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Effect of Soaking Solution Concentration on Resistant Starch and Oligosaccharide Content of Adzuki (*V. angularis*), Fava (*V. Faba*), Lima (*P. lunatus*) and Mung Bean (*V. radiata L.*)

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Abstract: Legume starches are typically more resistant to digestion than various other starches, rendering them low glycemic index foods, and potential substrates for beneficial colonic fermentation. The presence of oligosaccharides however reduces acceptability of various legumes. This study aimed to quantify resistant starch levels in some commonly consumed legumes - adzuki, fava, lima and mung bean - and assess the effect of varying soaking solution concentration on resistant starch and oligosaccharides. Legume samples were treated for twelve hours with either 1 % or 5 % citric acid or sodium bicarbonate solutions. A control sample was soaked in distilled water. These were autoclaved at 120 oC and freeze dried. Samples were analyzed for resistant starch by the procedure of McCleary and Monaghan 2002, and for oligosaccharides using a Megazyme® analysis kit. Adzuki bean had the lowest resistant starch content in the distilled water controls (2.94 g /100 g dry weight), while fava bean had the highest resistant starch content (5.15 g /100 g dry weight). Treatment with 5 % bicarbonate solution, decreased resistant starch in all legumes, while soaking in 5 % citric acid solution resulted in increases in resistant starch. Soaking in 5 % citric acid or sodium bicarbonate resulted in decrease in total starch, except for lima bean. There were minimal changes in oligosaccharide levels. Concentration of pre-process soaking solution therefore has considerable effect on resistant starch formation and retention, and a less noticeable effect on oligosaccharide levels.

Key words: Resistant starch, oligosaccharides, adzuki bean, fava bean, lima bean, mung bean, legumes, autoclaving, soaking solution

Introduction

Legumes are consumed as a major source of protein and energy in most parts of the world. Lima beans provide about 345 kilocalories per 100 g of dried seed. Some legumes contain up to 56.2 % starch (Lintas & Capelloni, 1992). Legumes such as mung bean (*Vigna radiate*) and adzuki bean (*Vigna angularis*) are major components of Oriental diets and are processed for use in multiple foods as pastes and fillings (Su *et al.*, 1997). Fava beans are commonly consumed in the Mediterranean regions as a staple.

Resistant starch - starch not digested in the gut - is fermented in the colon, producing short chain fatty acids that promote colonic health, particularly with regard to colon cancer protection (Muir *et al.*, 1993; Topping & Clifton, 2001). Resistant starch types include physically entrapped starch, ungelatinized starches, retrograded high amylose starch or chemically modified starches (Englyst *et al.*, 1992; Skrabanja & Kreft, 1998, Topping & Clifton, 2001). Legume starches are less digestible than some cereal starches and hence have lower glycemic indices (Foster-Powell & Brand Miller, 1995), They are therefore advantageous in protection and mitigation of incidence of diet-related diseases such as diabetes and obesity. The presence of various anti-nutritional factors such as phenolics and phytic acid, as well as the long cooking times required for legume preparation necessitates the use of pre-processing techniques. Procedures like autoclaving, fermentation, germination and roasting have been applied to reduce phytate in mung bean and other legumes (Chitra *et al.*, 1996). Steam is used in dehulling legumes (Tovar & Melito, 1996). Autoclaving and soaking are most effective in reducing anti-nutrient levels in lima beans (Ologhobo *et al.*, 1993; Adeparusi, 2001). Traditionally, beans such as *Phaseolus* varieties are soaked, then cooked for 1 - 2 hours (Cheung & Chau, 1998). Soaking in bicarbonate solution has been proposed by various researchers as a legume pre-treatment to facilitate reduction in anti-nutrient and phenolic levels (Bravo *et al.*, 1998; el-Adawy *et al.*, 2000; Siddhuraju and Becker, 2001).

