Chapter 5
(Part – I)

Effects of Processing on the Antinutritional Substances and Soluble Sugars of Mature seeds of *Parkia timoriana* (DC.) Merr

5.1a Introduction

The importance of legumes in nutrition seems to have some drawbacks like limitation of protein quality by a few amino acids and low starch digestibility (Negi *et al.*, 2001), poor mineral bioavailability (Kamchan *et al.*, 2004) and possession of high amounts of antinutritional factors (ANFs). Antinutrients have been defined as substances which by themselves or through their metabolic products arising in living systems, interfere with food utilization and affect the health and production of animals (Rasha *et al.*, 2011).

Legumes are usually cooked before being consumed as human diet. This improves the protein quality by destruction or inactivation of the heat labile antinutritional factors. However, cooking causes considerable losses in soluble solids, especially vitamins and minerals. Increasing the time and temperature of processing has been reported to reduce the nutritive value and available lysine of legumes (Saleh *et al.*, 2006).

Removal of undesirable components are essential to improve the nutritional quality of legumes and effectively utilize their full potential as human food. It is widely accepted that simple and inexpensive processing techniques are some effective methods of achieving desirable changes in the composition of seeds. Different authors have reported that soaking, cooking and germination improve the quality of legumes because of the removal of some antinutritional factors.
In many instances, usage of only one method may not effect the desired removal of antinutritional compounds and a combination of two or more methods are required (Vidal-Valverde et al., 1994). Soaking could be one of the processes to remove soluble antinutritional factors which had been eliminated with the discarded soaking solutions although some metabolic reactions were taken place during soaking (Vidal-Valverde et al., 1992). Honavar and Sohonie (1955) found that soaking prior to heat treatment was an essential step to eliminate the deleterious effects of *Phaseolus vulgaris*.

Most of the antinutrients are present in the seed coat, so dehulling improves the protein quality or the nutritional quality by reducing the antinutrients content. Soaking in salt solution reduces the cooking time and improves cooking quality.

Some authors opine that roasting reduce the levels of phytic acid and saponins in legumes and trypsin inhibitors in soybeans. Moreover, roasting also develops attractive flavours in the food. It was reported that dry heating inactivated trypsin inhibitor upto an extent of 40 to 50 percent (Lakra and Sehgal, 2005).

Cooking generally inactivates heat sensitive factors such as trypsin and chymotrypsin inhibitors. The cooking water may be discarded eliminating soluble compounds. Bressani and Elias (1980) observed that about 30 – 40% of polyphenols can be removed from *Phaseolus vulgaris* by cooking and discarding the cooking water solution. Trypsin inhibitor activity (TIA) in dry beans was appreciably destroyed by high temperature (Antunes and Sagarbieri, 1980) although resistance of trypsin inhibitors to heat has also been reported (Elias et al., 1979). Germination has been documented to be an effective treatment to remove antinutritional factors
in legumes, mobilizing secondary metabolic compounds which are thought to function as reserve nutrients e.g. phytates and raffinose and other oligosaccharides (Vidal-Valverde et al., 1994). Germination was also an effective means of processing to reduce levels of α-galactosides in faba beans (Vidal-Valvarde et al., 1998).

Among various treatments autoclaving was found to be more effective in reducing various antinutritional compounds of legumes without affecting the nutritional quality (Doss et al., 2011).

Soluble sugars present in the legumes may be affected by different processing methods. It was reported that cooking, autoclaving and germination increased the levels of total reducing sugars in chick pea and black gram whereas pressure cooking imparted increase in the levels of non-reducing sugars in green gram (Kakati et al., 2010).

From the above narration and that of chapter 1, it can be ascertained that various processing treatments are effective in reducing the levels of antinutritional substances of legumes. However, certain antinutritional substances can have increased levels during processing of legumes. However, same processing treatment does not produce same change in the seeds of different legume species and hence the mode of change is species dependent and even to variety too (Inaomacha, 2007). Thus, the effects of the adopted processing treatments on the antinutritional substances of *P. timoriana* mature seeds are envisaged in this part of study.

### 5.2a Materials and Methods

The samples prepared for analyzing processing effects on nutritional substances (chapter 4) were further stored inside dessicators and subjected to the determinations of antinutritional substances, reducing, non reducing and soluble sugars (as described in chapter 2).
5.3a Results and Discussion

Effects of different processing treatments on the antinutritional composition in mature seeds of *Parkia timoriana* are shown in tables 5.1 and 5.2.

Tannins

The tannins content of mature seed was observed to be 460mg/100g, a value noted to be lower to those of common legume seeds viz. 0.92% for *Pisum sativum* (Nikolopoulou *et al*., 2007); 1.7% for Phaseolus vulgaris and 1.4% for *Cajanus cajan* (Sangronis and Machado, 2007).

Tannins content became 350.14 mg/100g upon decortication of mature seed which contained 460mg tannins/100g (Table 2.3). This shows that tannins content is higher in the seed coat than in the cotyledon (P < 0.05). Similar finding of higher tannins possession in the seed coat was also reported by Ravindran and Ravindran (1988) and Josephine and Janardhan (1992) for Mucuna beans but with only traces of the compounds in the cotyledons. Predominance of the tannins in the seed coat of legumes was also reported by Reddy *et al*., (1984).

With the tannins value of decorticated seed, statistically comparable values of these compounds were noted for samples subjected to CAS + D + B + Dr, TS + D + B + Dr, JS + D, JS + D + B + Dr, GS + D, GS + D + B + Dr and CoS + D + B + Dr. The values left by these treatments were significantly lower from the control value and moreover statistically they are in parity. Bakr (1996) observed that dehulling generally reduced condensed tannins in faba bean and kidney bean. Moreover, literature records that dehulling of pulses viz. pigeon pea, chicken pea, black gram
and green gram resulted in 83–97% loss in tannin (Kakati et al., 2010). In the present case loss was found to be only 23.88%.

