Chapter V
General Discussion
5.1. Chemical composition, Phyto-chemical Screening and toxicity evaluation in *Parkia roxburghii* G. Don:

The determination of food composition is fundamental to theoretical and applied investigations in food science and technology. This is often the basis for establishing the nutritional value and overall acceptance of the food from the consumers' point of view (Wilson, 1979). In *Parkia* about 65% of the pod is edible and out of which 70% is represented by the pulp thereby indicating the advantage of using pods as vegetable (Figure 4.1.1 and 4.1.2).

5.1.1. Proximate composition: The chemical composition, otherwise known as the proximate composition, comprises protein, carbohydrate, ash, fat, crude fiber and moisture content of the food. For the legume seeds, the proximate composition is in the order carbohydrate > crude protein > crude fibre > moisture > ash > crude fat (Ifemeji (2004). However, in *P. roxburghii*, the order is moisture > carbohydrates > proteins > crude fibre > ash > crude fat in the tender pods and moisture > carbohydrates > proteins > crude fat > crude fibre > ash in the seeds. It showed the succulent nature of the *P. roxburghii* pods. Proximate composition of *P. roxburghii* pod in different growth stages is presented in Table 4.1.1.

5.1.1.1. Moisture: Water is often one of the major constituents in a food crop. It is held to other constituents by physical and chemical forces of diverse nature and strength. Water can be present in food crop in a variety of ways. These include, as a solvent or dispersion medium, as mono or polymolecular layer or in capillaries, held by molecular forces, or combined as water of hydration, either as true hydrate or held by hydrogen bonding within protein and carbohydrate molecules (Edeogu *et al.*, 2007). Generally it is determined to find out dry matter content of a particular food. The data in Table-4.1.1 showed the moisture percentage of *P. roxburghii* in different developmental stages of the pod. Moisture in the pod ranged from 73.23 to 81.93%, while it contained 59.35% in the seeds. The moisture per cent decreased with the age of the pods which was due to the fact that when they are tender, the pods are succulent and fleshy but as the pod advances, more cells are formed and its wall gets lignified.

5.1.1.2. Crude carbohydrates: Carbohydrate as a chemical component of a food crop occur as a group of chemical compounds formed by all green plants and are important to animals as food. They are composed of the elements, carbon, hydrogen, and oxygen. Carbohydrates are
essential for the nourishment of plants and animals which, is responsible for the growth of plants and animals by providing energy. Crude carbohydrate in *P. roxburghii* ranged from 59.26 to 67.82% in different stages of the pod while it contained 49.52% in the seeds. Increase in carbohydrate with maturity is also reported by Geervani and Devi (2006). Carbohydrate content in *P. roxburghii* is comparable with the reports of Horace and Ravo (1985) where they reported 63.7% in pigeon pea, 61.0% in chick pea, 61.9% in red bean, 61.3% in white bean, 64.0% in lima bean and 60.3% in mung bean. In other legumes, NAS (1983) reported 71.6% total carbohydrate in rice bean, while Apata and Ologhobo (1994) reported 66% in Bambara groundnut. Glycogen stores are also affected by the carbohydrate content of the food. Recent studies indicate that regular consumption of high-complex-carbohydrate with low fat diet during athlete training increases glycogen stores without the need to tricking the body with sudden dietary change (Probart *et al.*, 1993). Current recommendations for endurance athletes are to consume high carbohydrate with emphasis on complex carbohydrates during the training period (Conlee, 1987 & Probart *et al.*, 1993).

5.1.1.3. **Crude proteins:** Proteins are one of the chemical components of food crops and more complex than either carbohydrates or lipids in terms of both size (molecular weight) and variety of constituent units. It has diverse physiological and pharmacological roles in the biological systems: immunological protection, mechanical support, enzymatic catalysis, transport and storage functions, regulation of growth and differentiation of cells, hormonal action and source of fuel. In *P. roxburghii*, tender pods contain 17.51% protein while it contain 13.74 and 15.73% in the immature and mature pods (Table 4.1.1). In earlier reports, protein content in different stages of the *P. roxburghii* pod ranged from 13 to 19% (Longva and Deosthale, 1998). Similar findings in other legumes were also reported by Geervani and Devi (2006). Pods from beans of varying stages of maturity differ in protein content. Such variations in protein content with the developmental stages of the pod were also observed in *C. maritima* (Horace and Ravo, 1985). Protein content in *P. roxburghii* seeds was relatively high by containing 27.88% in the whole seeds, while it contains 39.7% and 48.9% in the cotyledons (without seed coat) under dry weight and dry and defatted weight basis, respectively. Protein content observed in the *P. roxburghii* whole seeds (27.88%) is almost in agreement with the earlier reports (29%) of Longva and Deosthale (1998). Protein content in other lesser known legumes are 29% in winged bean, 23% in mung bean and 19% in yam bean (Watson, 1977).
5.1.1.4. Crude fats: Fats are the best-known and simplest form of lipids. Like carbohydrate and proteins, lipids contain carbon, hydrogen and oxygen and some also have phosphorus, sulphur and nitrogen. Lipids are found widespread in nature and are insoluble in water but are highly soluble in non-polar solvents such as ether, chloroform etc. Fats and oils are sources of energy in the diet. They are the most concentrated form of energy in food (9 kcal, or 38 KJ per gram) yielding more than twice as much energy per gram as either carbohydrates or proteins. Data in Table 4.1.1 shows varying content of fat according to the maturity of the pods. It was lesser in the tender stage and increased with the age of the pods with its maximum found in the seeds. In *P. roxburghii*, fat content in the tender pods is 0.23%, while it contains 0.46% and 1.05% in the immature and matured pods. Increase in fat content with maturation was also reported by Geervani and Devi (2006). However, these reports are not in agreement with Longva and Deosthale (1998), who reported that fat content in different stages of *P. roxburghii* pods ranged from 1-16%. They also reported 34% fat content in the kernel (without seed coat), while in the present study it was 19.41% only in the seeds (whole). However, in this case the lower value may be due to inclusion of seed coats. Fat content in other legume seeds except groundnut, soybean and chickpea are less and generally ranges from 1.0 to 3.6 % depending upon the species (Takayama *et al.*, 1965). Legume fats have not been studied in detail because of their low lipid content and limited or little use as oil (Salunkhe *et al.*, 1982). Majority of the edible legumes contain fat in the range of 0.5 to 5.6 except in soybean and groundnut, where it contains 19.5 and 43.4%, respectively (Chatterjee and Bhattacharya, 1986). Among the lesser known legumes, winged bean reported to contain 17.7% fat (Watson 1977), while *Mucuna gigantea* contains 5.89% (Rajaram and Janardhan, 1991). *Parkia* seeds recorded high percentage of fat (19.41), which is fairly higher than most of the popular legumes so far studied, except groundnut.

Physico-chemical properties of the parkia oil is given at Table 4.1.2. The parkia seed oil is observed to have specific gravity of 0.9021 at 30° C. Its normality and acid value are observed to be 0.36N and 22.44. The high acid value is also confirmed by the presence of 25.6% free fatty acids. The saponification and iodine value of the oil are 182.33 and 21.6, respectively. The oil is thick and its specific gravity (0.9021) at 30° C has been very near to that of pure mustard oil (0.907). Acid value is very high (22.44) to compare with other edible oils like mustard (2.9). The occurrence of free fatty acids in the oils and fats is attributed to the action of air, moisture, light and enzymes. Freshly prepared animal oils and fats usually do not contain any free acid value, whereas vegetable oils from fresh seeds contain a small amount of it. Acid value is of importance in the selection of oils and fats for edible purposes.
and also in the case of lubricating oils. Acid value higher than 1.0 indicate high acidity (Iswaran, 1980). High acid value makes fats unpalatable. Presence of free acid also makes an oil unfit for lubricating purposes as they cause metal corrosion (Iswaran, 1980). *P. roxburghii* seed oil is very similar to the oils like groundnut except that it has high per cent of free fatty acids (25.6%) resulting in high acid value (22.44) and high normality of the oil (0.36N). Most of the edible oils and fats have saponification value in the range of about 190 (Iswaran, 1980). The saponification value of *P. roxburghii* is similar (182.33) to that. Iodine value of the fat or oil is the measure of nature and the extent of unsaturation of the fatty acids present in the oil. Iodine value in some of the oils like mustard, coconut, linseed and walnut are 104.2, 92.5, 200.0 and 142.0, respectively (Iswaran, 1980). Majority of the good quality edible oils like soybean, mustard groundnut, safflower, sesame and sunflower oil contains 86%, 92.4%, 82.0%, 89.0%, 86.0% and 88.0% unsaturated fatty acids, respectively (Swaminathan, 1987). Though parkia oil is poor in quality for edible purpose, the high oil content of the seeds should not be ignored. It may not be directly useful for soap or vanaspati ghee making (due to its pungency) or lubricating purposes (due to its high acid value) but similar oils like neem oil was reported to be used after proper treatments for lighting, heating and also as a lubricant for machinery. It is worthwhile to indicate that there are no data in the literature of the chemical and physical properties of *P. roxburghii* seed oil for comparison.

### 5.1.1.5. Crude fibre:

Fiber comprises those components of food crop that cannot be broken down by human digestive enzymes. The major types of fiber are cellulose, hemicellulose, lignin, pectin and gums (some hemicelluloses and storage polysaccharides). They help to lower serum cholesterol levels in humans (Jimoh and Oladiji, 2005). Cereals and legumes are the best sources of the water-soluble fibers. Since different types of fibers have different physiological roles, the fiber intake should come from a wide variety of fiber sources, including vegetables, fruits, legumes and cereals (Chaney, 2002). In *P. roxburghii*, Crude fibre in the pod ranged from 10.16% in the tender pod to 19.28% in the matured pod, while seed contained 9.03% of fibre (Table 4.1.1). Apata and Ologhobo (1994) reported fibre content in groundnut, kidney bean, lima bean, pigeon pea in the range of 3.2 to 5.4%, however, in jack bean, they reported it to be 9.5%. Among the lesser known legumes, *S. burseroides* contained 18.5% in the seed and 23.2% in the pod, while *A. bilimekii* contained 13.1 and 52.5%, respectively (Sotelo et al., 1999). The level of dietary fibre is generally high in lesser known legumes when compared with that of the most common legumes and seeds (Elegbede, 1998). Similar findings are also reported by Aletor and Aladetimi (1989). They observed that CF content of the under-utilized legumes has been generally higher than those
of the cowpea varieties. Although crude fibre enhances digestibility, the presence of high fibre levels in diet can cause intestinal irritation, lower digestibility and overall decreased nutrient utilization (Oyengu and Fetuga, 1975). Fibre content in *P. roxburghii* seed (9.03%) and pod (19.28%) is moderate, slightly higher than the common beans but much lower than the other legumes indicating that digestibility of associated nutrients in Parkia by monogastric animals is likely to be almost comparable with the other common legumes.

5.1.1.6. **Total ash:** The ash of an agricultural material is the inorganic residue remaining after the organic matter has been burnt off. It is also the inorganic residue left after the organic carbonaceous portion and other volatiles have been oxidized and evaporated. The importance of ash content is that it gives an idea of the amount of mineral elements present in the food sample. Total ash content in *Parkia roxburghii* (Table 4.1.1) seeds were to be in general agreement with those reported for other legumes (Platt, 1980). Ash content in *P. roxburghii* was higher in the matured pod (4.69%) compare to the tender (4.28%), immature pods (4.20%) and the seeds (4.10%). The ash content of 4.10% in *P. roxburghii* seed was comparable with that of other legumes which has been reported to range between 3.0 and 4.8% (Elegbede, 1998).

