

Effect of Soaking Time and Cooking Time on Qualities of Red Kidney Bean Flour

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ABSTRACT

Soaking time and cooking time of red kidney bean affected the reduction of raffinose and stachyose contents in its flour. Red kidney beans were soaked in distilled water for 6 and 12 h, and then cooked by steaming (30, 60, and 90 min) or boiled in pressure cooker (10 and 15 min). After extraction of raffinose and stachyose contents from flour made from soak-cooked beans, the results from HPLC showed an important decrease in oligosaccharides of soak-cooked beans. The highest decrease in raffinose and stachyose was to soak beans for 12 h and then boil in pressure cooker for 15 min which was 47% and 44%, respectively.

Key words: red kidney bean, oligosaccharides, raffinose, stachyose, HPLC

INTRODUCTION

Red kidney beans (*Phaseolus vulgaris*) are the whole grain consumed in the greatest quantity in the world (Carpenter, 1981). They are an important economical source of protein in the diet of many developed and developing countries (Nielsen, 1991); however, Thailand has exported most of the red kidney beans (Tharatthapan, 1996). Red kidney beans are rich in B-complex vitamins and minerals (Koehler *et al.*, 1987; Guzman-Maldonado and Paredes-Lopez, 1998). Moreover, red kidney beans are a very low in sodium, cholesterol, and saturated fatty acids but rich in unsaturated fatty acids such as linoleic acid (Barampama and Simard, 1994). They are not only a good source of both soluble and insoluble dietary fiber but also health benefits, including reduced risk of heart disease and colon cancer (Hughes, 1991; Guzman-Maldonado and Paredes-Lopez, 1998). Although they are advantages, several factors detract from their full nutritional potential such as the presence of

antinutritional factors that cause flatulence (Olson *et al.*, 1981) and low protein digestibility which may limit amino acid availability (Nielsen, 1991).

Raffinose oligosaccharides (e.g. raffinose, stachyose, and verbascose) have been called antinutritional factors that contribute to flatulence production (Reddy *et al.*, 1989; Guzman-Maldonado and Paredes-Lopez, 1998). They were not digested due to the lack α -1,6-galactosidase activity in the mammalian intestinal mucosa and cause intestinal microflora to produce flatus (Olson *et al.*, 1981; Sathe and Salunkhe, 1989). To improve nutritional quality of red kidney beans, soaking, cooking, germination, fermentation or irradiation treatments may be used to develop protein nutrition value (Sathe and Salunkhe, 1989; Guzman-Maldonado and Paredes-Lopez, 1998). Jood *et al.* (1985) and Barampama and Simard (1994) studied the effect of processing on oligosaccharides in legumes and found that soaking, cooking, germination or fermentation can be use to reduce antinutritional factors.

The objective of this research was to evaluate the effect of soaking and cooking (steaming and pressure cooker) at different length of time on the oligosaccharide contents in red kidney beans.

MATERIALS AND METHODS

1. Materials

Red kidney beans. Red kidney beans were purchased from local market and stored at room temperature (30°C).

Reagents and standards. The raffinose and stachyose standards were obtained from Fluga (Buchs, Switzerland). Acetonitrile and methanol were obtained from J.T.Baker (Philipsberg, New Jersey). All standards and solvents were HPLC grade.

2. Experimental design

A 2x3 factorial design with two levels (6 and 12 h) of soaking and three levels (30, 60, and 90 min) of steaming and a 2X2 factorial design with

two levels (6 and 12 h) of soaking and two levels (10 and 15 min) of boiling in pressure cooker were investigated.

3. Sample preparation

Raw red kidney bean flour. Red kidney beans were washed thoroughly with three times of distilled water at room temperature and dried using tray dryer (Model HA-40, BWS Trading, Ltd., Thailand) at 60°C for 1 h. After drying, they were reduced particle size by food processor and finally ground with a Hammer Mill (Model AP-S, Hosokawa Micron Corp., Japan) equipped with a 0.5 mm screen. Raw kidney bean flour was placed into plastic container and stored at 4°C during the period of analysis.