Treatment with CoS + D and G + D + B + Dr were noted to certainly retain the control level of tannins. Results of treatments viz. WS + D, WS + D + B + Dr, WS + D + PC + Dr, TS + D, CAS + D, SBS + D and SBS + D + B + Dr were uncertain as to producing values statistically comparable to that of control or decorticated seed.

Literature cited that tannins are water soluble (Reddy et al., 1984) and consequently leach into the liquid medium during soaking. The decrease in boiling may be due to their heat labile nature (Rakie et al., 2007) and thus degrade upon heat treatment. This finding was in conformity with that of contribution of Rehman and Shah (2005) who stated that tannins content of black gram, red kidney bean and white kidney bean were significantly reduced after ordinary cooking and pressure cooking at 121ºC for 20 minutes.

From the literature it could be viewed that factors that caused tannins reduction were heat degradation and solubilisation in water. It was ascertained that germination resulted in decrease of tannins of chick pea seeds (Frias et al., 2000). But what recorded in the present study was that tannins level raised above the level of control during treatments WS + D + F, DA + D, R + D, CS + D, CS + D + B + Dr and G + D against the expectation from the above reasons. Among these treatments, DA + D effected higher elevation of tannins level (574.95%). However, Sigauke et al. (2013) reported that tannins content decreased after roasting and boiling in Bauhinia petersiana seeds (a wild legume).
Nuzhat et al. (2008) observed that during the treatments of legumes by soaking in sodium chloride, acetic acid and sodium bicarbonate solutions and then by boiling, maximum reduction of tannins was caused by sodium bicarbonate soaking followed by boiling. This also showed that different treatments reduced tannins at different extents.

The condition of insignificant variation of tannins content of citric acid soaked + decorticated sample and sodium bicarbonate soaked + decorticated sample with that of decorticated sample deciphered incapability of tannins lowering due to citric acid soaking and sodium bicarbonate soaking.

For *P. timoriana* mature seed tannins content increased to 201.11% due to germinative treatment of six days and decortication. However, some authors have reported that in most legumes tannins content decreased after germination. In the present case it was found out that germination for 6 days increases the levels of tannins. Vidal-Valverde et al. (1994) also reported that in lentils tannins content increased after six days of germination. But due to attributes such as heat inactivation or solubilisation in water or both, the treatments G + D + B + Dr caused significant reduction of tannins from values given by G + D, but this level was of course in parity with the control value. D’Souza (2013) reported that germination of pre-soaked field beans showed the highest increase of tannins which might be due to solubilization of insoluble tannins that are brought to the surface.

It could be opined that because of above reasons coriander soaked + decoated + boiled + drained sample contained significantly lower amount of tannins than coriander soaked + decoated sample. On the other hand B + Dr followed after CAS + D, SBS + D, TS + D, JS + D, GS + D exerted no significant reduction of tannins as expected.
In lima beans tannins content decreased with the increase of cooking time (Egbe and Akinyele, 1990). Thus, there is need for furtherance of investigation upon processing change of tannins of *P. timoriana* mature seed by increasing cooking time. The study pointed out that irrespective of the soaking carried out in water, sodium bicarbonate and citric acid solutions, spice suspensions at room temperature, proceeding of treatments with D+B + Dr gave no discrimination of tannins level.

**Phytate**

Mature seed of *P. timoriana* was found to contain 851.58 mg/100g phytate (Table 5.1). Deshpande *et al.* (1982) reported that phytic acid contents of legumes vary from 0.40- 2.00 g/100g depending upon the variety and most of it is present in the outer aleurone layers.

About phytate it is observed that decortication does not change significantly phytate content of mature seed (Table 5.1). However, Bakr (1996) reported that decortication led to a significant increase in phytic acid content in faba bean. Mugendi *et al.* (2010) also observed that decorticated mucuna seed contained more phytate. Sashikala *et al.* (2013) also observed that accumulation of phytic phosphorus was in the cotyledons of green gram.