Detailed macro and micro element composition have been presented in table 4.1.3. Sodium (Na), calcium (Ca), potassium (K), and phosphorus (P) decreased as the pod matures and contained minimum in the seeds. Tender stage of the pod contained higher values of Na (200mg/100g), Ca (190mg/100g), K (2900mg/100g) and P (240mg/100g) than the other stages of the pod. Contrary to the above minerals, magnesium (Mg), iron (Fe), manganese (Mn) and copper (Cu) showed an increasing trend as the pod developed. It contained 32, 25, 13 and 0.9mg/100g in the tender stage of the pod, while in the mature pod it contained 47.1, 57.1, 25 and 2.7mg/100g, respectively. In all the minerals studied, *P. roxburghii* seeds were found to contain lesser values than the pods. Different legumes differed widely in their mineral composition. These findings were also in agreement with Apata and Ologhobo (1994). They found that potassium was the most abundant element ranging from 990mg/100g in jack bean to 1640mg/100g in lima bean followed by phosphorus (P) with values ranging from 240mg/100g in bambara groundnut to 460mg/100g in pigeon pea, while magnesium (Mg) was observed to be between 140mg/100g in jack bean to 220mg/100g in kidney bean. Sodium and calcium levels were generally low in all the legumes studied with mean values of 5mg/100g and 94mg/100g, respectively (Apata and Ologhobo, 1994). The amount of Ca, Fe, Cu, Zn and Mn in different strains of Bengal gram, black gram, green gram and lentil
varied from 109.6 to 281.9, 6.8 to 12.9, 0.6 to 1.9, 2.1 to 3.9 and 0.8 to 1.8 mg/100g material, respectively (Suchita, 1990). The very low amount of sodium in Parkia seeds (51mg/100g) may be good for health because of its relationship with hypertension in humans (Dahl, 1972). *P. roxburghii* is a good source of Ca, K, Cu and Zn by containing 170, 2780, 2.7mg/100g in the pods and 97.47, 2400, 2.3 and 2.77 mg/100g in the seeds, which are almost comparable to other legumes. Mg (34.7mg/100g) and P (160.0mg/100g) content in *Parkia roxburghii* seeds are comparatively low. Mg content in other legumes like rice bean, soybean and cowpeas are 272.2, 310.0 and 206.0 mg/100g, respectively (Suchita, 1990), whereas P content in chick pea, cowpea, green gram, horse gram, lentil and moth beans are 312, 414, 326, 311, 293 and 230 mg/100g (Gupta, 1982). Legume seeds are also an important source of dietary minerals, with the potential to provide all the 15 of the essential minerals required by man, although concentrations may vary in response to both genetic and environmental factors (Grusak, 2002). However, majority of the legumes were found to contain low concentrations of certain minerals especially Fe, Zn, and Ca relative to animal food products. Due to this, in regions where legumes are a significant component of the human diet, mineral deficiencies (especially Fe and Zn) can be quite prevalent (Jimoh and Oladiji, 2005). In spite of containing low values of Na, Mg, and P and comparable values of Ca, K, Cu and Zn, *P. roxburghii* is a good source of Fe and Mn by containing 57.1 and 25.0 mg/100g in the pod and 34.9 and 9.4 mg/100g in the seeds, respectively. The level of iron amongst all micro minerals analyzed was found to be the highest (57.1 mg/100g). This might be of nutritional importance especially in the part of the world where anaemia and iron deficiency is relatively rampant. Similar high content of Fe (78.27mg/100g) was also reported in *P. thonningii* (Jimoh and Oladiji, 2005). Iron, zinc and manganese are antioxidant micronutrients and their high presence in *P. roxburghii* could therefore boost the immune system (Talwar *et al.*, 1989). Potassium was the most abundant mineral in both the cowpea varieties and the under-utilized legumes with mean values of 1.45 and 1.66%, respectively, while P was the least abundant with 13.1 and 8.50 ppm, respectively (Aletor and Aladetimi, 1989). Calcium, magnesium, sodium and potassium contents of *Canavalia maritima* seed were lower than the pods, while with respect to phosphorus, the seeds contained them more. The minor elements, Cu, Fe and Mn were also lower in the seeds than the pods (Horace and Ravo, 1985). The present findings are in agreement with the above report except that in *P. roxburghii*, pods contained higher values in all the minerals studied.

**5.1.1.7. Dry matter and gross energy yield:** Dry matter and gross energy yield were shown in figure 4.1.3. Dry matter yield in the pod increased from 18.08% in the tender pod to
26.78% in the matured pod and 40.65% in the seeds. However, in case of gross energy yield, tender pods had higher energy yield (363.09kcals/100g) compare to other stages of the pod except seeds, which yield 444.55kcals/100g. Parkia seeds provided higher energy yield compare to other legumes like winged bean (400 kcals/100 g), the mung bean (310 kcals/100g) and the yam bean (327 kcals/100 g) (Watson, 1977). The calorific value of *P. roxburghii* was either comparable or had a margin over other lesser known legumes like *Mucuna gigantea* (374.91kcal/100g), *Mucuna monosperma* (408.19kcal/100g) and *Bauhinia racemosa* (407.64kcal/100g)(Rajaram and Janardhan, 1991; Arulmozhi and Janardhan, 1992; Mohan and Janardhan 1994).

5.1.1.8. **Acidity and ascorbic acid (Vitamin C) content:** Acidity and vitamin C content in *P. roxburghii* are illustrated at figure 4.1.4. Acidity was higher in the tender stage of the pod with 0.29%, which decreased with the development of the pod and was minimum in the seeds with 0.23%. The data on acidity showed a clear decreasing trend as the pod maturity advances. Such observations were also found in other fruits (Teotia *et al.*, 1978). It may be due to the reason that the organic acids are converted to sugars or ultimately to starch because it is the predominant form of storage food in plants. Likewise higher concentration of vitamin C (58.98mg/100g) was found in the tender stage of the pod which decreased to 45.11mg/100g in the immature stage and 49.65mg/100g in the matured stage of the pod while it further decreased to 26.42mg/100g in the seeds. Ascorbic acid in other legumes like lablab bean contains the same in the range of 11.67 to 14.30 mg/100g (Sharaf Uddin *et al.*, 2002). In Parkia, seeds contained lesser quantity of ascorbic acid (26.0mg/100g) than the pods thereby showing majority of the ascorbic acid concentrated in the pods. Though no consistent trend was observed in vitamin C content in the pods (Figure 4.1.4.), there was a marked increase in ascorbic acid with the advancement of maturity. Decrease in ascorbic content in the later stage (Seeds) might be due to enzymic loss of alpha-ascorbic acid through oxidation or less accumulation of the same in the seeds than in the pods (Sharaf Uddin *et al.*, 2002).

5.1.2. **Chemical composition in different parts of the pod:**

5.1.2.1. **Total sugar, free amino acids (FAA) starch and proteins:** Figure 4.1.5 presented total sugar, free amino acids, starch and protein content in different parts of the pod. Cotyledons contained 5.19 per cent soluble sugar which was higher than the pulp (3.73) and testa (0.87). Free amino acid (FAA) content in the cotyledon was 4.04 per cent, while it was 2.89 in the pulp and 3.08 per cent in the testa (seed coat). Pulp contained major portion of the
starch (14.36%) than the cotyledon (5.04%) and testa (3.5%). Total soluble sugar and total free amino acids are almost in agreement with other reports in kidney beans (Jasvinder et al., 1994), pigeon pea (Singh, et al., 1980) and chick pea (Singh, et al., 1981). P. roxburghii pods contained appreciable amounts of starch in the pulp. In humans, starch is normally consumed as part of cooked or processed food. Grain legumes are characterized by a relatively low glycaemic index (the blood Glc-raising potential) that is about one-half that of white bread. Foods with a low glycaemic index are considered to be beneficial in reducing postprandial blood glucose and insulin responses; therefore, it is especially useful to include legumes in the diet of people with insulin-dependent diabetes (type 2). Vegetarian diets that are high in grain legumes reduce the incidence of digestive tract cancers by reducing the consumption of saturated fats and increasing the content of unavailable carbohydrates in the diet (Aranda et al., 2001). Starch is the primary energy source in many animal diets, but legume starch generally provides less available energy, especially in monogastric animals, than do cereals because of its high amylose content (almost double) and the properties of the granules (Aranda et al., 2001). Starch content in other legumes is 66% in black gram (Raghuvansi, et al., 1993), 52.9 to 70% in different mung genotypes (Malewar, et al., 1990) and 41.63% in kidney beans (Mankolia and Modgil, 2004). Starch content in P. roxburghii was very less compared with the other legumes, which might be due to its high oil content. Starch content in other oil seed legumes like soybean ranged from 6 to 8% (aob.oxfordjournals.org/content/40/745/full.pdf.). Distribution of proteins in different parts of the P. roxburghii pod was shown in Figure 4.1.5. Cotyledon recorded 34.1% protein, while testa and pulp recorded 31.5 and 18.5% only. In Parkia roxburghii, proteins were more concentrated in the cotyledon than the testa and the pulp. Protein content in other lesser known legume pods are 3.5% in Styphonolobium burseroides, 8.6% in Acacia bilimekii (Sotelo et al., 1999) and 8.69% in Canavalia maritima (Horace and Ravo, 1985), while seeds contained 14.3%, 35.5% and 27.43% protein, respectively. Protein content in P. roxburghii pulp (18.5%) was higher than the above mentioned legumes, while the seeds contained almost comparable values.

Storage protein fractions of the seed play an important role in determining the nutritive value of seed proteins. Legume seeds characteristically contain storage proteins of the legumin and vicilin types. These are salt-soluble globulins of which the legumins are formed by two chains, linked by disulfide bonds, while vicilins are usually single-chain proteins without disulfide bonds. They aggregate to form trimers of subunits with varying molecular masses (Shewry et al., 1995). The nutritive value of pulses is determined by studying the protein fractions, since globulin is known to be resistant to digestion (Liener, 1980). Bajaj et al.,
(1971) were able to demonstrate the importance of water soluble proteins as indicators of protein quality as these contributed to superior nutritional value. Figure 4.1.6 showed the protein fractions in different parts of the seeds. Cotyledon contained 8.14% albumin, 13.05% globulin, 6.86% prolamn and 5.96% glutelin, whereas testa contained 0.73%, 9.47%, 1.15 and 2.38%, respectively. In the present study albumin and globulin were found to be the two major protein fractions which agreed with the earlier observations reported in black gram (Bera and Bera, 1990) and green Gram (Dana, 1991). In these pulses globulin fraction was the highest and prolamn was the least. Similar low prolamn content was also reported in Cajanus cajan (Pandey and Pant, 1978), rice bean (Suchita, 1990). In most of the popular legumes, albumin fraction is very less compared with the globulins. The average ratio of globulin to albumin in majority of the popular legumes is around 4 (Singh et al., 1985). However, in case of Parkia seeds the ratio was 1.60 thereby showing higher contents of albumins (8.14%) compared with globulin (13.05%), which was about 23.87% and 38.27% of the total recovered protein in the cotyledon (Figure 4.1.7). Such variable levels of protein fractions would be a useful indicator for their seed protein quality. It has been suggested that higher albumin would ensure improved nutritional quality in pulses due to its better amino acid composition as albumin contains 2 to 3 times the lysine and sulphur amino acids as compared to other protein fractions (Roy, et al., 2003). It was also reported that albumins have higher biological value than globulins as demonstrated from the microbiological assays (Bajaj, 1973). In this context, Parkia roxburghii proteins, because of their relatively high albumin are likely to provide better quality proteins. Of all the legumes, soybean is the richest in terms of protein content (43%), while others have protein content ranging from 20 - 25% (Apata and Ologhobo, 1994; Swaminathan and Jain, 1973) The high protein content in the cotyledons of P. roxburghii speciality (34.1%) coupled with the fact that it is abundant in this part of the world may encourage its use as high protein source in some food formulations.