Cooked red kidney bean flour. The method of preparation as given in Figure 1 was followed in this investigation. Red kidney beans were washed thoroughly with three times of distilled water at room temperature, and then were soaked in distilled water at a ratio of 1:10 for 6-h and 12-h at room temperature.

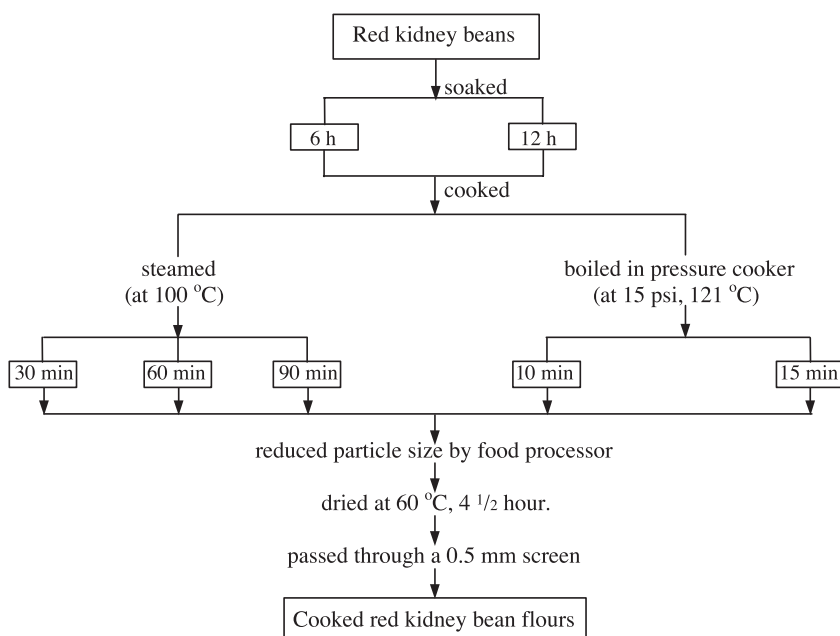


Figure 1 Preparation steps of cooked red kidney bean flours.

The soaked samples were cooked by steaming at 100°C for 30 min, 60 min, and 90 min or boiled in pressure cooker at 15 psi for 10 and 15 min (1:1.5 red kidney beans to water ratio). After the processed samples were reduced particle size by food processor, they were dried at 60°C for 4 1/2 h and finally ground with a Hammer Mill equipped with a 0.5 mm screen. All the soak-cooked bean flours were placed into plastic containers and stored at 4°C during the period of analysis. Concentration of oligosaccharides were calculated in a dry basis.

4. Raffinose and stachyose oligosaccharide analysis

Raffinose and stachyose standard solution. Stock standard mixture solution was prepared by accurately weighed raffinose and stachyose standard and dissolved in deionized water. The other four concentrations were prepared from stock standard mixture solution diluted with deionized water. The resulting peak areas were plotted against concentrations for the calibration curve by using the external standard method.

Extraction. Based on a modification procedure of Barampama and Simard (1994), sample was weighed about 1.5 g in a 20-ml test tube, added 10 ml of 80% ethanol : water (v/v) for first extraction. The sample was thoroughly mixed using a vortex mixer, and then was shaken in a boiling water bath at 80°C for 15 min. The mixture was centrifuged for 5 min at 2,800 rpm using a centrifuge (Model Universal 16R, Andreas Hettich GmbH&Co., Germany), and the supernatant was transferred to another 50-ml test tube. For the sample residue in the first centrifuge tube, added 5 ml of 80% ethanol : water (v/v) and repeated extraction as in the previous described. Finally, 10 ml of 80% ethanol : water (v/v) was added to the residue and the extraction repeated as in previous step. The three collected supernatants were combined in a 5-ml polyethylene centrifuge tube, then added 2 ml of 10% lead acetate : water (w/v)) to deproteinize the solution. The mixture was shaken using a vortex

mixer until it was homogeneous, and then centrifuged for 20 min at 2,800 rpm. The supernatant was transferred to another centrifuge tube, then added 0.5 ml of 10% oxalic acid : water (w/v). The supernatant was centrifuged for 20 min at 2,800 rpm to remove the excess lead acetate in the sample, the clear extract was quantitatively transferred to a 25-ml volumetric flask and made up volume with deionized water. It was stored immediately at -20°C for further analysis. All experiments were conducted in duplicate.