Table 5.1 displays that phytate levels of C and D, those recorded after treatments such as WS + D, WS + D + B + Dr, WS + D + PC + Dr, R + D, G + D, JS + D, GS + D, GS + D + B + Dr and CoS + D do not vary significantly at 5% level. In case of treatments done as TS + D, TS + D + B + Dr, JS + D + B + Dr, DA + D and CoS + D + B + Dr enhancing of phytic acid level was recorded in the range of 18.52–58.71%.
However, due to treatments such as WS + D + F, CS + D, CS + D + B + Dr, G + D + B + Dr, CAS + D, CAS + D + B + Dr, CS + D + B + Dr, SBS + D and SBS + D + B + Dr phytate levels have been significantly reduced below the control value. Higher reduction (79.58%) was ensued due to WS + D + F and it was followed with the impact of SBS + D, SBS + D + B + Dr, CAS + D + B + Dr and CAS + D which gave matching values of phytate. Igbedioh et al. (1994) mentioned that boiling of soaked pigeon pea seeds caused a lowering of the phytic acid content. Tinsely et al. (1985) reported that due to boiling phytate content of tepary beans, faba bean, cowpea and chickpea had been reduced by 38%, 41%, 53.7% and 16% respectively. Kakati et al. (2010) reported that pressure cooking caused 35.2% decrease of phytic acid content compared to the control (unprocessed value). Phytic acid in the seeds of Bauhinia petersiana seeds (a wild legume) decreased after roasting and boiling (Sigauke et al., 2013). The observed reduction in phytic acid content of leguminous seeds during heat treatments may be partially due to the heat labile nature of phytic acid and the formation of insoluble complexes between phytate and other components (Udensi et al., 2007). In faba bean also dry heating brought about a large reduction in the phytic acid content which may be due to insoluble phytins formed between phytic acid and some minerals (Crean and Haisman, 1963). Further, these workers found that during cooking of dried peas phytic acid combined with calcium and magnesium in the seeds to form an insoluble calcium and magnesium phytate. Leaching out of the antinutrients into the soaking medium under the influence of concentration gradient which governs the rate of diffusion might also contribute to the reduction of phytate. The decrease of phytic acid content of germinated legumes have been reported earlier by other
workers also (Ibrahim et al., 2002; Ghavidel and Prakash, 2007 and Khattak et al., 2007). The reduction could be due to increase in endogenous phytase activity as opined by Khattak et al. (2007) and Shimelis and Rakshit (2007) depending on types of legumes. During germination Kakati et al. (2010) also reported decrease in phytate content from the control value of green gram and black gram. The present study envisages that germinative treatment causes no significant reduction of phytate. As observed by Vidal-Valverde et al. (1998) pre-soaking the faba bean in citric acid before cooking led to 35% reduction of phytic acid. Phytic acid seems to be soluble in an acidic medium and cooking led to a large reduction in its content (Ford et al., 1978). These workers further found that in soybeans largest reduction of phytic acid content took place during soaking at pH 5.5. Table 5.1 displays that significant reduction of phytate takes place during citric acid soaking. D’Souza (2013) observed that all methods of traditional processing of field beans resulted in lowering of the phytic acid content, the most significant reduction seen upon germination.

The reduction caused by B + Dr subsequent to G + D might be due to reasons cited above. But B + Dr followed to WS + D, CAS + D, TS + D, CS + D, GS + D, SBS + D did not exhibit significant change of phytate relative to the values produced by these soaking + decoating treatments as noted similarly for PC + Dr followed to WS + D. But B + Dr done after CoS + D elevated significantly phytate level from the level left by latter treatment.

The cases of elevation of phytate level from the control level had been previously mentioned. Herein, it was pertinent to mention that boiling of faba bean followed by draining resulted to increase of phytate (Vidal-Valverde et al., 1998).
Thus, it could be inferred that the way of change of phytate depended upon the legume and otherwise on the processing treatment.

**Trypsin Inhibitors**

The effect of different treatments on trypsin inhibitor activity (TIA) of *P. timoriana* seeds is shown in Table 5.1. Unprocessed seed is found to contain 7.44 TI units/mg sample. Soni et al. (1978) and Hove and King (1979) indicated TI values of raw fababean seeds from 1.60 – 2.30 TI units/mg dry matter. For faba bean seeds, Valdebouze et al. (1980) found TI units ranging from 4.1 – 4.5/mg dry matter and Arntfield et al. (1985) reported values of 5.9 TI units/mg dry matter depending on the type and variety of the legume studied. The value increased to 9.93 TI units/mg after decortication of mature seed (Table 5.1) Regarding effect on TI activity due to treatments, only the values recorded with G + D and SBS + D were noted to be statistically comparable to the value of decorticated seed. But, in case of Dolichos germination, decrease of TIA by 19.3% was noted (Osman, 2007). It means that relative to TIA of decorticated seed all treatments except the above impacted affection on TI activity. Because of the treatments such as WS + D, DA + D, R + D, TS + D, TS + D + B + Dr, CS + D, GS + D, SBS + D + B + Dr, CAS + D, JS + D, TIA obtained had not been noted to significantly vary with the control value. Contrary to our finding, Sigauke et al. (2013) reported that treatment by roasting was effective in inactivating the trypsin inhibitors in *Bauhinia petersiana* seeds as they are heat labile and can be partially or completely denatured when exposed to elevated tempereature. Moreover, Mubarak (2005) noted decrease of TIA due to soaking and dehulling.
Table 5.1: Changes of antinutritional substances of mature seed following different processing methods.

<table>
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<tr>
<th>Treatments</th>
<th>Tannins (mg/100g)</th>
<th>R/I(%)</th>
<th>Phytate (mg/100g)</th>
<th>R/I(%)</th>
<th>Trypsin inhibitor activity (unit/mg sample)</th>
<th>R/I(%)</th>
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<td>_</td>
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<td>D</td>
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* Values with same superscript in the same column do not differ significantly (P > 0.05); R = Reduction, I = Increase; (+) Indicates increase in change; (-) Indicates decrease in change; Values are means of three replications ± SD. Abbreviations are same as given in table 4.1. Percent changes are calculated from mean values and relative to control value.

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Compared to control value treatments viz. WS + D + F, WS + D + PC + Dr, CS + D + B + Dr, WS + D + B + Dr, JS + D + B + Dr, CoS + D, CAS + D + B + Dr had given outcome as reduction with values of 19.62%, 29.3%, 30.11%, 34.01%, 35.89%, 39.92% and 42.47% respectively. However, treatments which impacted drastic affection as compared with the value of control were GS + D + B + Dr (53.63%), CoS + D + B + Dr (63.86%) and G + D + B + Dr (68.33%). Boiling of Bauhinia petersiana seeds decreased trypsin inhibitor activity (Sigauke et al., 2013). Rasha et al. (2011) reported that dehulling after soaking 24 hrs. caused loss in TIA by values of 22.3%, 42.3% and 47.4 % in soybean, mungbean and kidney bean respectively. Similar result as obtained in the study carried out by Mubarak (2005) as TI activity decrease upto 15.8% with soaking and dehulling of mungbean seeds was noted. In this contribution it was observed that such reduction determined relative to control value was exhibited by CoS + D only. Doss et al. (2011) also found that after soaking + dehulling + boiling TIA was decreased in Canavalia ensiformis seed.