5.1.2.2. Phenolics: Figure 4.1.8 showed the phenolics present in different parts of the pod. Pulp contained 1.65% total phenols (TP), 0.32% orthodihydric phenols (ODHP) and 0.3% bound phenols (BP), while cotyledon contained 0.37, 0.21 and 0.07%, respectively. TP, ODHP and BP content recorded in the testa were 0.59, 0.25 and 0.42%. Figure 4.1.9 showed the phenolic pigments in different parts of the pod where higher levels of anthocyanin (50.55mg/100g) were found to concentrate in the testa then the cotyledon (27.2mg/100g) and pulp (22.4mg/100g). Leuco-anthocyanin ranges from 0.45 to 3.5mg/100g. It contained 0.45mg/100g in the cotyledon and 0.55mg/100g in the testa, while maximum concentration was recorded in the pulp (3.5mg/100g). These phenols in different varieties of Glycine max
ranged from 1.98 to 5.89 in the seeds and 1.59 to 3.58 in the pulp (Brahma et al., 2005). Total phenols and orthodihydric phenols were higher in the pod pulp compare to the cotyledon and testa. However, in case of bound phenols, testa showed higher concentration of bound phenols than the pulp and the cotyledon. Presence of appreciable amounts of phenolics in the legumes imparts their partial resistance to pests and disease causing organisms (Mahadevan, 1982). Phenolics are also often found to be associated with antioxidants. Most of them showed biological activity as antibacterial, anti-carcinogenic, anti-inflammatory, anti-viral, anti-allergic, estrogenic and immune-stimulating effects (Larson, 1988). The antioxidant activity of phenolics is mainly due to their redox properties, which allow them to act as reducing agents, hydrogen donors, and singlet oxygen quenchers. In addition, they have a metal chelating potential (Akula and Udhav, 2008). Synthetic antioxidants such as butylated hydroxyanisole (BHA), butylated hydroxytoluene (BHT) and tertbutylhydroquinone (TBHQ) have been used widely as antioxidants in foods, but concerns over the safety of use have led towards interests in natural anti-oxidants (Wanasundara and Shahidi, 1998). These synthetic antioxidants are substituted phenolic compounds, and subsequently much of the research on natural anti-oxidants has also focused on phenolic compounds, in particular the flavonoids and hydroxycinnamic acids (Martínez-Valverde et al., 2002). Phenolic compounds also possess an array of potentially beneficial lipoxygenase inhibitory and anti-oxidant properties and reported to have been used for the treatment of inflammatory diseases (Sreejayan and Rao, 1996).

5.1.2.3. Minerals in different parts of the pod: Composition of different minerals in different parts of the pod was shown at table 4.1.4. Potassium was the most abundant mineral in different parts of the pod by containing 3200, 2850 and 2450mg/100g in the pulp, testa and cotyledon, respectively. Almost all the macro elements studied were found to be more concentrated in the pulp portion of the pod. Among the micro elements, pulp contained significantly higher amounts of Fe (47.93) and Mn (36.05mg/100g) than testa (27.47 & 20.74mg/100g) and cotyledon (22.02 & 12.04mg/100g) though pulp recorded lesser values of Zn (1.65) and Cu (1.47mg/100g). Cu was the only element found concentrated in the cotyledons (2.37mg/100g), while Zn was found to be concentrated in the testa (6.43mg/100g), which was significantly higher than the other parts of the pod. However, unlike other elements, no significant difference was observed in case of Mg in different parts of the pod. Distribution of elements in different amounts in different parts of the pod is also reported by Jai et al. (1996), Elegbede, (1998) and Horace and Ravo, (1985). Co was not detected in all the parts of the *P. roxburghii* pods studied. These observations were in
agreement with the reports that legumes are seldom found to have cobalt. In majority of the common legumes like lima bean, mung bean, navy bean, red kidney bean, white beans, yellow beans, broad beans and chick pea, Co was not detected. (http://www.aob.oxfordjournals.org/content/40/745/fijll.pdf).

5.1.3. Phyto-chemical screening of Parkia roxburghii:

The phytochemical screening of the seed (Table 4.1.5) showed presence of saponins, flavonoids, tannins while alkaloids and cyanogenic factors were absent. Some of these chemical compounds have been reported to have inhibitory effects on some gram-negative bacteria such as *Escherichia coli* and *Bacillus subtilis* amongst others. They also have prominent effects on animal systems and microbial cells (Liu *et al.*, 1990; Topcu *et al.*, 1993; Oyagade *et al.*, 1999). The presence of high concentration of these chemical compounds, therefore, suggests the pharmacological activities of *P. roxburghii* and this necessitates detailed investigation.

Flavonoids are a group of compounds widely distributed in the plant kingdom. Chemically, flavonoids contain 2 benzene rings with a 3-carbon bridge. The true flavonoids consist of the anthocyanins, anthozanthins, the catechins and the leuco-anthocyanins (Mahadevan, 1982). Flavonoids after acid hydrolysis give flavones. Flavones isolated from plants are large but number of flavones isolated from plant foods are relatively small (Seshadri, 1951). Among the natural compounds flavones form the major part of the nature's contribution to mankind and these are used for their therapeutic properties both in allopathic as well as in the ayurvedic systems of medicine (Mayer, 1987). All the plants contain some type of flavones or its derivatives. Out of thousands of plant flavones, rutin (vit P) is the one which is in clinical use both as 3-rutinoside as well as its aglycon (Quercetin). These two are used in combination with vitamin C to treat capillary bleeding due to increase capillary fragility. Apart from these uses, rutin and its aglycon are used as protectant against nuclear hazards (Sharma, 1982). Though, *Parkia* pods contain flavonoids in appreciable amounts as seen in Table 4.1.5, which is also evident from anthocyanin and Leuco-anthocyanin content (Figure 4.1.9), the use of *P. roxburghii* flavonoids for therapeutic purpose is not well established. However, there are reports of using *P. roxburghii* extracts for various medicinal purposes. The seeds as well as tender pods are known to cure stomach disorders and regulate liver functions (Sharma, *et al.*, 1993). The fruits are used in bleeding piles. The bark is prescribed in dysentery and diarrhoea by the Paite tribe and Meiteis. Pods pounded in water are used in washing the head and the face (Burkill, 1935; Quisumbing, 1951). Bark and leaves are used
in making lotions for skin diseases and ulcers. The fomentation of decoction of leaves to the rheumatic affected parts is beneficial (Bose, 1991). Another most widely distributed chemical compound among the plants are the flavonoid pigments, anthocyanins, flavonones and flavonols. Several leuco-derivatives of these are also present and are considered effective inhibitors of parasites (Taliyeva, 1954). Flavonoids play a fundamental role, being excreted by the plant in response to nodulation factors produced by the bacteria. Isoflavones, which are molecules mainly found in legumes, may have beneficial effects on human health, and their use can be of help in the fight against many diseases, including several types of cancer and cardiovascular disorders (Velazquez et al., 2010). Many of these alleged effects of flavonoids have been linked to their known functions as strong antioxidants, free radical scavenger and metal chelators (Torel et al., 1986; Nakayama et al., 1993). Even in very high amounts (for example, 140 grams per day), flavonoids do not appear to cause unwanted side effects. When raised to the level of 10% of total caloric intake, flavonoid supplementation has been shown to be non-toxic. Studies during pregnancy have also failed to show problems with high level intake of flavonoids (http://www.whfoods.com/genpage.php?tname=nutrient&dbid 119#impact cooking storage processing).

_P. roxburghii_ seeds also contained appreciable quantity of saponins and tannins (Table 4.1.5). The medicinal value of _P. roxburghii_ may also partly be due to the presence of saponins. Saponins are naturally occurring compounds that are widely distributed in all cells of legume plants. Saponins, which derive their name from their ability to form stable soap-like foams in aqueous solutions, constitute a complex and chemically diverse group of compounds. In chemical terms, saponins contain a carbohydrate moiety attached to a triterpenoid or steroids. Their aglycons are called sapogenins. Related to this group are the cardiac glycosides. Saponins are attracting considerable interest as a result of their diverse properties, both deleterious and beneficial (Shi et al., 2004,). Clinical studies have suggested that these health-promoting components, saponins, affect the immune system in ways that help to protect the human body against cancers, and also lower cholesterol levels. Saponins decrease blood lipids, lower cancer risks, and lower blood glucose response (Shi et al., 2004). A high saponin diet can be used in the inhibition of dental caries and platelet aggregation, in the treatment of hypercalciuria in humans, and as an antidote against acute lead poisoning. In epidemiological studies, saponins have been shown to have an inverse relationship with the incidence of renal stones (Shi et al., 2004,). These also bear a structural resemblance to the steroid saponins but they are distinguished from other steroid glycosides by an unsaturated lactone ring attached at C-17, a cis-juncture of rings C and D, a 14β-
hydroxyl group and by the particular sugars composing them. Saponins occur in a wide variety of food plants, bengal gram, soybean, navy beans, haricot beans and kidney beans being relatively rich (Fenwick and Oakenfull, 1983; Oakenfull, 1981; Varshney, 1969). The presence of saponins in potatoes when eaten in large quantity causes abdominal pain, vomiting and diarrhea. Saponins are also important in human diet as they reduce the risk of heart diseases (Potter et al., 1979). It has also been reported (Applebaum et al., 1969) that they induce resistance among legume seeds against insect attack.

Another important constituent found in higher quantity has been tannin (Table 4.1.5). Tannins are polymeric phenols and hydrolysis of some of the tannins yields seven carbon gallic acid; others give ellagic or phenolic acids. Condensed tannins are composed of catechins and phenols. Most of these exist in the form of glycosides which are less toxic compared to the aglycones. Evidently, the active group is the OH group and as long as a molecule contains these groups, it is biochemically active. This polar OH group will attach itself to water and the substituted benzene ring will have an affinity for the lipid material. Introduction of more hydrophilic groups (-COOH etc.) or more lipophilic (aromatic) groups will drastically affect the solubility thereby influencing the orientation of the molecule and consequently toxic action (Mahadevan, 1982). Tannin also complexes proteins, divalent metals, cellulose, hemicellulose, pectin and other carbohydrates (Mahanato, et al., 1982). High consumption of tannin is dangerous to health, being a phenolic secondary plant metabolite with one or more hydroxyl substitutes bonded to aromatic ring, it produces anthrocyanides, another toxic product on acid degradation (Gatachew et al., 2000; Waterman and Cole, 1994). Another danger of consumption of high concentration of tannins is that it is not normally extracted either with solvents or detergents thus tannin-protein complexes cannot easily be broken down or digested (Perez Maldonado, et al., 1996). Tannin toxicity for fungi, bacteria and yeasts is reviewed and compared to toxicity of related lower molecular weight phenols (Scalebert, 1991). The different mechanisms proposed so far to explain tannin antimicrobial activity include inhibition of extracellular microbial enzymes, deprivation of the substrates required for microbial growth or direct action on microbial metabolism through inhibition of oxidative phosphorylation (Scalebert, 1991).