The analytical method for sample cleanup was modified by the procedure of Barampama and Simard (1994) and Sánchez-Mata *et al.* (1998). The prepared extract was purified by filtered through a Sep-Pak C₁₈ cartridge (Waters Associates, Milford, MA), which was prewetted with 5 ml of methanol followed by 5 ml of deionized water. Before injection, the sample was filtered through a 0.45 µm cellulose acetate filter (Sartorius, AG). The eluent was collected in a 4-ml clear shell viol for raffinose and stachyose analysis by HPLC.

HPLC Quantification. Aliquots of filtered sample (20 µl) was injected to the reverse phase HPLC system with Waters Associates Liquid Chromatograph (Waters, Milford, MA), equipped with a Water™ 600 controller, Waters™ 600 pump and a Waters 410 differential refractometer. The separation of oligosaccharides was achieved by a 4 × 250 mm, 5 mm (Merk, Darmstadt, Germany) LiChrosphere NH₂ column, connected to a µBondapak NH₂ guard column (Waters, Milford, MA) using a 10-µl microsyringe (Model MS-NR 10, Ito Corp., Fuji). The isocratic mobile phase contained acetonitrile : water (70:30) at 0.8 ml/min flow rate. The mobile phase was filtered using a 0.45 µm filter membrane. The column temperature was controlled at 30°C and the temperature of RI detector was set at 35°C with high sensitivity (512). A completed analysis was carried out about 13 min. Data were collected and processed by Millenium version 2.10 program on a PC 130-486 DX2 computer connected to HPLC apparatus. The

concentrations of raffinose and stachyose in the samples were calculated using the average peak areas compared with the standard, and expressed as g/100g of dry beans.

5. Statistical analysis

Analysis of variance (ANOVA) was done to determine and compare differences in raffinose and stachyose contents. Duncan's Multiple Range Test was performed for post hoc multiple comparisons. Significant differences were established at $\alpha = 0.05$.

RESULTS AND DISCUSSION

The chromatograms of raffinose and stachyose in mature red kidney bean separated by LiChrosphere NH₂ Column are shown in Figure 2. According to the chromatogram, the retention time of raffinose and stachyose are 8.25 and 11.50 min, respectively. The effect of various soaking times and steaming times on raffinose and stachyose contents are shown in Figure 3 and 4, respectively. The increase in soaking time and steaming time significantly ($P \leq 0.05$) decreased raffinose. The extent of loss was increased as the time of soaking

increased from 6 to 12 h or as the time of steaming increased from 30 to 90 min. After being soaked for 6 h, stachyose contents were decreased significantly ($P \leq 0.05$) as the steaming time increased from 30 to 60 min. However, at the 60-min and 90-min steaming, stachyose content was not significantly different ($P > 0.05$) for 6-h soaking. At 12-h soaking, the increase in 60-min to 90-min steaming was significantly ($P \leq 0.05$) affected the reduction of stachyose from 2.22 to 1.90 g/100 g of dry beans. Soaking for 12 h followed by steaming for 90 min represented the lowest raffinose and stachyose contents with 0.63 and 1.90 g/100 g of dry beans, respectively. Therefore, soaking time and cooking time affected the reduction of raffinose oligosaccharides in red kidney beans. The result was agreed with the study of Jood *et al.* (1985), and Barampama and Simard (1994) who found on that soaking and cooking reduced oligosaccharides in legumes.

The effect of soaking and boiling in pressure cooker on raffinose and stachyose in red kidney bean flour are showed in Figure 5 and 6, respectively. Raffinose and stachyose were decreased significantly ($P \leq 0.05$) after soaking time increased.