Osman (2007) reported that in Dolichos lablab bean trypsin inhibitor activity was significantly reduced due to different treatment methods with cooking being the most effective. Soaking of the bean overnight reduced the trypsin inhibitor activity by 6.3% and cooking the soaked beans further reduced the TIA content by 66.7%. The report of Osman (2007) agreed with that of Marquiz and Alonso (2002) who reported a reduction of TIA during soaking and boiling of chickpea. Kadam et al. (1986) observed a significant decrease in TIA in winged bean after cooking of the pre-soaked bean. Cooking generally inactivates heat sensitive factors such as trypsin
inhibitors as a result of denaturation of heat labile proteins (Vidal-Valverde et al., 1994).

Table 5.1 reveals that all soaking treatments except in sodium bicarbonate produced significant reduction of TI activity in cotyledonous portion. It was then affirmed that B + Dr eventually reduced TIA significantly from values left by different soaking treatments and decortication. In cotyledonous portion the calculated reduction values produced from JS, CAS, TS, GS, CS, WS, CoS were respectively 18.53%, 20.74%, 25.07%, 26.89%, 27.79%, 32.73% and 54.98%. During boiling and draining practised after SBS + D, TS + D, WS + D, CS + D, CoS + D, JS + D, CAS + D and GS + D, TI activity had been reduced by values of 23.60%, 26.21%, 26.50%, 27.48%, 39.82%, 41.04%, 45.62% and 52.47% respectively. Marquiz and Alonso (2002) also reported that in chickpea TIA after soaking the seeds in deionized water, citric acid and sodium bicarbonate solution were lowered but did not completely remove the activity irrespective of the pH of the soaking solution. The trypsin inhibitor activity was effectively reduced on boiling the seeds in water. However, El-Adawy et al. (2000) noted maximum reduction of TIA while soaking in 0.5% sodium bicarbonate for soybean, mungbean and lupin seeds. Iyer (1980) observed that no modification of TIA level was found in citric acid soaking as acid hardened the seed coat during soaking thereby less diffusion of chemical compounds like TIA to the soaking liquid took place. Same result was produced in the present study.

Osman (2007) reported that roasting and autoclaving Dolichos lablab bean significantly reduced TIA by 23.05% and 12.09% respectively. Ramakrishna et al. (2006) also reported 45% reduction of TIA due to
roasting of dry Indian bean seeds. Though TI units/mg value of control seed did not differ significantly with the values obtained for DA + D and R + D, these latter values were of decorticated forms. From this it could be mentioned that accountable reduction of TI units/mg sample might have taken place in cotyledonous portion during autoclaving and roasting. Processing by germination for 6 days led to an increase in TIA to 16.26% relative to the control value. However, Siddhuraju and Becker (2001) reported a reduction of TIA level after 3 days germination in mucuna bean by 44% and attributed it to mobilisation and enzymatic degradation of proteins including protease inhibitors in seeds during germination. Germination is mainly a catabolic process as the reserved substances present in the cotyledon are used for the development and growth of the embryo. El-Adawy (2002) believed that germination is not an effective process to remove trypsin inhibitor and there are conflicting reports by many authors. Burbano et al. (1999) opined that there is possibility about the trypsin inhibitors for utilizing as an energy source during the early stage of germination but as germination time is prolonged TIA increased. Rasha et al. (2011) observed that in kidney bean, mung bean and soybean significant decrease in TIA was ensued by germination for 24 and 48 hrs but TIA value increased by 120 hrs germination in soybean and kidney bean. However, for mungbean no significant difference was observed for TIA value of raw mungbean and that measure after 12 hrs. germination.

Ramakrishna et al. (2006) found that the raw dry Indian bean had a very high trypsin inhibitor activity which progressively decreased to 51% during the 12 hrs. soaking period which decreased further to 17%
of the basal value of dry seeds at 32 hrs germination. But table 5.1 shows that 6 days germination resulted no accountable change of TI activity in the cotyledonous mass of seed. Upon treatment of such mass by B + Dr, reduction of TI activity by 72.83% was found out.

Alaa et al. (1982) reported that the inhibitory activity of TI of broad bean, chickpea, black eye pea, lentil and mung bean seeds decreased in most of them after soaking in water at room temperature for 24 hrs. and pressure cooking the soaked legume seeds at 121°C for 30 min. Increasing the time of boiling caused greater loss in trypsin inhibitor activity as noted by Egbe and Akinyele (1990) in cooking lima bean (Phaseolus lunatus L.) therein cooking for 60 min resulted in 100% loss in TIA. Similar contribution (Rasha et al., 2011) showed that mungbean, soybean and kidney bean produced 96.8%, 87.8% and 86.5% TIA losses respectively after boiling for 30 min. Devaraj and Manjunath (1995) found that D. lablab proteinase inhibitor activity was completely lost by 60 min cooking.