Some of these procedures, however, have an effect on nutrient levels as well. Inadequate cooking - undercooking or overcooking- could result in leaching and loss of starch and other nutrients, which in turn would affect product quality. Adzuki paste quality for instance, is influenced by cooking time as well as bean physical and chemical characteristics (Baik *et al.*, 1998). Pre-processing possibly results in alteration of the physical and chemical properties of the plant cell wall polysaccharides, as has been reported in peas (Periago *et al.*, 1996). Legume starches are generally high in amylose, which is susceptible to retrogradation with processing, decreasing susceptibility to enzyme hydrolysis and increasing conversion to resistant starch (Tovar *et al.*, 1991). Processes that augment and retain resistant starch in legumes will increase their utilization as sources of minimally digestible, physiologically beneficial carbohydrates.

The objective of this study was to quantify resistant starch in various commonly consumed legumes and assess the effect of varying concentrations of pre-process soaking solution on resistant starch and oligosaccharides. Total starch was quantified to evaluate any concomitant changes with processing. These insights will be useful in developing optimal pre-process conditions that enhance resistant starch levels in legumes, while improving their acceptability by depleting levels of oligosaccharides. This would in turn increase their utility and application as high value foods.

Materials and Methods

Sample preparation: Samples were prepared by an adaptation of soaking procedures described by Bravo *et al.* (1998), and Siddhuraju & Becker (2001).

Four legume varieties- adzuki bean (*Vigna angularis*), fava bean (*Vicia faba*), lima bean (*Phaseolus lunatus*) and mung bean (*Vigna radiate L.*) - were soaked for twelve hours in either 5 % citric acid, 1 % citric acid, 1 % sodium bicarbonate solution, or 5 % sodium bicarbonate solution. A control sample was soaked in distilled water. Bean to solution ratio was 1:3 (w/v). The soak solutions were drained and the beans dried with a paper towel. These were then transferred to Teflon tubes and autoclaved in 1:3 (w/v) ratio of distilled water for 60 minutes at 120 oC. Autoclave solution was drained and the beans freeze-dried at - 40 oC for 72 hours. The samples were pulverized prior to analysis.

Determination of Oligosaccharide content: Oligosaccharide (raffinose-series) levels were determined using an analytical test kit from Megazyme International Ireland Ltd. Sample material (500 mg) was weighed out into glass tubes, treated with 95 % ethanol, and refluxed at 88 oC for 5 minutes. The mixture was transferred to volumetric flasks, made up to 50 ml with sodium acetate buffer (pH 4.5), and allowed to extract over 15 minutes. Aliquots (5 ml) of the slurry were treated with chloroform and centrifuged for 10 minutes at 1,000 g. Aliquots (0.2 ml) of the supernatant were treated with either invertase (Megazyme International Ireland Ltd) or μ-galactosidase (Megazyme International Ireland Ltd) and invertase, and then incubated for 20 minutes at 50°C. Glucose oxidase peroxidase reagent (Megazyme International Ireland Ltd.) was added and the tubes, along with a reagent blank and glucose standards, were incubated for another 20 minutes at 50 oC. Absorbance of all tubes was read at 510 nm and oligosaccharide content computed by a reference formula according to the kit instructions.

Determination of Resistant Starch and Total Starch: These were determined by the procedure developed by McCleary and Monaghan, (2002). Sample material (100 mg) was weighed out into screw cap tubes and sodium maleate buffer (pH = 6.0) containing pancreatic amylase (Megazyme International Ireland Ltd) and amyloglucosidase (3 U/mL, Megazyme International Ireland Ltd) added. Samples were thoroughly mixed and incubated in a shaking water bath for 16 hours at 37 OC. The tubes were then treated with 50 % ethanol, and centrifuged for 10 minutes at 1,000 g. The supernatants were decanted, the pellets re-suspended in 50 % ethanol, and centrifuged. The process was repeated and the supernatant collected.

Determination of Resistant starch: The pellets from centrifugation were treated with potassium hydroxide (2 M) in an ice bath with stirring for 20 minutes. Sodium acetate buffer (pH = 3.8) followed by amyloglucosidase (3300 U/mL, Megazyme International Ireland Ltd) were added and the tubes incubated for 30 minutes at 50 OC. Aliquots (0.1 mL) were removed and treated with glucose oxidase peroxidase reagent (Megazyme International Ireland Ltd.). These were incubated at 50 OC for 20 minutes and the absorbance read at 510 nm, against a reagent blank. Glucose solution (1 mg/ mL) was used as a standard.