5.1.4. Toxicity evaluation of P. roxburghii: Parkia extracts was evaluated for toxicity against cabbage aphids, Aphis craccivora Koch. The mean mortality percentages of aphids obtained with various concentrations of P. roxburghii under laboratory conditions has been presented in Table 4.1.6. For all the concentrations, the percentage mortality was
significantly higher than the two controls maintained. The percentage kill increased with increase in time and concentration, and vice versa. The highest kill was obtained with 2.0% concentration which reached 100% after 96 h of its application and differed significantly from the other concentrations, except from 1.5% concentration. The ether extracts of *Parkia roxburghii* seeds were highly poisonous to the aphids and as little as 2% of the crude extract could kill all the aphid population within two days. Joymati *et al.*, (2004) also reported the potentiality of dried parkia leaves and flowers for the control of root knot nematode, *Meloidogyne incognita* infesting faba bean.

Pulses have evolved a large array of anti-nutritional compounds to protect their seeds against insects (Janzen, 1976). Chickpea, *Cicer arietinum* (L.) (Fabaceae), have endogenous natural insecticides produced in the seed that are active against *C. maculates*, (Mouhouche and Fleurat-Lessard, 2004). There are several examples of legumes being a source of natural insecticides against other stored-product insects (Jouvensal *et al.*, 2003; Louis *et al.*, 2004; Taylor *et al.*, 2004). Toxicity of *P. roxburghii* to aphids is therefore not surprising. Presence of appreciable amounts of saponins and tannins in *P. roxburghii* (Table 4.1.5) may relate to its possible toxic effects on lower organisms. Saponins are found to be toxic to lower organisms like earthworms and fishes (Sharma, 1982). For optimum toxic activity, sapogenins require a polar group in ring A and a moderately polar group in ring D or E (Schlosser and Wulff, 1969). Compounds containing a 16-α-OH or 16-keto group together with a 3-β-OH are known to be highly active. Most steroid saponins inhibit microorganisms by a common mechanism involving the formation of a complex with cholesterol and cell membrane (Tschesche and Wulff, 1964). However, saponins differ in their capacity to complex which is thought to be due to the number of sugar molecules in the saponin. Saponins with 2-3 sugar molecules complex cholesterol in less effectively than saponins with 4 or 5 sugar molecules (Wolters 1968). Price *et al.*, (1987) observed an irreversible combination of saponins with membranes in animals and cells, thus rendering the membrane non semi-permeable. When given orally in high doses (300mg/kg body weight) to rats, saponin causes diarrhoea, restlessness and histopathological changes in liver and kidney, ultimately leading to death (Lalitha *et al.*, 1990). They are known to occur in many of the popular legumes but are eliminated by soaking (Banerjee, 1978).

Seeds, particularly those of the Leguminosae, are also rich sources of lectins. Insecticidal potential of certain bean varieties may be related to the presence of hydrophilic protein such as lectins that could prevent egg hatching and larval development of *Canavalia maculates* as
previously observed by Janzen et al., (1976), Gatehouse et al., (1995), Gatehouse and Gatehouse (1998), Okeola et al., (2002), and Boleti et al., (2007). These authors found that high rates of lectins in certain species of legume such as *Dolichos lablab* (L.) and *Rhycocea saucia* prevent the development of *C. maculatus*. Various plant lectins have shown entomotoxic effects when fed to insects from Coleoptera, Homoptera, and Lepidoptera orders. Some lectins are highly toxic when ingested by mammals inducing a variety of systemic effects. Among them are the lectins from the common bean (*Phaseolus vulgaris*), soybean (*Glycine max*), wheat germ (*Triticum vulgaris*) (Pusztai et al., 1986, 1993; Bardocz et al., 1995; Kordas et al., 2000) and jack bean (*Canavalia ensiformis*) (Udedibie and Carlini, 1998). Sales et al., (2000) and Carlini and Grossi-de-Sá (2002) have demonstrated feeding inhibition by legume seed vicilin compounds and arcelin that may be used for the defense of legumes against bruchid beetles. From a nutritional point of view, the legume lectins are part of the human diet. Surprisingly, these highly antinutritional compounds are resistant to proteolytic degradation during their transit through the human gut (Vasconcelos and Oliveira, 2004). Earlier work had also identified the lectins as biochemical factors in plant resistance to insects, mainly against coleopteran species. Another possible constituent for toxicity of *P. roxburghii* to the lower organisms may be the α-Amylases (Discussed ahead). They catalyze the initial hydrolyses of α-1,4-linked sugar polymers, such as starch and glycogen, into shorter oligosaccharides, an important step towards transforming sugar polymers into single units that can be assimilated by the organisms. These widely distributed molecules are the most important digestive enzymes of many insects that feed exclusively on seed products during larval and/or adult life. When the action of the amylases is inhibited, nutrition of the organism is impaired causing shortness in energy. α-Amylase inhibitors occur in many plants as part of the natural defense mechanism (Marshall and Lauda, 1975; Ishimoto et al., 1996).

5.2. Organoleptic taste and Effects of processing and cooking methods in *Parkia roxburghii* G. Don:

5.2.1. Organoleptic Taste: *Parkia roxburghii* is a widely used pod vegetable in Manipur. It is hardly known to be used in other parts of India, except the north eastern states. In Table 4.2.1., the different physical characters, pigments and flavour values are presented. Out of the thirteen cultivars studied (Photo Plate 3.9.1), 3 pods were green (1, 8 and 9), 5 pods light green (2, 3, 5, 10 and 11), 1 olive green (6), 1 dark green (12), 1 light yellow (4) and 2 yellow (7 and 13). Pod length and stalk length ranged from 25 to 53.3cm and 5 to 14.2 cm while pod weight and seeds per pod ranged from 42 to 119.3g and 10 to 19.3, respectively. Highest pod
length (53.3cm), seeds per pod (19.3) and stalk length (14.2cm) were recorded in cultivar 7, while the highest weight (119.3g) was recorded in cultivar 6. Significant differences were observed among different characters (Table 4.2.1). Carotinoid was the major pigment in *P. roxburghii* pods, which ranged from 10.1 to 75.8 mg/L. Total chlorophyll content ranged from 0.9 to 2.1 mg/L. Chlorophyll 'b' was the main component of chlorophyll in all the cultivars except PRB-6. These are the pigments which directly influenced the colour of a pod according to its composition. Closer the values of chlorophyll 'a' and chlorophyll 'b' along with lesser values of carotinoids, the pods become darker in colour and poorer in flavour (Table 4.2.1). Moderate chlorophyll content (1.1 to 1.35 mg/L) and moderate carotinoid content (33.9 to 42.2 mg/L) make the pod yellow and less in flavour. Higher chlorophyll 'a' (0.9mg/L), 'b' (1.2mg/L) and carotinoids (75.8mg/L) was found in cultivar 2 which was followed by cultivar 3 (0.8 and 1.2mg/L) and 10 (0.9 and 1.0mg/L) in case of chlorophyll 'a' and 'b', while cultivar 10 (60.9mg/L) and 3 (57.7mg/L) in case of carotinoids. In Table 4.2.2, the correlation matrix of 12 parameters has been presented. Though the characters like pod weight, pod breadth, pod thickness, stalk length, number of seeds/pod, pod length breadth ratio were not significantly correlated with flavour rather these characters had a negative tendency so far as flavour of the pods are concerned (Table 4.2.2). Flavour was positively correlated with the pigments, chlorophyll 'a', chlorophyll 'b' and carotinoids. Pod breadth, pod length and pod thickness were observed to be highly correlated with pod weight, while Stalk length and number of seeds were highly correlated with the pod length. In case of pigments, chlorophyll 'b' was highly positively correlated with chlorophyll 'a' while carotinoid was found to be positively correlated with the total chlorophyll content. In spite of the correlation existed among them, all the pigments were also observed to be positively correlated with flavour of the pods. Flavour values out of 5 point scale ranged from 1.7 to 4.4, the maximum point being recorded in cultivar 2. When the total chlorophyll contains around 2 mg/L, chlorophyll 'b' more than 1mg/L with fairly high values of carotinoids made pods light greenish in colour and imparts superior flavour than broad, heavy, thick with yellow or green to dark green cultivars. No attempt so far seemed to have been made to evaluate palatability of *P. roxburghii* on the basis of morphological characters. Meitei and Singh (1990) evaluated palatability on the basis of varieties and reported 'Thangmai' variety to have superior taste than the others. The word 'Thangmai' assigned to the name of a variety is very vague as it denotes the place of collection. In fact, *P. roxburghii* pods with varied morphological characters and distinguishable features are available at Thangmai. Moreover, in another observation, variety 'Pari' was reported to be the tastiest (Anon (1981a). However, the authors failed to give the salient features of the said variety.
Parkia roxburghii is one of the legumes which has varied colours and different physical characters of pods, may be due to genetic factors or due to environmental factors or both. Therefore, it has reasons to test any relationship between the physical appearance of a pod and its flavour. In the present work, the effect of pod colours on the palatability of P. roxburghii pods has been reported for the first time.

5.2.2. Processing and Cooking Effects: Though, large quantities of proteins and minerals are present in legumes, their availability sometimes affected by methods of preparation and processing. In the raw state legumes contain substances which are indigestible or antagonistic to digestion such as phenolics, saponins, glycosides, alkaloids, conjugates of proteins with phytin or hemicellulose and substances which inhibit the action of the digestive enzymes (Savelkoul et al., 1992). The effect of processing and cooking is discussed under the following headings:

5.2.2.1. Changes in proximate composition: The proximate composition of the Parkia seeds after processing and different cooking methods are given in Table-4.2.3. Moisture content was observed to have significantly increased (P<0.05) in both the ordinary and pressure cooked samples compared to the raw sample. Onyeike and Omubo-Dede, (2002) reported that autoclaving and cooking slightly increased the moisture level in yam bean. Boiling in water softened the cell tissues of the kernels, increased water-absorbing and water retention capacities of the seed due to the increased permeability of the cell membrane to water (Kingsley, 1995). Parkia seed also lose weight significantly due to both the processes may be due to leaching of water soluble and heat labile components and also through osmotic gradient. The ash content (Table-4.2.3) decreased significantly in both the cooking processes. Loss in ash is mainly due to leaching of the soluble inorganic salts into the processing water (Mbajunwa, 1995). Parkia seed contained higher amounts of crude protein (27.79%) which was significantly reduced by the different cooking methods. Loss of protein may partly be due to leaching of soluble proteins into the processing water especially albumins and globulins. Crude protein and ash contents in yam bean were also reported to have decreased by cooking and autoclaving (Onyeike and Omubo-Dede, 2002).The oil content of the raw Parkia seed is observed to be high (19.29%) compared with other legumes except ground nut. There is significant increase in the crude fat content of ordinary cooking and pressure cooking methods. Cooking may have enhanced the efficiency of oil extraction from the samples by the solvent (Achinewhu, 1983). The high oil content of these seed makes it a good source of vegetable oil for nutritional and industrial purposes. Raw Parkia seed also has
a high fibre content (9.08%), which was found to be not affected by both the cooking processes. The results are in agreement with the reports of Barampama and Simard (1995) in cooked beans. It may therefore be a good source of dietary fibre which may improve large bowel functioning and reduce plasma cholesterol. Raw *Parkia* seed showed a crude carbohydrate content of 39.74% (Table-4.2.3) which increased significantly (P<0.01) due to both the cooking processes. Similar findings have also been reported by Bhagya *et al.*, (2006) in wild legume, *Canavalia cathartica*.