Reducing Sugars, Non-reducing Sugars and Total Soluble Sugars

The effect of different treatments on reducing sugars, non-reducing sugars and total soluble sugars of dry mature Parkia timoriana seed is narrated in table 5.2. The value of reducing sugar in unprocessed seed of P. timoriana was found to be 912.53 mg/100g. Undehulled seeds of African locust bean and melon seeds were found to contain 11.85 and 4.9% reducing sugars (Omafuvbe et al., 2004). In green gram and black gram reducing sugars content were 727.97mg/100g and 742.45 mg/100g respectively (Kakati et al., 2010). The reducing sugar content as observed by Chakraborty (1993) in the five cultivars of green gram ranged from 641.61mg/100g to 794.50mg/100g. Relative to control level, reduction of
reducing sugars was caused by TS + D (29.14%), R+D (39.47%) and WS+D+F (40.08%). The remaining treatments except G + D + B + Dr, CAS + D + B + Dr, TS + D + B + Dr, CS + D, CS + D + B + Dr and DA+D raised the level of reducing sugars with higher values given by JS + D (149.59%) followed by JS + D + B + Dr (78.37%), WS + D + B + Dr (65.34%) and G + D (57.80%). Kakati et al. (2010) noted that in black gram and green gram reducing sugar increased after soaking in water, pressure cooking and germination. Sood et al. (1988) observed that cooking, autoclaving and germination increased the levels of total reducing sugars in chick peas and black gram. Gloria et al. (2005) reported that in peas, germination caused a significant increase in glucose and fructose. Chavan et al. (1981) noted a ten fold rise of some reducing sugars of sorghum during sprouting. Vidal-Valverde et al. (1998) observed disappearance of certain reducing sugars and appearance of other reducing sugars during soaking treatment of faba bean. Contrary to our result reduction of reducing sugars (88%) was also recorded during soaking of faba bean in sodium bicarbonate solution (Vidal-Valverde et al., 1998).

Mature seed of P. timoriana was found to contain 6853.34mg/100g non-reducing sugars (Table 5.2). Inaomacha (2007) observed that in faba bean non-reducing sugar content was 5.8%. Majority of the treatments viz. D, WS + D, WS + D + B + Dr, WS + D + PC + Dr, WS + D + F, G + D + B + Dr, DA + D, R + D, CAS + D + B + Dr, TS + D + B + Dr, CS + D, CS + D + B + Dr, JS + D, JS + D + B + Dr, CoS + D, CoS + D + B + Dr and SBS + D imparted insignificant change of non-reducing sugars as compared with control value. From the control value only a few of the treatments elaborated non-reducing sugar level, the values being 30.99% (CAS + D), 32.51% (GS + D + B + Dr), 35.26% (TS +
D) and 63.05% (GS + D). Reduction of non-reducing sugars relative to control value had been ensued with the treatments such as SBS + D + B + Dr (24.79%) and G + D (65.37%). Inaomacha (2007) reported decrease of non-reducing sugars after soaking and pressure cooking in cotyledonous portion of faba bean but increased the level after boiling the soaked cotyledonous mass when cooking liquid was not discarded in both cases. Kanno et al. (1982) reported a partial loss of oligosaccharides (non-reducing sugars) in water soaking and steaming of soybean for natto production. Decrease in non-reducing sugars after water soaking + boiling and water soaking + pressure cooking might have association with the decrease of α-galactosides (Devindra et al., 2003). Vidal-Valverde et al. (1998) found that germination caused significant reduction of non-reducing sugars (α-galactosides) in faba beans. Data regarding the effect of processing on the α-galactosides content in some other legumes have been displayed and many authors agree that germination can be considered a bioprocess for reducing flatus potential although reductions vary depending on the germination procedure and also on the type of legume being used (Rao and Belavady, 1978; Sood et al., 1988; Kataria et al., 1989 and Vidal-Valverde et al., 1992). It can be mentioned that germination metabolises oligosaccharides and hence does not produce gas or flatulence. During envisaging with different soaking treatments, Vidal-Valverde et al. (1998) observed that citric acid soaking + boiling produced the maximum reduction (96%) of non-reducing sugars. Devindra et al. (2003) reported that soaking of red gram seed reduced α-galactosides content due to leaching into water. Moreover, boiling of faba bean not followed with draining of liquid was not accompanied with reduction of α-galactosides (Vidal-Valverde et al., 1998). However, present study revealed that none of the soaking treatments affected reduction of non-reducing sugars. Instead, as pointed out above, increase of non-reducing
Table 5.2: Changes of soluble sugars of mature seed following different processing methods