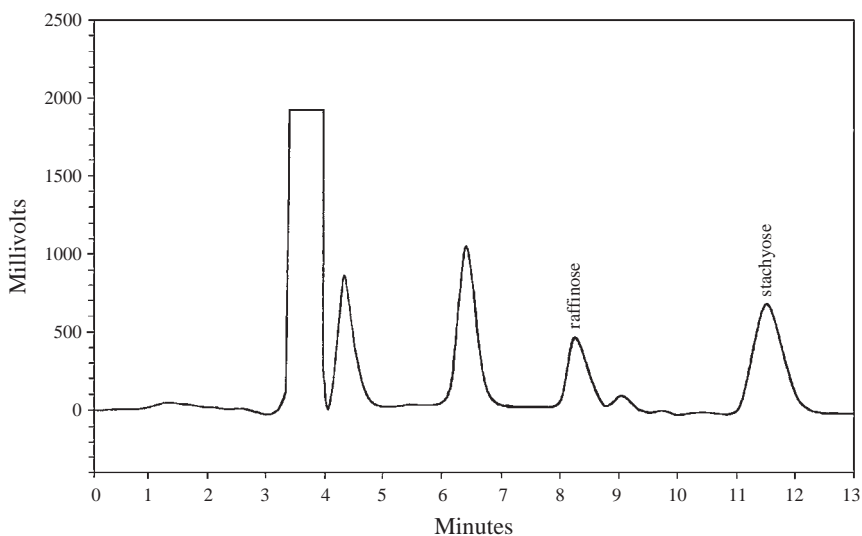


Figure 2 HPLC chromatogram of raffinose and stachyose in raw red kidney bean.

The increased in cooking time was not significantly ($P>0.05$) affected raffinose content ; however, the significant ($P\leq 0.05$) reduction was found in stachyose contents. It was indicated that the 12-h soaking followed by boiling in pressure cooker for 15 min present the highest decrease of raffinose and stachyose by 0.53 and 1.60 g/100 g of dry beans, respectively.

The results of raffinose and stachyose content and the percent loss under different treatments are summarized in Table 1. Stachyose content was higher than that of raffinose in raw red kidney beans which was supported by Salunkhe *et al.* (1989) who studied the oligosaccharide compositions of raw red kidney bean and found that raffinose and stachyose contents were 0.93% and 2.44%,

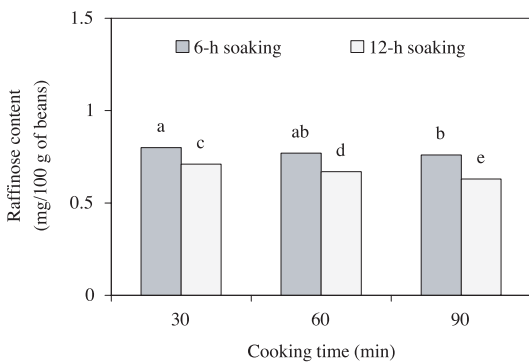


Figure 3 Raffinose content (g/100 g of dry beans) of red kidney bean flour after soaking and steaming. abcde Means with different letters are significantly different ($P\leq 0.05$)

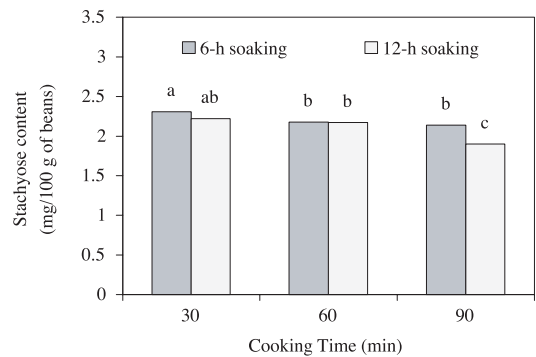


Figure 4 Stachyose content (g/100 g of dry beans) of red kidney bean flour after soaking and steaming. abc Means with different letters are significantly different ($P\leq 0.05$)

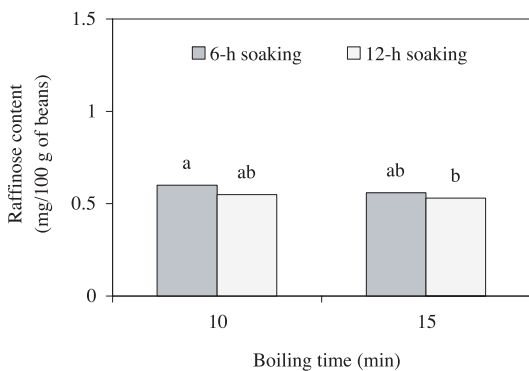


Figure 5 Raffinose content (g/100 g of dry beans) of red kidney bean flour after soaking and boiling in pressure cooker. ab Means with different letters are significantly different ($P\leq 0.05$)

