (0.5–10.1%). The variability in the observed amount of saponins reduction might be attributed to differences in the bean tissues, pressing treatments, and/or to the initial saponin component compositions of these raw bean seeds.

3.2. Effects of different cooking media on the stability of saponins B

Navy beans are commonly cooked in water or cooked with meat for meals. Therefore, the distilled water and water–oil mixture were selected to study the effects of different cooking media on the kinetic degradation of saponins B for the unsoaked and soaked beans (soaked for 6 or 12 h at the seed-to-water ratio of 1:3) under ordinary cooking conditions. Statistical results shows that the seeds cooked in the distilled water or the water–oil mixture significantly reduced ($p < 0.05$) saponins B contents in all the cooked seeds excepted for the samples (7.59 ± 0.17 mg/g) that are the unsoaked seeds cooked in the distilled water for 15 min, as compared to the initial saponins B contents (7.62 ± 0.19 mg/g). A combination of soaking and cooking processes caused a high loss of saponins B contents in the processed beans. The thermal treatments have greater effects on saponins degradation because saponins B are a monodesmoside saponin and have a sugar chain linked to the C-3 position of its aglycones (sapogenol B) with a hydrogen atom at C-21 position (Heng et al., 2006). The linkage bond between sugar chain and sapogenol B would be broken, and the aglycones may also be decomposed when high energy is applied, such as heating at higher temperatures. Therefore, thermolabile saponins degraded during cooking (Shi et al., 2004; Heng et al., 2006). The water-soluble and oil-soluble characteristics of saponins B is another reason to cause more saponins to be released from the seed tissue matrix into the cooking media, resulted in a high degree of degradation of saponins B. Therefore, the prolonged time and excess thermal energy induced more saponins degradation in the seeds. The cooking media also carried more saponins out of the seed's matrix during the cooking processes, especially when the soaked seed was subjected to heating because of the softness of the bean tissue permits the heat to more easily penetrate inside of the seed's matrix to degrade saponins B.

Fig. 3 shows the reductions of saponins in cooked bean seeds under different cooking media. For the unsoaked seeds subjected to ordinary cooking, the water–oil mixture showed more pronounced effects on the reduction of saponins B as compared to the water media. The oil media could maintain a higher temperature in the cooking media, enhancing the cooking media's perme-

![Fig. 2](image-url)  
**Fig. 2.** Effects of soaking conditions on the kinetic degradation of saponins B in various soaked seeds (○ is beans soaked at the seed-to-water ratio of 1:3, □ is beans soaked at the seed-to-water ratio of 1:7).
ability into the matrix, and to drive degradation of saponins B during the thermal process. However, progressive decreases in saponins B contents were found in the soaked seeds when cooked in distilled water. One possible explanation for these phenomena lies in the saponins B chemical structure. Saponins possess both lipophilic (aglycones) and hydrophilic (sugar chain) components. However, the saponins are more soluble in water than in lipid when the sugar-chains are attached to the aglycones. The lipophilic structure of the aglycone is also more stable than the sugar chain under thermal treatment and hydration (Oakenfull and Sidhu, 1990; Oda et al., 2003). Therefore, more water-soluble saponins have been leached into the media. Simultaneously, products of decomposed saponins that were subsequently oxidized by oxygen in the water media caused a high degradation of saponin. More oxygen is present in water than in oil media. The results suggest that the oil media is more favourable toward stabilizing saponins B in the beans, as compared to water media traditionally used in the cooking process.

The degradation of saponins B in different cooking media under thermal processes also followed the first-order kinetic degradation model with a correlation coefficient ($R^2$) greater than 0.94 (Figs. 4 and 5). The values of kinetic degradation rate constants ($k$) were determined as the slope of the linear regression line from the plot of the degradation of saponin contents in the samples vs. cooking times. The values of $k$ increased with increasing soaking time and the cooking times. The results agreed with the conclusion that the length of soaking and cooking are important factors that greatly influenced the saponins B stability in the beans during the process. Saponins degraded rapidly in the seeds after soaking and were then subsequently subjected to thermal processes. In comparing the kinetic degradation rate of saponins B in those seeds cooked in water or a water–oil mixture, the values of the kinetic degradation rate constants were higher in the seeds first soaked when followed by cooking in water than by cooking in a water–oil mixture. These phenomena confirmed that oil media enhanced saponins B stability in beans during the cooking processes. Similar results were noted in the saponin contents in moth bean (Khokhar and Chauhan, 1986), edible dry beans (Oda et al., 2003), soybean flour (Tarade et al., 2006), and kidney beans (Shimelis and Rakshit, 2007) during bean processing.