<table>
<thead>
<tr>
<th>Treatments</th>
<th>Total soluble sugars(mg/100g)</th>
<th>R/I(%)</th>
<th>Reducing sugars(mg/100g)</th>
<th>R/I(%)</th>
<th>Non-Reducing sugars(mg/100g)</th>
<th>R/I(%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>C</td>
<td>7765.87±219.66</td>
<td>_</td>
<td>912.53±12.40</td>
<td>_</td>
<td>6853.45±163.45</td>
<td>_</td>
</tr>
<tr>
<td>D</td>
<td>8052.00±187.93</td>
<td>+3.68</td>
<td>1154.91±40.06</td>
<td>+26.56</td>
<td>6897.09±41.76</td>
<td>+0.64</td>
</tr>
<tr>
<td>WS+D</td>
<td>8656.87±185.16</td>
<td>+11.47</td>
<td>1294.80±77.75</td>
<td>+41.89</td>
<td>7362.07±59.92</td>
<td>+7.42</td>
</tr>
<tr>
<td>WS+D+B+Dr</td>
<td>6900.00±34.86</td>
<td>-11.15</td>
<td>1508.80±95.61</td>
<td>+65.34</td>
<td>5391.20±57.09</td>
<td>-21.33</td>
</tr>
<tr>
<td>WS+D+PC+Dr</td>
<td>7680.00±158.64</td>
<td>-1.10</td>
<td>1365.33±78.20</td>
<td>+49.62</td>
<td>6314.66±224.98</td>
<td>-7.86</td>
</tr>
<tr>
<td>WS+D+F</td>
<td>7292.80±125.29</td>
<td>-6.09</td>
<td>546.75±36.45</td>
<td>-40.08</td>
<td>6746.05±153.71</td>
<td>-1.56</td>
</tr>
<tr>
<td>CS+D</td>
<td>9250.00±290.56</td>
<td>+19.11</td>
<td>1060.00±65.49</td>
<td>+16.16</td>
<td>8190.00±290.77</td>
<td>+19.50</td>
</tr>
<tr>
<td>CS+D+B+Dr</td>
<td>8084.00±99.20</td>
<td>+0.01</td>
<td>957.46±60.40</td>
<td>+4.92</td>
<td>7126.53±119.83</td>
<td>+3.99</td>
</tr>
<tr>
<td>G+D</td>
<td>3813.33±181.13</td>
<td>-50.89</td>
<td>1440.00±73.62</td>
<td>+57.80</td>
<td>2373.33±109.73</td>
<td>-65.37</td>
</tr>
<tr>
<td>G+D+B+Dr</td>
<td>6760.00±286.78</td>
<td>-12.95</td>
<td>849.33±65.43</td>
<td>-6.92</td>
<td>5910.66±274.40</td>
<td>-13.75</td>
</tr>
<tr>
<td>DA+D</td>
<td>7968.00±111.23</td>
<td>+2.60</td>
<td>975.05±142.96</td>
<td>+6.85</td>
<td>6992.90±74.52</td>
<td>+2.04</td>
</tr>
<tr>
<td>R+D</td>
<td>6186.66±141.96</td>
<td>-20.33</td>
<td>552.30±45.73</td>
<td>-39.47</td>
<td>5634.44±142.38</td>
<td>-17.78</td>
</tr>
<tr>
<td>TS+D</td>
<td>9916.31±503.76</td>
<td>+27.69</td>
<td>646.60±55.52</td>
<td>-29.14</td>
<td>9269.71±165.97</td>
<td>+35.26</td>
</tr>
<tr>
<td>TS+D+B+Dr</td>
<td>7663.33±112.33</td>
<td>-1.32</td>
<td>682.00±50.40</td>
<td>-25.26</td>
<td>6981.33±459.41</td>
<td>+1.87</td>
</tr>
<tr>
<td>JS+D</td>
<td>9152.00±1485.34</td>
<td>+17.85</td>
<td>2277.60±138.88</td>
<td>+149.59</td>
<td>7637.00±160.72</td>
<td>+11.43</td>
</tr>
<tr>
<td>JS+D+B+Dr</td>
<td>8213.33±123.98</td>
<td>+5.76</td>
<td>1627.73±84.90</td>
<td>+78.37</td>
<td>6585.60±55.52</td>
<td>-3.91</td>
</tr>
<tr>
<td>GS+D</td>
<td>1245.61±878.90</td>
<td>+60.39</td>
<td>1281.60±64.35</td>
<td>+40.44</td>
<td>11174.52±873.95</td>
<td>+63.05</td>
</tr>
<tr>
<td>GS+D+B+Dr</td>
<td>10470.60±1417.87</td>
<td>+34.82</td>
<td>1388.83±108.63</td>
<td>+52.19</td>
<td>9081.77±1512.82</td>
<td>+32.51</td>
</tr>
<tr>
<td>Co+S+D</td>
<td>8802.00±108.00</td>
<td>+13.34</td>
<td>1351.35±80.46</td>
<td>+48.08</td>
<td>7450.65±1105.20</td>
<td>+8.71</td>
</tr>
<tr>
<td>Co+S+D+B+Dr</td>
<td>8649.33±572.78</td>
<td>+11.38</td>
<td>1289.60±58.37</td>
<td>+41.32</td>
<td>7359.73±1628.83</td>
<td>+7.39</td>
</tr>
<tr>
<td>CAS+D</td>
<td>10146.50±1711.75</td>
<td>+30.65</td>
<td>1169.07±104.09</td>
<td>+28.11</td>
<td>8977.43±396.35</td>
<td>+30.99</td>
</tr>
<tr>
<td>CAS+D+B+Dr</td>
<td>7854.00±575.37</td>
<td>+1.13</td>
<td>1072.00±106.58</td>
<td>+17.47</td>
<td>6782.00±587.63</td>
<td>-1.04</td>
</tr>
<tr>
<td>SBS+D</td>
<td>8403.50±809.61</td>
<td>+8.21</td>
<td>1309.19±180.00</td>
<td>+43.47</td>
<td>7094.31±1827.02</td>
<td>+3.52</td>
</tr>
<tr>
<td>SBS+D+B+Dr</td>
<td>6421.27±274.08</td>
<td>-17.31</td>
<td>1267.20±69.51</td>
<td>+38.87</td>
<td>5154.07±252.93</td>
<td>-24.79</td>
</tr>
</tbody>
</table>

* Values with same superscript in the same column do not differ significantly (P > 0.05); R = Reduction, I = Increase; (+) Indicates increase in change; (-) Indicates decrease in change; Values are means of three replications ± SD. Abbreviations are same as in table 4.1. Percent changes are calculated from mean values and relative to control value.
sugars took place during CAS, TS and GS. On the other hand among the
treatments consisting of draining of cooking liquid, lowering of non-
reducing sugars was noticed only with SBS + D + B + Dr.