**5.2.2.2. Changes in dry matter, total sugar and flavonoid content in the pods:** Figure 4.2.1 and 4.2.2 shows dry matter loss due to different processing and cooking methods in *P. roxburghii*. Dry matter loss in different stages of the pod ranged from 4.72% (tender) to 5.8% (mature) in ordinary cooking (OC), while the range being 5.76% (immature) to 8.23% (mature) in pressure cooking (PC). Loss of dry matter increased with the age of the pod. Dry matter loss in the whole seed ranged from 1.64% (OC) to 2.24% (PC), while dehulled and soaked seeds (DSS) ranged from 6.42% in ordinary cooking to 9.02% in pressure cooking (Figure 4.2.2). Dehulling increased dry matter loss during cooking. Figure 4.2.3 shows changes in total sugar content in different stages of the pod. Total sugar (%) in different stages of the pod decreased due to different cooking methods. The extent of loss from the original content in tender stage was 40.03% in ordinary and 62.86% in pressure cooking, while in mature pods it has been 74.81 and 78.36% (Figure 4.2.4). In the seeds, total sugar was reduced from 3.39% to 2.03% and 1.48% due to OC and PC in the whole seeds, while it reduced from 3.3% to 0.65% and 0.48% in the DSS (Figure 4.2.5). The extent of loss recorded was 40.12% in OC and 80.83% in PC in whole seeds, while DSS lost up to 2.65%, 56.24% and 85.84% due to soaking, ordinary cooking and pressure cooking, respectively (Figure 4.2.6). Decrease in soluble sugar in cooked beans is also reported by Barampama and Simard (1995). Cooking as well as autoclaving brought about slight decrease in glucose, fructose and sucrose levels and increased oligosaccharide content of all the varieties of lima bean (Ologhobo and Fetuga, 1988). Figure 4.2.7 showed changes in flavonoid content in different stages of the pod due to different cooking methods. Flavonoid content (%) in different stages of the pod decreased due to different cooking methods, the maximum loss being recorded in pressure cooking. The percentage removed in different stages (Figure 4.2.8) has been 93.18 and 98.48 in tender stage, 94.39 and 98.13 in immature and 82.11 and 88.42 in mature stage of the pod due to ordinary and pressure cooking methods, respectively. Flavonoid in the seeds was also affected by cooking. Flavonoid in the raw whole seed recorded 1.14%, which decreased to 0.18% and 0.15%, while DSS (0.65) decreased to 0.15%
and 0.08% in ordinary and pressure cooking (Figure 4.2.9). Dehulling and soaking removed flavonoids up to 42.98%. The percentage of removal from the original content in whole seed was 84.21 and 86.84, while in dehulled and soaked seeds it was 86.84 and 92.98, respectively (Figure 4.2.10). Heat, degree of acidity (pH), and degree of processing can have a dramatic impact on the flavonoid content of food. For example, in fresh cut spinach, boiling extracts 50% of the total flavonoid content. With onions (a less delicate food), boiling still removes about 30% of the flavonoids (http://www.whfoods.com/genpage.php?tname=nutrient&dbid=119#impactcookingstorage processing). Processing methods (soaking and roasting) also influenced total phenolic, flavonoid and antioxidant contents (DPPH, FRAP) in selected dry beans (Boateng et al., 2008). In comparison to the original raw beans, all processing methods caused significant decreases in total flavonoid content (TFC), DPPH free-radical scavenging activity, ferric-reducing antioxidant power (Xu and Chang, 2009a).

5.2.2.3. Effects on mineral composition in the pods: Table 4.2.4 and 4.2.5 indicated changes in the major and minor element composition in different stages of the pod. Ca, Mg, K and Zn in different stages affect significantly by different methods of cooking, while no such changes were observed in the case of S, P, Fe, Mn and Cu. Loss of minerals was not related with the age of the pod in almost all the elements tested, except in case of Ca and Zn. Though both cooking methods did not differ significantly, the degree of loss was often found to be more in case of ordinary cooking, which can be clearly seen in Figure 4.2.11. The extent of reduction of Ca, Mg and K being ranged from 26.88 to 37.27%, 25.51 to 37.57% and 3.95 to 13.04% in ordinary cooking, while it was 18.69 to 25.24%, 8.39 to 33.69% and 6.72 to 11.59% in pressure cooking. Similarly, Zn and Mn loss was up to 49.51% and 66.67% in different stages of the pod. However, contrary to the above, S and Fe has been recorded gain up to 6.58 and 12.33% in OC, while 5.38 and 20.54% in PC. Similarly, a slight gain in P (0.69%) was also observed in case of pressure cooking.

The mineral content of raw, soaked, ordinary cooked and pressure cooked seed samples of the Parkia roxburghii are presented in Table-4.2.6. Potassium (K) was the only element which has been lost significantly due to the cooking methods in both the two seed groups, whole seeds and soaked and dehulled seeds (DSS). It was the only element which was lost significantly due to soaking and dehulling, among the elements studied. As in the case of pods, S and P were also found not to be affected by the processing and different cooking methods. Parkia seeds contained 154.57 and 211.35 mg/100g phosphorus and calcium which was unaffected by cooking methods in the whole seed but reduced significantly (P<0.05) in
dehulled and soaked seeds in case of calcium. The low level of phosphorus in the raw seed was unaffected by processing and cooking methods. Mg content of the whole seed has been found to have reduced significantly (P<0.01) by ordinary cooking however, no such trend was observed in other processing and cooking methods. Figure 4.2.12 shows percentage loss/gain out of the total content in major elements due to processing and cooking methods. Ca in the whole seeds recorded a loss of 13.89% in ordinary cooking and 8.68% in pressure cooking, while dehulled and soaked seeds recorded 25.09 and 28.71%, respectively. Mg recorded loss up to 12.32% in OC in whole seeds while in DSS, it recorded gain of 0.13% in OC and 4.1% in PC. K suffered loss in both the seed types and cooking methods. S recorded gain up to 7.96% in whole seed, however, the gain was lesser (5.6%) in DSS seeds. A loss of 14.17% of P was recorded in DSS under OC but it registered 5.65% gains in pressure cooking. These results were in agreement with the various reports on legumes. Chitra et al., (1996) studied effect of processing treatments on various pulses and reported to have little effects on calcium, magnesium and iron contents. Similar findings were also reported by Kingsley, (1995). Raw Parkia seeds were a good source of Zn, Fe, Mn and Cu and contained 6.02, 39.53, 1.67 and 4.9mg/100g, respectively. Fe content reduced significantly (P<0.05) by pressure cooking method in the dehulled and soaked seeds, however, other processes did not affect any significant reduction. In case of Zn, ordinary cooking reduced significantly (P<0.01) then the pressure cooking both in the whole seed and dehulled and soaked seeds. Figure 4.2.13 shows per cent loss/gain in case of minor elements. Zn recorded loss of 22.92% in OC and 12.79% in PC in whole seeds, whereas, it recorded loss of 22.76% in OC and 9.47% in PC in dehulled and soaked seeds. Cu recorded gain in different methods in both the seed types. Mn gain in whole seed ranged from 13.77 to 23.95%, while in DSS, it recorded loss up to 38.32%. Apata and Ologhobo (1994) also reported all the cooked legumes having lower mineral concentrations compared to raw legumes. Meiners et al., (1976) had also observed a decrease in the mineral constituents of seeds after cooking and it is likely that the decrease was due to leaching of minerals on account of the enhanced permeability of the seed coat by the process of cooking. The Parkia seed was a fairly good source of dietary manganese (1.67mg/100g) and copper (4.9mg/100g), which was also not affected by processing and cooking methods rather it increased, may be due to leaching of water soluble ingredients, thereby allowing it to concentrate. Such observations were also reported by Kingsley, (1995). Cobalt was not detected in any of the tested seeds.

5.2.2.4. Anti-nutritionals: Cooking and soaking brought about a considerable decrease in the level of phenolic and other anti-nutritional compounds in P. roxburghii pods (Table
Total phenols in tender (61.42mg/g), immature (52.25mg/g) and mature raw pods (37.70mg/g) decreased to 16.72, 17.82 and 13.58mg/g in ordinary cooking and to 20.10, 18.71 and 15.72 mg/g in pressure cooking. The same trend was observed in orthodihydric phenols (ODHP) and tannins. ODHP in the raw tender (3.34mg/g), immature (2.27mg/g) and mature pods (1.08mg/g) decreased to 0.63, 0.54 and 0.52mg/g in ordinary cooking, while it decreased to 0.74, 0.35 and 0.52mg/g in pressure cooking. Likewise, tannin in tender (98.30mg/g), immature (86.47mg/g) and mature raw pods (56.27mg/g) reduced to 12.81, 14.31 and 6.64mg/g by ordinary cooking and to 16.69, 18.84 and 8.92mg/g by pressure cooking. Phytate phosphorus (PP) in different stages of the pod (mg/100g) ranged from 30.9 to 40.5mg/100g, which reduced to 25.2mg/100g (tender pod), 24.8mg/100g (immature pod) and 34.2mg/100g (mature pod) by ordinary cooking and to 20.7, 17.1 and 25.2mg/100g by pressure cooking. Percentage removal out of the original content is presented in figure 4.2.14. Total phenol was found to have removed up to 72.8%, 73.8% and 23.16% of the original content in tender, immature and mature stages of the pod, respectively (Figure 4.2.14A). Tannins were removed greater than the total phenols and it ranged from 91.56% in tender to 90.98% in immature and 87.76% in mature stage of the pod (Figure 4.2.14B). Loss of Phytate P was lesser compared with total phenols and tannins and accordingly its removal out of the total content was also lesser and ranged from 33.01% to 53.28% in different stages of the pod (Figure 4.2.14C).

Table 4.2.8 indicated changes in anti-nutritionals in the whole and dehulled seeds. TP, ODHP and Tannins were found to contain 11.76, 1.63 and 4.68mg/g in the raw seeds, while it decreased to 10.77, 0.58 and 2.54 mg/g in ordinary cooking and to 11.16, 1.14 and 3.39 mg/g in pressure cooking. The same trend was also observed in the case of dehulled and soaked seeds (DSS), though the degree of loss was more in the latter case. In both the cases, ordinary cooking had more effect in removing the anti-nutritionals. When pressure cooking removed 5.10% TP, 30.06% ODHP and 27.56% tannins in whole seeds and 5.95% TP, 59.51% ODHP and 42.52% tannins in dehulled seeds, ordinary cooking removed 8.42%, 64.42% and 45.73% in whole seeds and 13.18%, 56.44% and 53.42% in DSS, respectively (Table 4.2.8). In case of Phytate phosphorus (PP), loss of PP due to ordinary cooking and pressure cooking has been recorded to be 26.69% and 29.80% of the total content in the whole seeds and 40.20% and 35.80% in DSS seeds. Highest degree of loss was observed in ODHP when it was removed up to 64.42% by OC in whole seeds and 59.51% by PC in dehulled seeds. The mean values of all the anti-nutrients decreased due to processing and cooking methods as
compared to raw seeds. Different cooking methods have varied effects on reducing total phenolics, saponins, phytic acids, and individual phenolic compounds (Xu and Chang 2009).