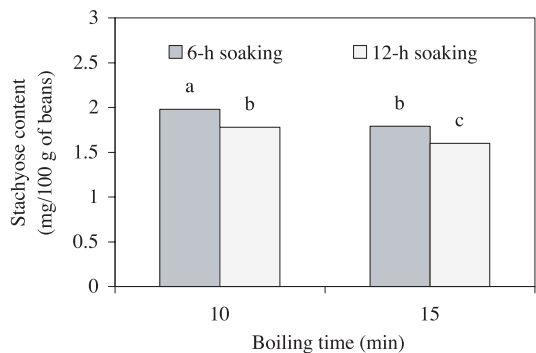


Figure 6 Stachyose content (g/100 g of dry beans) of red kidney bean flour after soaking and boiling in pressure cooker. abc Means with different letters are significantly different ($P< 0.05$)

Table 1 Raffinose and stachyose content (g/100 g of dry beans) in red kidney beans and flour subjected to various treatments.

Treatments	Raffinose	Stachyose
1. Raw red kidney beans	1.00±0.03	2.87±0.19
2. 6-h soaking and boiling in pressure cooker for 10 min	0.60±0.02 (-40) ^a	1.98±0.00 (-30)
3. 6-h soaking and boiling in pressure cooker for 15 min	0.56±0.00 (-44)	1.79±0.02 (-37)
4. 12-h soaking and boiling in pressure cooker for 10 min	0.55±0.00 (-45)	1.78±0.01 (-38)
5. 12-h soaking and boiling in pressure cooker for 15 min	0.53±0.03 (-47)	1.60±0.00 (-44)
6. 6-h soaking and steaming for 30 min	0.80±0.01 (-20)	2.31±0.03 (-20)
7. 6-h soaking and steaming for 60 min	0.77±0.00 (-23)	2.18±0.02 (-24)
8. 6-h soaking and steaming for 90 min	0.76±0.01 (-24)	2.14±0.00 (-25)
9. 12-h soaking and steaming for 30 min	0.71±0.02 (-29)	2.22±0.01 (-23)
10. 12-h soaking and steaming for 60 min	0.67±0.02 (-33)	2.17±0.03 (-24)
11. 12-h soaking and steaming for 90 min	0.63±0.01 (-37)	1.90±0.09 (-34)

^aValues in parentheses represent variation (%) in reference compared to raw beans.

respectively. Soaking and cooking reduced raffinose and stachyose in all treatments. For steaming process, the highest reduction of raffinose (37%) and stachyose (34%) were conducted by soaking for 12 h followed by steaming for 90 min. For boiling in pressure cooker, soaking for 12 h followed by cooking for 15 min was the highest decreased in raffinose and stachyose by 47% and 44%, respectively. Salunkhe *et al.* (1989) also reported that Great Northern, kidney, and pinto beans which were soaked in either distilled water or a mixed salt solution for 18 h at 22°C reduced raffinose oligosaccharides within the range of 32.77–51.02%.

The combination of soaking and cooking reduced raffinose oligosaccharides which causes flatulence. The reduction was caused by a loss of sugar to water during diffusion (Barampama and Simard, 1994); therefore, the soaking water should be discarded prior to cooking. However, the nutritional quality such as some vitamins and minerals may also be lost in the soaking water along with flatulence-causing oligosaccharides (Deshpande *et al.*, 1989). In addition, prolonged cooking time may even reduce the nutritive quality

of beans; however, quick-cooking beans such as pressure cooking seems to offer not only a decrease in cooking time and fuel requirement, but also appear to have as good nutritional qualities as the standard cooked beans (Salunkha *et al.*, 1989).

CONCLUSION

Soaking and cooking is an appropriate combination processing to improve nutritional quality of red kidney bean flour by reducing the amounts of flatus-producing oligosaccharides. The red kidney bean flour that was processed by soaking raw red kidney beans for 12 h and boiling in pressure cooker for 15 min could decrease the raffinose and stachyose contents by 47% and 44%, respectively.

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