The value of total soluble sugar in unprocessed seed is 7765.87mg/100g (Table 5.2). Content of total soluble sugars of the treated sample
should be sum of the amounts of reducing and non-reducing sugars.
Thus, during treatment the former will have value according to available
values of latter two. Table 5.2 displays that decortication of mature seed
is not associated with significant change of total soluble sugars content.
Only the treatment G + D lowered the total soluble sugar content as
measured from the value of control. Treatments such as CAS + D, TS +
D, GS + D and GS + D + B + Dr elevated the content of total soluble
sugars from the control value. Zacharie and Sinard (1995) reported a
dercrease of soluble sugars content after soaking of common beans.
However, increase of soluble sugar contents was reported after soaking
of faba bean by Vidal-Valverde et al. (1998). Inaomacha (2007) also
observed a decrease in total soluble sugars of cotyledonous portion of
faba bean after soaking + pressure cooking and soaking + boiling. Frias
et al. (2000) studied about changes of total soluble sugars of chickpea
seed during different processing methods. They observed that soaking +
cooking brought about a larger decrease in available carbohydrate (23 –
24%) and dry heating also caused 24% reduction. A reduction of 50.89%
was observed during germination in the present study as the only case of
reduction. Vidal-Valverde et al. (1998) and Mubarak (2005) also reported
a significant reduction of total soluble sugars during germination which
might be due to simpler monosaccharide used as an energy source during
the process of germination.
Chapter 5  
(Part II)  

Effects of Processing on the Oligosaccharides of Mature Seed of  
*Parkia timoriana* (DC.) Merr  

5.1b Introduction  

From table 5.2 it can be accounted that non-reducing sugars constitute 88.23% of soluble sugars of mature seed of *P. timoriana*. Among the soluble sugars, oligosaccharides of the raffinose family are found in most legumes and account for 31 – 76% of the total sugars (Reddy *et al.*, 1984). $\alpha$-Galactosides, also called as raffinose family oligosaccharides (RFOS) belong to the low molecular weight, non-reducing sugars, soluble in water and are widely distributed in the plant kingdom. Raffinose (trimer) is a representative of this group. Apart from raffinose, this group also includes stachyose (tetramer), verbascose (pentamer), ajugose (hexamer) and unnamed longer chain oligosaccharides up to nanosaccharides (Cerning-Bernard and Filiatre-Verel, 1980). $\alpha$-Galactosides are considered as antinutrients since they are thought to be the major producers of flatulence due to the absence of $\alpha$–galactosidases in the human intestine; consequently undergoing bacterial fermentation. These oligosaccharides accumulate in the lower intestine and undergo anaerobic fermentation by bacteria with gas expulsion ($H_2$, $CO_2$ and $CH_4$), causing the flatus effect and sometimes diarrhoea and abdominal pain (Reddy and Salunkhe, 1980) and a factor which has tendency to render legumes less acceptable. Flatulence is also due to components present in the cell wall fibre. Although components
of cell wall fibre and starch are also possible flatulence causing agents, most workers take interest on envisaging the fate of $\alpha$-galactosides during different processing treatments. It has been found out that soaking in water significantly decrease the $\alpha$-galactoside content in lentils (Frias et al., 1995). In addition, it has displayed that cooking by either boiling or pressure cooking also decreases $\alpha$-galactosides content of legumes such as chick pea, lentils and kidney bean (Vidal-Valverde et al., 1993).

The present study is an attempt for studying the effects of a few common processing treatments such as decortication, water soaking + decortication + boiling + draining and water soaking + decortication + pressure cooking + draining on certain oligosaccharides (including $\alpha$-galactosides of $P.\ timoriana$ mature seeds.

5.2b Materials and Methods

Decorticated, water soaked + decorticated + boiled + drained and water soaked + decorticated + pressure cooked + drained samples of mature seeds of $P.\ timoriana$ were prepared as described in chapter 4. The mature seeds and processed seed samples were sun dried and ground upto 60 mesh size. They were stored inside desiccators for complete drying up and analysis of individual oligosaccharide contents afterwards. Each of these samples (0.5g) was blended in 80% ethanol (40ml) for 45 min at $57\pm 2^\circ$C and mixed with another 40 ml of 80% ethanol. The homogenate was centrifuged for 30min at 1500g and supernatant was collected and filtered through filter paper (Whatman 40). The extract was concentrated using a rotary vacuum evaporator at 50$^\circ$C by the removal of the ethanol. Then, these samples were air transported to Sophisticated
Analysis Instrument Facility (SAIF), Bose Institute, Kolkata under ice cold condition for analysis of individual levels of certain oligosaccharides. The volume of concentrate was made upto 15ml with distilled water. Standards of raffinose, stachyose, verbascose and sucrose were procured from Sigma Chemical Company. A standard solution of these oligosaccharides was prepared in 90% acetonitrile in water (v/v) containing 10μg of each of them per ml. The flow rate of the HPLC system was 10ml/min. At the beginning, the standard solutions and extracts were filtered through a 0.45μ nylon membrane filter. Then 500μl of each of the filtered extract and standard solution was taken in a small vial tube of 1.2ml capacity and placed into the instrument (Shimadzu-Prominence Series). Then, 30μl of each of the standard and extract was passed through amine C18 column (Phenomenax luna column). Analysis was done within 72 hrs after preparation of extracts. After passing through the column each of the standard and extract was passed through the refractive index detector and the retention time was given to the monitor of the computer. Then, using the relation between the concentration and the area in the HPLC chromogram, individual level of an oligosaccharide of a sample was produced. Triplicate results taken were statistically analysed for variation of means adopting ANOVA and Duncan Multiple test range (Stephen and Ruth, 2000).