Cooking had been reported to be effective in reducing tannin content of winged bean (Tan et al., 1984); soya bean (Bressani et al., 1982) common beans (Elias et al., 1979). Loss of tannins may be due to its solubility in water as tannins are known to be water soluble. Tannin reduction in Parkia seeds may improve its nutritional value by increasing protein digestibility. Consumption of high concentration of tannins is not desirable as it is not normally extracted either with solvents or detergents thereby forming tannin-protein complexes that cannot be easily broken down or digested (Perez Maldonado et al., 1996). Though presence of high amounts of tannin interferes with the digestive system (Savelkoul et al., 1992), presence of tannin in food sometimes gives body and fullness of flavour to the food. The greatest difference in composition between cider apples and culinary apples is in the tannin content (Mayer, 1987). Naturally high tannin content is desirable for slight astringency (Valier, 1951).

Cooking methods significantly (P<0.05) reduced phytate phosphorus in P. roxburghii. This observation agrees with the results of Ologhobo and Fetuga (1984) and Sutardi and Buckle (1985). The effects of four thermal processing methods on phyto-chemical profiles in green pea, yellow pea, chickpea, and lentil, were investigated. As compared to the original raw legumes, all the processing methods caused significant reduction in total phenolic content, procyanidin content, total saponin content and phytic acid content (Xu and Chang 2009). A decrease in the phytate content of the beans with use of soaking was observed. Soaking, autoclaving and toasting significantly (P < 0.05) reduced the levels of phytin, tannin and cyanide. Except for tannin, autoclaving for 20 min was found to eliminate all the other anti-nutrients in lima bean (Adeparusi, 2001). Autoclaving and roasting were more effective in reducing phytic acid in chickpea and pigeonpea than in urd bean, mung bean, and soybean (Chitra et al., 1996). Loss of phytate is due to its solubility in processing water during cooking operations and also partly due to the activities of endogenous phytases (Lolas and Markakis 1975). Reduction of Phytate may increase the bioavailability of protein and minerals of Parkia seeds. Phytic acid is a chelating agent and is considered as an anti-nutrient factor because it can decrease the bioavailability of essential elements such as Ca, Fe, Mg, Zn. It is the main reservoir of P and other minerals in the seed that are mobilized with germination (Lott et al., 1995).
Presence of these groups of anti-nutritive substances was reported to protect from oxidative stress. They have the ability to quench oxygen-derived free radicals by donating hydrogen atom or an electron to chelate redox-active metals and inhibit lipoxigenase (Raghuvanshi et al., 1993). Phenolics and phytic acid are also now thought to have beneficial biological effects in the diet, such as lowering blood cholesterol or preventing cancer (Anderson and Wolf, 1995).

5.2.2.5. Enzyme inhibitors and Toxic substances: Though legumes are an excellent source of proteins and essential dietary minerals but its utility to human is limited by the presence of anti-nutrients such as trypsin inhibitors, chymotrypsin inhibitors, amylase inhibitors (Savage and Deo, 1989). Heat inactivation of enzyme inhibitors in legumes has been reported by many workers (Ravindran and Ravindran, 1988; Ogun et al., 1989). Soaking, autoclaving and toasting completely eliminated trypsin inhibitor in lima bean (Adeparusi, 2001). Table 4.2.9 shows the enzyme inhibitors and toxic substances in different stages of the *P. roxburghii* pod. Trypsin inhibitor units (TIU)/mg in tender (13.37), immature (7.77) and mature (6.94) raw pods decreased to 6.02, 5.81 and 4.05 TIU/mg due to ordinary cooking and to 4.41, 3.88 and 3.02 TIU/mg by pressure cooking. Likewise, amylase inhibitor units (AIU) in tender (4.70mg/g), immature (7.90mg/g) and mature raw pods (8.40mg/g) reduced to 3.40, 6.40 and 6.80mg/g in ordinary cooking and to 3.90, 5.90 and 6.90mg/g, respectively in pressure cooking. Saponin content in tender (25.20mg/g), immature (26.20mg/g) and mature raw pod (28.95mg/g) decreased to 16.20, 16.70 and 17.0mg/g due to ordinary cooking and to 15.20, 15.80 and 16.50mg/g by pressure cooking. No significant differences were observed in TI and saponin content in different stages of the pod, however, AI increased with the age of the pod. Per cent destruction of enzyme inhibitors and toxic substances are presented in figure 4.2.15. Per cent destruction of TI recorded up to 67.02%, 45.24% and 32.3% of the original content in tender, immature and mature stages of the pod (Figure 4.2.15A). Destruction of AI was comparatively lesser and recorded loss of 27.76% in tender, 25.32% in immature and 17.86% in mature stages (Figure 4.2.15B), whereas per cent removal of saponin out of the original content ranged from 41.28% to 43.01% in mature pods and 36.26% to 39.69% in both the other two stages of the pod (Figure 4.2.15C).

Trypsin inhibitor activity (TIA) and amylase inhibitor activity (AIA) in *P. roxburghii* seeds decreased from 7.86 to 2.17 units/mg (P<0.01) and 5.07 to 1.38 units/g (P<0.01) respectively due to processing and cooking methods (Table 4.2.10). Bishnoi and Khetapaul (1994) observed that soaking of peas in water for 6 hr or longer could marginally reduce TIA and
AIA, may be due to leaching against concentration gradient. However, in contrast to it, no reduction in TIA was observed even after 12 hr of soaking in Parkia seeds. TIA and AIA decreased from 7.86 to 4.56TIU/mg and 5.07 to 3.03AIUmg/g in whole seeds due to ordinary cooking, while it decreased to 2.47TIU/mg and 4.48AIUmg/g in pressure cooking, which were 58.05% and 50% destruction, respectively (Table-4.2.10). Destruction of TI and AI were more in case of DSS seeds reaching up to 61.34% in trypsin inhibitor and 72% in amylase inhibitor, respectively. Trypsin inhibitors and amylase inhibitors are heat labile which may explain the destructive effect of cooking in diminishing TIA and AIA. Similar results were also reported by Manorama and Sarojini (1982) and Rekha et al., (2005). No earlier information is available on the saponin content of Parkia seeds to compare with. However, the values obtained for saponin content (23mg/100g ) are lower than the values reported for other popular legumes like Faba bean (Sharma and Sehgal, 1992), Rice bean (Kaur and Kapur, 1990) and Soybean (Grewal, 1992). Moreover, processing reduced P. roxburghii saponins up to 22.09% in whole seeds and 35.09% in DSS seeds. Seigler (1998) reported that saponins have anti-carcinogenic properties, immune modulation activities and regulation of cell proliferation as well as health benefits such as inhibition of the growth of cancer cells. Besides, saponins have been reported to assert a physiological effect in lowering the level of plasma cholesterol (Oakenfull, et al., 1979).Dehulling and Soaking significantly (P<0.01) reduced saponin content. This reduction may possibly be due to leaching during soaking. Reduction of saponin due to soaking was also reported by Rekha et al., (2005). Similarly, reduction in saponin content during soaking and dehulling had also been reported earlier (Khokhar and Chauhan, 1986; Jood et al., 1986). Ordinary cooking of unsoaked as well as soaked-dehulled Parkia seeds reduced the saponin content, significantly (P<0.01), by 22.09 to 29 %, respectively. Pressure cooking of unsoaked and soaked-dehulled seeds brought higher reduction in saponin content than that observed during ordinary cooking. Pressure cooking seemed to have more beneficial effect. Loss during ordinary and pressure cooking may be attributed to the thermo labile nature of saponin. The results obtained in the present study conforms with those reported by Jood et al., (1986) and Khokhar and Chauhan (1986). No cyanide was detected in the raw, cooked and pressure cooked seeds. Trypsin inhibitors, saponins and isoflavones are now thought to have beneficial biological effects in the diet, such as lowering blood cholesterol or preventing cancer (Anderson and Wolf, 1995).
5.3. Relationship between agro-climatic zones and quality parameters in *Parkia roxburghii*:

Parkia is a genus of approximately 31 species of leguminous trees. The genus is taxonomically most diverse in the rainforest of the Amazon basin (Hopkins, 1986), but four species are found in Africa and Madagaskar (Hopkins, 1983), and about ten in the Indo-Pacific region (Hopkins, 1994). Knowledge of the level, structure and origin of genetic variation within and between plant populations is important for the effective utilization and conservation of species (Hamrick, 1987). Factors determining the level and structure of genetic variation within plant species include evolutionary history characteristics, population density, mating system and mechanism of gene flow (Hamrick et al., 1991). The aspect which is of particular interest concerns the chemical composition of the seed and the properties of the various constituents. The influence of genetic variation on the chemical composition of pulses has been reported by many workers (Lai et al., 1963a & b; Gupta et al., 1976a).

5.3.1. Chemical composition:

5.3.1.1. Moisture, acidity and ascorbic acid: Table 4.3.1 shows moisture, acidity and ascorbic acid (vitamin C) content in different agro-climatic zones under different stages of pod development. Moisture in different agro-climatic zones ranged from 80.20% to 84.30% in tender stage, 75% to 78.5% in immature and 69.5% to 76.10% in mature stage. Moisture in the seed ranged from 58.20% to 60.10%. Significant differences in moisture content were observed in different stages of the pod however, no such differences among the agro-climatic zones were found. The same trend was found in case of acidity also. Acidity ranged from 0.28% to 0.30% in tender, 0.26% to 0.30% in immature and 0.26% to 0.29% in mature stages of the pod while it ranges from 0.20% to 0.25% in the raw seeds. Like in moisture, no significant differences in acidity were observed among the agro-climatic zones. Vitamin C content was found higher in the tender stage of the pod, which ranged from 38 to 60.80mg/100g in tender, 19.60 to 33.20mg/100g in immature and 24.40 to 40.80mg/100g in mature stage of the pod. Vitamin C in tender stage under sub-tropical plain zone (STPZ) recorded 40.80(mg/100g), which decreased to 25.60(mg/100g) in immature stage but accumulated again to 28.0 (mg/100g) in the mature stage of the pod. Higher amount of vitamin C was observed in tender stage that significantly decreased to immature stage of the pods in all the agro-climatic zones. The same trend was observed in different stages of the pod in all the agro-climatic zones. The observations revealed that vitamin C content was
higher in the pods than the seed. Among the agro-climatic zones, Temperate sub-alpine zone (TSAZ) recorded significantly higher values of vitamin C in all the stages of the pod and seeds, while the least being found in Mild tropical hill zone (MTHZ). Differences in moisture and vitamin c with respect to genotypes and environments are also reported by some workers (Amir et al., 2007).