### 3.3. Effects of cooking methods on stability of saponins B

Table 2 shows a comparison of the cooking methods’ effects on the kinetic degradation of saponins B in navy bean seeds during thermal processing. The kinetic degradation of saponin did not follow the first order kinetic model ($R^2 < 0.83$) when the beans

![Fig. 3. Effects of cooking media on the reductions of saponins B in cooked bean seeds.](image)

![Fig. 4. Kinetic degradation of saponins in different combined soaking and ordinary cooking in water cooking media (▲ uns soaked seeds, □ soaking for 6 h, △ soaking for 12 h).](image)
were cooked using an autoclave. The results showed that the degradation of saponins B increased with increasing cooking time. For the soaked and cooked beans in the ordinary cooking process, enhanced permeability of the seed membrane as a result of the soaking processes improved the cooking process, which also had a limitation on increasing the leached saponins in the boiling water. The highest reduction in saponin contents in ordinary cooked samples was 70.03% when the seeds were first soaked for 12 h and then subjected to cooking for 35 min. However, there were still some saponin B contents remaining after 35 min cooking time. The lowest content in the ordinary cooked seeds was 2.14 ± 0.11 mg/g when the beans were soaked for 12 h and then subjected to cooking for 35 min. For autoclave cooking, no detectable amounts of saponin B were found when the seeds were autoclaved for 25 min or longer. Simultaneously, mashed cooked seeds were obtained after autoclaving for 35 min because longer times under high pressure and temperature caused the destruction of the navy bean seed matrix. The thermally induced reduction of saponin contents was 73.49% for an unsoaked bean autoclaved for 15 min, and 92.32% reduction was found in the beans soaked for 6 h and then autoclaved for 15 min. The results suggested that autoclaving could destroy most of saponins B in the beans, especially for the long-time-soaked beans, even if only autoclaved for 15 min. The soaking process enhanced the effectiveness of the reduction of saponins B on the subsequent ordinary cooking method. However, this effectiveness was not as pronounced for the autoclave process. The differences in saponin B degradation between ordinary cooking and autoclaving might be attributed to the high energy level in the combination of high pressure and high temperature in the autoclave, causing those components such as aglycones and sugar to decompose, resulting in a serious degradation of saponins in the beans. Because aglycones possess a heat stable structure, they did not totally decompose during ordinary cooking. Therefore, there were still some amounts of saponin B that remained in the ordinary cooked samples. These results are consistent with the reported studies by Duhan et al. (2001), Sharma and Sehgal (1992), and Drumm et al. (1990), all of whom reported that pressure cooking had a greater reducing effect than ordinary cooking on the saponin contents of pigeon peas and faba beans.

### 4. Conclusion

Processing methods and conditions greatly change the saponin contents in beans during food preparation and processing. Kinetic
analysis results showed that the soaking times and seed-to-water ratios significantly influenced the quantity of saponins B leached out from the beans’ matrix during the soaking processes. Short soaking times with less soaking media probably favoured stabilizing the saponins B in the beans. Cooking media and methods significantly changed the saponin contents in different cooked beans. Oil media seem to be less harmful on the degradation of saponins B during the cooking processes. The pre-soaking process enhanced subsequent thermally induced degradation of the saponins and caused a high loss of the contents during the cooking processes. Autoclaving destroyed most of the saponins B in processed beans. The lowest amount of saponin contents were found in processed beans when beans that had been soaked for 6 h were subjected to autoclaving for less than 15 min. These data would be helpful for the food industry in finding a suitable method for maintaining the content of health-promoting components or reducing antinutrient substances in processed legume products.

References