Determination of Solubilized starch: The supernatants were made up to volume in 100 mL volumetric flasks. Aliquots (0.1 mL) were treated with 3 mL glucose oxidase peroxidase reagent (Megazyme International Ireland Ltd.), incubated for 20 minutes at 50 OC and the absorbance read at 510 nm against a reagent blank.

Total starch was calculated as the sum of solubilized starch and resistant starch.

Data Analysis: Duplicate samples were analyzed. Absorbance data was converted by a reference formula from McCleary & Monaghan (2002), into g/100 g dry weight for resistant starch and solubilized starch. Results were expressed as a mean ± standard deviation.

Results and Discussion

Oligosaccharide Content of Legumes Soaked in Solutions of Varying Concentration: There were minimal differences in oligosaccharide levels among the various legumes (Table 1). With the exception of mung bean, concentration of the soaking solution did not appear to greatly affect oligosaccharide levels, as there seemed to be no consistent trends between concentration of soaking solution and legume oligosaccharide content. Soaking of raw legumes is recommended to reduce oligosaccharides and the attendant physiological effects of flatulence with legume consumption. The elevated heat and moisture of autoclaving would possibly contribute to some solubilization of oligosaccharides, but there are no discernible differences with changes in concentration of the soaking solution.

Resistant starch Content of Legumes Soaked in Solutions of Varying Concentration: There were varietal differences in resistant starch levels among the various legumes (Table 2). Adzuki bean soaked in distilled water (control) had the lowest resistant starch content (2.94 ± 0.2 g/ 100g), while fava bean had the highest content of resistant starch (5.15 ± 0.0). These differences could be due to varietal variations in starch structure, legume seed coat and amylose: amylopectin ratios which influence resistant starch levels in processed foods. Starch susceptibility to digestion is greatly affected by the source of the starch, and its composition. Mung bean starch is reported to have an amylose content of 34.5 g/100 g, while adzuki bean starch has an amylose content of 27.9 g/100 g (Su *et al.*, 1997). This significant difference could therefore possibly account for the corresponding difference in resistant starch content in the autoclaved legumes pre-soaked in distilled water.

These inherent differences would in turn have an effect on the development of resistant starch, which has been described as retrograded amylose (Bravo *et al.*, 1998). Steam processing, a common pre-treatment in most legumes, results in increases in resistant starch content. This is possibly as a result of retrograded starch, commonly found in processed foods. Bravo and colleagues (1998) and Siddhuraju and Becker (2001) have also demonstrated a modification in starch susceptibility to digestion with processing in legumes. Multi-cycle autoclaving of starchy products facilitates retrogradation, decreasing starch digestibility and increasing resistant starch content (Skrabanja & Kreft, 1998; Escarpa *et al.*, 1996). Overall however, legume starches do have higher amylose levels than cereal starches, which with processing and subsequent retrogradation result in the formation of resistant starch (Cheung & Chau, 1998). The chemical composition and granular structure of legume starches render them more predisposed to restricted swelling and solubility, and they therefore have a higher tendency toward retrogradation and syneresis (Doublier, 1987, Eliasson, 1988, Bogracheva *et al.*, 1998).

Treatment with solutions of varying concentrations resulted in changes in the levels of resistant starch (Table 1). Soaking in a high concentration (5 %) of sodium bicarbonate solution resulted in decreased levels of resistant starch in all four of the legume varieties, while treatment with 5 % citric acid, resulted in the highest levels of resistant starch. This corresponds to work by Siddhuraju and Becker (2001) demonstrating improved starch digestibility (decrease in resistant starch) in the legume *Mucuna pruriens* with pre-soaking in bicarbonate followed by autoclaving. Increasing the concentration of sodium bicarbonate soaking solution therefore would seem to correspond to decreases in resistant starch levels in legumes. It would appear that the high alkaline concentrations facilitate solubilization of resistant starch, and hence increases starch digestibility.