5.3.1.2. Proximate composition: Table 4.3.2 showed the proximate composition in different agro-climatic zones under different stages of the pod development. Significant differences in different stages and zones were observed in crude protein. Crude protein in TSAZ recorded 19.68% in tender stage which decreased to 15.31% in the immature stage but increased again to 17.93% in the mature stage of the pod. The same trend was observed in all the agro-climatic zones. Crude protein in tender stage in different zones ranged from 15.5 (MTHZ) to 19.68 (TSAZ), the highest being found in TSAZ and the least being in MTHZ. The same trend was observed in all the stages including the seeds. Among the different stages of pod, tender stage, in all the regions was found to have more proteins than other stages of the pod. The protein yields of plants are reported to vary widely with different species, different extraction methods, different stages of maturity and also soil conditions (Matai et al., 1970). The considerable variation in protein may partly be attributed to variation in genetic makeup of the genotypes studied. In addition, variation in environmental factors (soil, temperature, etc.) of the study sites may also contribute for the variation in the protein content of the genotypes (Alamerew, 2003). Swaminathan and Jain, (1973) also reported that differences in nutrient contents of legumes are dependent on variety and location. Smirnova-Ikonnikova (1962) observed significant variations in the accumulation of proteins in the seeds of different varieties of cowpeas and soybeans and suggested that the nature of these changes depend on specific peculiarities and growing conditions of the legumes. The variation in grain protein content, which can be attributed to genetic difference, is generally smaller in magnitude than the variation caused by environmental influence (Walker, 1982). The usefulness of seed protein variability for discriminating among cultivars and wild accessions as well as for studying the genetic relationships among lines have been widely reported for legume and cereal crops (Wrigley et al., 1982; Brown et al., 1982 and Romero-Andreas and Bliss, 1985). Crude fibre was another constituent which was found to differ significantly in different zones and stages. Crude fibre increased as the pod advances. Higher range in crude fibre was obtained in MTHZ which ranged from 12 (tender) to 23 (mature), while the minimum range being found in TSAZ which ranged from 7.85 to 15.80. The same trend was observed in the seeds also. Similar observations with respect to crude fiber and ash content
were also reported in different varieties of lentils (Karadavut and Jenk, 2010). Environmental conditions exert significant influences on chemical composition of legumes (Al-Karaki & Ereifej, 1997). Significant differences in total carbohydrate and crude fat reported among legume varieties have been attributed to climatic and varietal differences (Karadavut and Genc, 2010; Kaya & Yalçın, 1999). However, in the present study, no significant differences were observed in case of crude oil, crude carbohydrate, total ash and gross energy (GE) in different agro-climatic zones. This may be due to the fact that the data shown here are the pooled values of six genotypes. Higher crude oil content in the pod was observed in TSAZ which ranged from 0.25% to 1.30% and the least being found in MTHZ ranging from 0.22% to 0.93%. However, higher crude oil content in the seeds was found in STPZ (20%) to compare with other zones. Sub tropical plain zone comprises the valley portion of the state which is having higher temperature than the other zones. These results are in conformity with the findings that temperature has effect on total oil and protein content of soybeans. Wolfe et al. (1982) reported the presence of positive correlation between maximal temperature and oil percentage. The oil content in all the agro-climatic zones increased gradually from tender stage to the mature stage (Table 4.3.2). Among the agro-climatic zones, temperate sub-alpine zone and sub-tropical plain zone have a margin over other zones in oil content of the seed. GE (kcal/100g) in the seeds recorded higher values than the three stages of the pod. The GE values in different agro-climatic zones were very narrow and recorded to be 448.90, 443.30, 437.25 and 448.80 (kcal/100g) in TSAZ, STHZ, MTHZ and STPZ, respectively. Similar calorific values of 100 g dry matter of Mucuna monosperma seed material in different places were 408.19 kcal (Kerala) and 378.60 kcal (Tamilnadu) (Arulmozhi and Janardhan, 1992), whereas calorific values of Bauhinia racemosa were 407.64 kcal (Ayyanarkoil Forest) and 402.90 kcal (Mundanthurai Wildlife Sanctuary) (Mohan and Janardhan 1994).

5.3.1.3. Free amino acids, soluble sugar (TSS) and starch in different agro-climatic zones: Table 4.3.3 indicated free amino acid (FAA), total soluble sugar (TSS) and starch content in the seeds in different agro-climatic zones. FAA values were not significantly differed among the four zones while TSAZ recorded significantly higher values of TSS (4186.57mg/100g) than the other 3 zones though no significant differences were observed among STHZ, MTHZ and STPZ. Similarly, TSAZ recorded significantly higher values of starch (2047.17mg/100g) than 1617.68(mg/100g) in STHZ, 1693.67(mg/100g) in MTHZ and 1606.95(mg/100g) in STPZ, respectively. In most of the alpine plants, the storage compounds are carbohydrates in their shoots and roots and lipids in the leaves (Billings and
Mooney, 1968). It was reported that increase in temperature decreases photosynthetic activity (Pushpalatha et al., 2008). Nautiyal (1983) has also reported increase in sugar with increasing altitude in Artemisia. Soluble sugars are well known to lower the freezing point and thus possible resistance to low temperature in the cold climates. Starch content increased with increasing altitude (Prakash et al., 2008), while free amino acid content in the leaf showed increasing trend in all the Polygonum species with increase in altitude although in some species, it decreased (Prakash et al., 2008). The TSAZ being the highest (altitude) agro-climatic zone among the four zones, significantly higher values of TSS, FAA and starch were in agreement with the above reports. Lignifications of tissues, conversion of starch into sugars and high soluble sugar contents have been reported to have an adaptive significance at high altitudes (Levitt, 1980 a, b; Nautiyal, 1983). Starch content changes leading to a decrease in the conc. of soluble carbohydrates during chilling are regarded as a factor increasing the chilling tolerance of the plants (Ashworth et al., 1993). Russel (1940) reported accumulation of soluble carbohydrates that results in higher osmotic value in plants growing in alpine and sub alpine areas, thereby increasing their cold tolerance. Seed characteristics at harvest are also determined by the cultivar genotypes and abiotic factors acting during plant growth and seed development. Overview of the work published indicated environmental effects on the quality of bean seeds (Shellie and Hosfield, 1991; Santalla et al., 1995).

5.3.1.4. Storage protein fractions: Table 4.3.4 indicated storage protein fractions in the seeds of P. roxburghii in different agro-climatic zones. Albumin content in TSAZ (7.27%) was significantly higher than the STHZ (5.45%) and MTHZ (4.81%). Globulins were found to represent the major component of storage proteins in the seeds and glutelin did not show any significant differences among the 4 agro-climatic zones. The highest concentration of globulins was found in STHZ (14.11%), while the least being recorded in STPZ (12.93%). Prolamin fraction in STHZ was found to be significantly higher (2.50%) than all the 3 agro-climatic zones. Globulin to albumin ratio was determined as a measure of the nutritive value, where the least is the better. Globulin/albumin ratio in P. roxburghii seeds in the 4 agro-climatic regions ranged from 1.82 to 2.78, while the highest value 2.78 recorded in MTHZ differed significantly from the remaining 3 zones STPZ (1.82), STHZ (2.59) and TSAZ (1.86). However, no significant difference was observed among STPZ, STHZ and TSAZ respectively. Bajaj et al., (1971) were able to demonstrate the importance of water soluble proteins as indicators of protein quality as these contributed to superior nutritional value. In the present study albumin and globulin were found to be the two major protein fractions which agrees with the earlier observations reported on black gram (Bera and Bera, 1990) and
green ram (Dana, 1991). Recovery of protein fractions out of the total content revealed that STPZ and TSAZ had higher extractability in albumin while STHZ and MTHZ in globulins. Overall extractability as per the method used ranged from 82.86% in STPZ to 87.57% in STHZ (Figure 4.3.1). Differences at the rate of protein extraction depending upon the methods used are evident. In addition, there are reports of environmental influences on nutritional composition of legumes. The protein content of Faba bean seeds grown in a location with high nitrogen content could be higher compared to the protein content of Faba bean seeds grown in a location with soil that has low nitrogen content (Alamerew, 2003). Environmental conditions have a marked effect on accumulation of proteins and hence on composition of seed proteins (Kumar and Matta, 1997).

### 5.3.1.5. Phenolic substances:

Table 4.3.5 presents different phenolic substances in the seeds of *P. roxburghii* in different agro-climatic zones. Total soluble phenols (TSP), orthodihydric phenols (OP) and bound phenols (BP) did not show any significant differences among the agro-climatic zones. However, higher values of TSP and OP were recorded in TSAZ, while higher values in BP recorded at MTHZ. Tannin was the only phenolic substance found to be significantly different among the agro-climatic zones. Higher content of tannin was recorded in TSAZ (2909.20mg/100g) which significantly differed from STHZ (1684.93mg/100g) and MTHZ (2131.37mg/100g) but no significant difference was observed from STPZ (2393.73mg/100g). Figure 4.3.2 indicated phenolic pigments in the seeds of 4 agro-climatic zones. Leuco-anthocyanin content in the seeds ranged from 3.8(λ550 x 1000) in STPZ to 4.4(λ550 x 1000) in TSAZ, while anthocyanin ranged between 27.82(mg/100g) in TSAZ to 43.6(mg/100g) in MTHZ. High concentration of anthocyanin was recorded in all the zones to compare with leuco-anthocyanins. The present findings were in agreement with a number of reports as several factors such as plant type, age of the plant or plant parts, stage of development, and environmental conditions were reported to govern the polyphenol contents in plants (Salunkhe *et al.*, 1971). Several phenolics are present in legume seeds, but practically nothing is known on environmental effects on their levels in the seeds. Phenolic compounds increased during seed development until 30 days after anthesis, but decreased thereafter in parallel to polymerization of tannins (Coelho and Lajolo, 1993). No information is available on environmental effects on these changes during seed development and in mature seeds. Tannin content, as well as polyphenol oxidase activity and associated changes in seed colour, also change during storage at rates that depend on storage temperature (laderoza *et al.*, 1989). The effect of environment on gene expression is evident. In addition, environmental influence on nutritional composition of many legumes was reported.
5.3.2. Mineral composition:

Table 4.3.6 showed the major element composition in the pods under different agro-climatic zones. Na and Ca varied significantly according to the stage of the pod and in different zones. However, no significant difference in Na content in the seeds was observed among the zones. Ca was another element which showed differences according to the stage and in different zones. Ca content in STPZ showed significantly higher values in immature and mature stages of the pods than TSAZ. However, no significant differences were observed in immature stage among STHZ, MTHZ and STPZ and between MTHZ and STPZ in mature stages, respectively. Ca content decreased from the tender stage to the mature stage of the pod. Seeds recorded lesser amount of Ca and ranged from 100 to 120 (mg/100g) in the 4 agro-climatic zones. Observations on K showed no significant differences among the zones. However, significant difference was observed in different stages of the pod. As in the case of Na and Ca above, K concentration was also found to be higher in the tender stage that decreased towards maturity in all the agro-climatic zones. Contrary to K, Mg showed significant differences among the zones but not in stages. Higher concentration of Mg in the tender stage of the pod and seeds was recorded in MTHZ (190.3mg/100g), which significantly differed from the other zones. In case of P, no significant differences either in stages of the pod or in different agro-climatic zones were recorded. Table 4.3.7 showed minor element contents in P. roxburghii under different agro-climatic zones. No significant differences in stages as well as in zones were observed in Fe content. However, appreciably higher amounts of Fe was observed in all the agro-climatic zones which ranged from 39.4 to 66.5(mg/100g) in TSAZ, 45 to 62(mg/100g) in STHZ, 41.5 to 57.1 (mg/100g) in MTHZ and 45 to 77.1(mg/100g) in STPZ in the pods, respectively. Mn content increased significantly with the maturity of the pod in TSAZ, MTHZ and STPZ but no significant differences were observed among the agro-climatic zones. Zn content in all the agro-climatic zones significantly increased from the tender stage to immature stage but decreased significantly as the pod matures. Zn content in TSAZ was found to have significantly higher values among the 4 zones, while STHZ recorded the least. Zn is the only element which was found significantly different in different stages of the pod and in different zones. Similar trend as with Zn was found in Cu by significantly increasing from the tender stage to the immature stage of the pod in all the agro-climatic zones. Cu was found to be significantly different in different stages of the pods but no such observations were found among the agro-climatic zones. Composition evaluation of leguminous seeds such as soybean, cowpea, groundnut, chickpea and red gram has been carried out in different locations by many investigators (Mba
et al., 1974; Ologhobo and Fetuga 1984 a and b; Newman et al., 1987; Singh et al., 1990). Such variation in the content of minerals for the same legume species may be related to genetic origin, geographical conditions and the level of soil fertility (Apata and ologhobo, 1994). In general, analysis of field-grown material has demonstrated comparable ranges of mineral concentrations among seeds of most legume species. Recently, seed mineral levels were characterized in the 500-accession *Pisum* Core Collection (part of the U.S. Department of Agriculture’s germplasm holdings), using plants grown under controlled, nutrient depleted conditions. In this study, broad genetic diversity was observed for both seed micronutrient and macronutrient concentrations (http://www.ars-grin.gov/cgi-bin/npgs/html/crop.pl?177). Mineral composition of legumes is also affected by the availability of one mineral or in other words one mineral influences the concentration of another. Correlation analysis of seed minerals in recombinant inbred lines of bean (*Phaseolus vulgaris*) has shown positive associations between most minerals (Beebe et al., 2000).