5.3b Results and Discussion

It was found that among oligosaccharides of unprocessed P. timoriana mature seed so far known to occur, stachyose exhibited higher content (2.48%) followed by those of raffinose (1.72%), sucrose (0.51%) and verbascose (0.36%). The total content of the four
oligosaccharides was recorded to be 5.07% (Table 5.3). Muhammed et al., (2012) studied about oligosaccharides of different varieties of mung bean and produced the range of oligosaccharide contents to be 4.6 – 5.42 g/100g and verbascose was found to be most abundant followed by sucrose, stachyose and raffinose. Maria et al. (2006) reported that ciceritol and stachyose were the main α-galactosides for chick pea and lentil respectively. Khattab and Arntfield (2009) reported that stachyose was the predominant oligosaccharide followed by verbascose in both cowpea and kidney bean. This contribution found out that decoated seed of P. timoriana contained less raffinose (1.43%) and more of sucrose (0.91%) and stachyose (2.99%) than control. Kaur and Kawatra (2000) reported that dehulling caused a significant reduction in the raffinose and stachyose content in rice bean. Doss et al. (2011) observed regarding Canavalia ensiformis L.D.C seed that soaking, boiling and autoclaving caused reduction of raffinose by 58%, 66%. 75%, stachyose by 52%, 70%, 82% and verbascose by 45%, 69% and 80% respectively for each case. Table 5.3 is evidential that WS + D + B + Dr reduced significantly the contents of stachyose, sucrose, raffinose and verbascose by 17.34%, 23.53%, 29.65% and 63.89% respectively. Whereas WS + D + PC + Dr evented reduction of raffinose and verbascose by 33.14% and 66.67% respectively. It seems that WS + D + B + Dr can reduce content of the four oligosaccharides by 25.44 % while WS + D + PC + Dr does it by 17.95%. Sigauke et al. (2013) observed that raffinose content decrease to 9.45% after boiling Bauhinia petersiana seed. Onigbinde and Akinyele (1983) reported that in cowpea cooking decreased its total oligosaccharides by 48%. In agreement with this report Udensi et al. (2007) noted that oligosaccharides of cowpea could be reduced by 60.65% after boiling
Fig. 7. HPLC chromatogram for detection of oligosaccharides of seed
(Peak : 2 = Sucrose, 7 = Raffinose, 8 = Stachyose, 9 = Verbascose)
Table 5.3: Content of oligosaccharides of mature seeds following different processing treatments

<table>
<thead>
<tr>
<th>Treatments</th>
<th>Sucrose (g/100g)</th>
<th>R/I%</th>
<th>Raffinose (g/100g)</th>
<th>R/I%</th>
<th>Stachyose (g/100g)</th>
<th>R/I%</th>
<th>Verbascose (g/100g)</th>
<th>R/I%</th>
<th>Total (g/100g)</th>
<th>R/I%</th>
</tr>
</thead>
<tbody>
<tr>
<td>C</td>
<td>0.51±0.31</td>
<td>-</td>
<td>1.72±0.23</td>
<td>-</td>
<td>2.48±0.65</td>
<td>-</td>
<td>0.36±0.02</td>
<td>-</td>
<td>5.07</td>
<td>-</td>
</tr>
<tr>
<td>D</td>
<td>0.91±0.11</td>
<td>(+)</td>
<td>1.43±0.03</td>
<td>(-)</td>
<td>2.99±0.12</td>
<td>(+)</td>
<td>0.37±0.01</td>
<td>(+)</td>
<td>5.70</td>
<td>12.43</td>
</tr>
<tr>
<td>WS+ D+ B+Dr</td>
<td>0.39±0.14</td>
<td>(-)</td>
<td>1.21±0.24</td>
<td>(-)</td>
<td>2.05±0.04</td>
<td>(-)</td>
<td>0.13±0.01</td>
<td>(-)</td>
<td>3.78±0.21</td>
<td>25.44</td>
</tr>
<tr>
<td>WS+D+PC+Dr</td>
<td>0.47±0.07</td>
<td>(-)</td>
<td>1.15±0.05</td>
<td>(-)</td>
<td>2.42±0.43</td>
<td>(-)</td>
<td>0.12±0.03</td>
<td>(-)</td>
<td>4.16±0.24</td>
<td>17.95</td>
</tr>
</tbody>
</table>

* Values with same superscript in the same column do not differ significantly (P>0.05), R=Reduction ;I=Increase; (+) indicates increase in change; (-) indicates decrease in change; values are means of three replications+SD; Abbreviations are same as given in table 4.2A. Percent changes are calculated from mean values and relative to control values.
for 45 min. Vidal-Valverde et al. (1998) measured the effect on α-galactosides of faba bean as due to certain processing method as maximum reduction of verbascose followed by extents of reduction of stachyose and raffinose. It can be asserted that heat hydrolysis of the oligosaccharides lead to formation of simple di and mono saccharides from which other compounds can be formed (Onigbinde and Akinyele, 1983). In case of Czech grain pea, soaking partially reduced α-galactosides by enzyme cleavage and cooking did it by extraction only (Zatopkova et al. 2000). This reduction of α-galactosides during WS + D + B + Dr and WS + D + PC + Dr could be due to these reasons.

The study thus affirmed that WS + D + B + Dr could reduce raffinose, stachyose and verbascose contents of P. timoriana mature seed by 29.65%, 17.34% and 63.89% respectively while WS + D + PC + Dr, reduced verbascose by 66.67%. Thus it seemed that WS + D + B + Dr and WS + D + PC + Dr could reduce the total content of these α-galactosides. Vidal-Valverde et al. (1998) also reported that boiling of bean followed with draining of cooking liquid was associated with reduction of α-galactosides.

5.4 References of I & II


and anti-nutritional factors in faba beans as affected by processing. Z. Lebanon Unters Forsch., 207 : 140 – 145.