Total starch Content of Legumes Soaked in Solutions of Varying Concentration: Total starch levels in legumes decreased with treatment (Table 3). Total starch content in distilled water controls were generally comparable to values reported in the literature, particularly for adzuki and mung bean (Lintas & Capelloni, 1992; Su *et al.*, 1997). Even though there were no discernible trends in changes in total starch content of adzuki, fava and mung bean, treatment with 5 % citric acid resulted in minimal changes in total starch content compared to treatment with 5 % sodium bicarbonate. This corresponds to studies by Tovar and Melito (1996) which indicate that total starch content does not change considerably in black beans and lima beans cooked for 90 minutes, but results in increases in resistant starch. The decrease in total starch content has been suggested to be as a possible result of transglycosidation reactions (Tovar & Melito, 1996). Total carbohydrates are also reduced by soaking bean seeds in 0.5 % sodium bicarbonate solution (el-Adawy *et al.*, 2000).

Total starch content of lima beans seems to increase with soaking in citric acid or sodium bicarbonate solutions. This is similar to reports by Barampama & Simard (1995) indicating that starch content of common beans is increased by soaking. These would probably be relative increases, reflecting possible leaching of other nutrients into soaking solutions, and

Table 1: Oligosaccharide levels in various autoclaved legumessoaked in solutions of varying concentration

Been variety	Soaking Solution				
	Control (Distilled water) mg/100g dry weight	5% citrate mg/100g dry weight	1% citrate mg/100g dry weight	1% bicarbonate mg/100g dry weight	5% bicarbonate mg/100g dry weight
Adzuki bean	0.7±0.01	0.6±0.00	0.5±0.001	0.5±0.03	0.7±0.05
Fava bean	0.7±0.09	0.9±0.01	1.0±0.1	0.4±0.09	0.7±0.03
Lima bean	0.5±0.04	0.4±0.04	0.6±0.005	0.3±0.09	0.4±0.02
Mung bean	0.7±0.1	0.2±0.00	0.2±0.02	0.2±0.00	0.2±0.03

Means of two samples. Data expressed as mean ± standard deviation

Table 2: Resistant starch levels in various autoclaved legumes soaked in solutions of varying concentrations

Been variety	Soaking Solution				
	Control (Distilled water) g/100g dry weight	5% citrate g/100g dry weight	1% citrate g/100g dry weight	1% bicarbonate g/100g dry weight	5% bicarbonate g/100g dry weight
Adzuki bean	2.94±0.2	7.82±3.2	3.19±0.1	3.58±0.6	2.45±0.1
Fava bean	5.15±0.0	5.16±0.3	4.63±0.7	4.70±0.2	3.65±0.1
Lima bean	3.71±0.3	4.19±0.2	4.44±0.4	3.75±0.3	3.31±0.1
Mung bean	3.52±0.2	8.40±1.2	3.95±0.1	4.16±0.0	3.05±0.2

Means of two samples. Data expressed as mean ±standard deviation.

Table 3: Total starch content of various autoclaved legumes soaked in solutions of varying concentrations

Been variety	Soaking Solution				
	Control (Distilled water) g/100g dry weight	5% citrate g/100g dry weight	1% citrate g/100g dry weight	1% bicarbonate g/100g dry weight	5% bicarbonate g/100g dry weight
Adzuki bean	41.5	34.3	44.6	47.8	39.7
Fava bean	42.1	37.6	42.3	40.0	38.0
Lima bean	30.3	34.9	38.7	33.2	34.7
Mung bean	41.2	29.5	40.2	39.6	42.3

Means of two samples. Data expressed as mean ± standard deviation

modifications in legume structural components to make starch more accessible and hence more quantifiable. Electron microscopy indicates that legume starches are embedded in a protective, protein matrix (Marconi *et al.*, 2000). Soaking and the ensuing heat processing degrade and break down this intact cell wall structure even though the overall regular form of the bean is generally maintained.

Resistant starch levels in autoclaved legumes are influenced to a great extent by concentration of the pre-autoclave soaking solution. High concentrations of sodium bicarbonate solution enhance starch digestibility, reducing resistant starch formation, while higher levels of citric acid result in considerable increases in resistant starch. Total starch levels, while corresponding to reported values in the literature, are not influenced by the concentration of the soaking solution, with the exception of lima bean. Altering concentration of the soaking solution does not result in any note-worthy changes in oligosaccharide content for most of the legumes assessed.

Concentration of the pre-process soaking solution is therefore a relevant and pertinent consideration in developing and establishing processes that improve and optimize the utilization of legumes and increase their potential for application as high value foods.

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