Seed mineral composition based on genetic diversity has been studied in several legumes (Meiners et al., 1976). Sharma et al., (2001) reported increase in the levels of soil pH resulted increase in Sodium, calcium and magnesium concentration in all the genotypes. Significant environmental effects on the quality of bean seeds have also been found in several studies (Shellie and Hosfield, 1991; Santalla et al., 1995). Environment has influence on nutritional composition of Faba bean. The Genotypes investigated gave different percentage of protein and fat ranges for all sites. However, in case of mineral composition, significant values were recorded in one site only, among all the sites studied (Alamerew, 2003). The present findings agreed with this by getting significant values in respect of crude protein and crude fibre, whereas other values were not significant among the 4 agro-climatic zones.

5.4. Genetic Diversity in *Parkia roxburghii* G. Don:

*Parkia roxburghii* is a highly polymorphic species regarding the pattern of variation in the morphological characters as well as in biochemical characters. Morphological, agronomic, as well as biochemical parameters have been widely used in the evaluation of plants for genetic diversity, breeding value and yield potential (Singh and Sahu, 1998; Parthasarathy et al. 2002; Ahmed et al., 2005). The growth, yield and biochemical analysis techniques were utilized for the documentation of germplasm and determination of variability in different cultivars of tomato (Fehmida and Ahmed, 2007). The present investigation on morphological and biochemical qualities were conducted on 24 different *P. roxburghii* cultivars collected
from different agro-climatic zones of Manipur (Figure 3.12.1). The characterization thus will help in the identification of varieties and their future utilization for varietal improvement programme using conventional techniques of selection and hybridization. Altogether 14 different morphological characters and 15 biochemical parameters were studied to understand the nature of variability among the genotypes (Annexure I and II).

5.4.1. Distinguishing characters: Table 4.4.1 to 4.4.4 shows the distinguishing characters of the 24 P. roxburghii cultivars. The cultivars were morphologically different to each other with one respect or the other (Photo Plate 3.12.1 and 3.12.2).

5.4.2. Mean performance of the cultivars: The mean performance of the 24 cultivars is shown in the Tables 4.4.5 and 4.4.6. The analysis of variance revealed significant differences among the cultivars for all the characters indicating the presence of wide genetic variation amenable for breeding programmes (Revathi et al., 2010). The variability estimates, in general, revealed that the phenotypic variation (PCV) was higher than the corresponding genotypic variance (GCV) for different characters though the extent of difference between the two was relatively low (Table 4.4.7 and 4.4.8). The estimates of PCV and GCV indicated the existence of fairly high degree of variability for seed weight, stalk length, pod weight and seed thickness in terms of morphological characters (Table 4.4.7), whereas acidity, total phenols (TP), bound phenols (BP), total soluble sugar (TSS) and prolamin in case of biochemical parameters (Table 4.4.8). Moderate variability was observed for seed to seed distance, pod length, stalk diameter, pod thickness (Table 4.4.7), tannins, starch and albumin content (Table 4.4.8). The genotypic coefficient of variation ranged from a minimum of 6.31 for seed L/B ratio to a maximum of 44.27 for seed weight in case of morphological characters, while it ranged from 4.76 in orthodihydric phenols to 65.5 in TSS in case of biochemical qualities. The seed weight showed the highest PCV value of 45.72 in comparison to GCV of 44.27 suggesting less environmental influence on this character, which was also supported by its high (88.29%) heritability (Table 4.4.7). Likewise, biochemical parameters like TSS, acidity, tannins and free amino acids recorded very high values of heritability thereby indicating that these biochemical qualities are less influenced by the environment (Table 4.4.8). Similar results have also been reported by Bagrecha et al., (1972) and Ghosh and Gulati (2001). The estimate of GCV were slightly lower in magnitude than the magnitude of phenotypic coefficient of variability (PCV) due to partial interaction of the genotypes with the environment or other environmental factors which influenced the expression of these characters (Table 4.4.7 and 4.4.8). As all the traits showed a narrow
difference between GCV and PCV, phenotypic selection could be made effectively. Highest GCV and PCV for traits like seed weight, pod weight, seed thickness, TSS, acidity and total phenols indicated the presence of diversity and offer good scope of enhancing productivity and quality by selecting individuals for these traits (Walia, 2002). High heritability coupled with high genetic advance as per cent mean was noticed for pod breadth, seed weight, TSS and free amino acids suggesting involvement of genetic factors. The occurrence of such values might be due to the presence of additive gene action (Panse, 1957). The above results are in agreement with the findings of Lal and Seth (1979). Estimate of heritability coupled with genetic gains would be useful for selection of hybridization programmes (Johnson et al., 1955). For a clearer understanding about the relationship among GCV, PCV and heritability of the characters in both the cases, two figures have been shown in figure 4.4.1 and 4.4.2.

5.4.3. Phenotypic and genotypic correlation: Table 4.4.9 presents genotypic and phenotypic correlation matrix based on 14 morphological characters. Genotypically stalk diameter was found to be highly positively correlated with pod breadth, pod thickness, pod weight, seed length and seed breadth, while pod breadth was found to be positively correlated with pod thickness, pod weight, seed length, seed breadth and seed weight. Phenotypically, the same trend was observed in all the characters studied though with lesser values.

Genotypic correlation in case of biochemical qualities (Table 4.4.10) revealed vitamin C to be positively correlated with TP and albumin content while negatively correlated with BP and prolamin. BP is also negatively correlated with albumin content. On the other hand, albumin is positively correlated with TSS and total protein content. The usefulness of seed protein variability for discriminating among cultivars and wild accessions as well as for studying the genetic relationships among lines have been widely reported for legume and cereal crops (Brown et al., 1982 and Romero-Andreas and Bliss, 1985). The usefulness of seed-protein profiles in taxonomic and evolutionary studies as well as in discriminating wild and cultivated accessions of legume species have been well established (Ladzinsky and Hymowitz, 1979; Gepts et al., 1986 and Lioi, 1987). However, phenotypically, none of the biochemical qualities are positively correlated to each other (Table 4.4.10).

5.4.4. Performance of the cultivars: The range along with the lowest and the highest cultivars in respect of morphological characters can be seen from Table 4.6.5. Highest stalk length was recorded in PRB 24 and highest pod length and pod weight in PRB 16, while highest stalk diameter, pod breadth, seed length and seed breadth has been observed in PRB 1. The range of variability indicated the existence of variability for all the characters. The
present study indicated the presence of wide range of variability for stalk length, pod length, pod breadth, pod weight, seed thickness and seed weight out of which stalk length, pod breadth and seed thickness highly contributed (61.96%) towards divergence (Table 4.4.11). Therefore, selection should be based on these characters in order to achieve greater productivity in this tree crop.

The performance of the cultivars with respect to biochemical qualities are seen in table 4.4.6. Highest acidity was observed in PRB 6 and highest vitamin C and total phenol (TP) were recorded in the single cultivar PRB 23. Similarly, highest values in total soluble sugar (TSS) and total protein were observed in the cultivar PRB 24 while highest values in total bound phenol, tannins and free amino acids were recorded in the cultivars PRB 13, PRB 20 and PRB 21, respectively. This indicates the existence of variability of the biochemical parameters studied. Moreover, the three parameters, TSS, acidity and tannins contributed about 66.66% towards divergence (Table 4.4.12).

5.4.5. Clustering: The clustering pattern base on morphological characters by Tocher's method revealed that the genotypes originated from different agro-climatic zones can be grouped in Cluster I, II and IV which indicated that there has been no association between genetic diversity and geographical diversity (Table 4.4.13). The same clustering was also observed by the Ward's minimum variance dendrogram (Figure 4.4.3). The genotypes that originated in one region had been distributed into different clusters, indicating that genotypes with the same geographical origin could have undergone changes for different characters under selection. Similar results in some crops have been reported by Sankarapandian, et al., (1996). This could be due to genetic drift, selection pressure and environment, which creates greater diversity rather than genetic distance (Murthy and Arunschalam, 1966). The graphical features of the cultivars in each cluster are shown in the figure 4.4.4.

The Tocher's method of clustering based on the biochemical parameters revealed the 24 cultivars to be into 5 groups. Maximum number of cultivars (8) belonged to the Cluster 1 while 7 cultivars belonged to cluster 3. The remaining 3 clusters have 3 cultivars each. As shown above, the genotypes originated from different agro-climatic zones are grouped together in Cluster 1, 2 and 4, which indicates that there has been no association between quality of the cultivar and geographical distribution (Table 4.4.14). The same is also evident from the Ward's minimum variance dendrogram presented in figure 4.4.5.
5.4.6. **Intra-cluster and inter-cluster distances:** The quantum of genetic divergence has also been assessed using Mahalanobis’s Euclidean Square distances. In case of morphological study, low intra-cluster $D^2$ values were recorded for Cluster III and V as they included only single genotype in each cluster (Figure 4.4.6). However, cluster II with 8 genotypes exhibited maximum intra-cluster distance (112.7) indicating that the genotypes in this cluster are more diverse than the other clusters (Table 4.4.15). The maximum inter-cluster distance was observed (508.81) between cluster I and cluster V indicating wider genetic diversity between the genotypes in these groups. Since these clusters have more inter-cluster distance among them, selection of parents from such clusters for hybridization programme would help to achieve novel hybrids as greater the genetic distance between the clusters, wider is the genetic diversity between genotypes. Inter-cluster distance was found to be minimum (160.61) between cluster II and IV indicating close relationship and similarity for most of the characters of the genotypes in these two clusters, hence, selection of parents from these two clusters should be avoided (Sridhar et al., 2002).

In the $D^2$ analysis based on biochemical qualities (Figure 4.4.7), low intra-cluster $D^2$ values were recorded for Cluster 5 (167.43) while maximum intra cluster distance was observed in cluster 4 (382.69) with 3 genotypes (Table 4.4.16) indicating that the biochemical qualities of these genotypes in this cluster are more diverse than the other clusters. The maximum inter-cluster distance was observed between cluster 1 and cluster 5 (1062.47) indicating wider genetic diversity between genotypes in these groups.

5.4.7. **Cluster mean Values:** Data on cluster means are summarized in Table 4.4.17 and 4.4.18. Cluster mean values on morphological characters revealed that the different clusters exhibited marked differences in respect of all the 14 characters. Cluster II exhibited higher mean values for pod length (40.13cm) and pod L/B ratio (11.68). Cluster III has higher mean values for Stalk length (15.50cm) and seed to seed distance (2.90cm), while cluster V exhibits maximum values on Stalk diameter (0.71cm), pod breadth (4.62cm), pod thickness (0.56cm), pod weight (113.03g), number of seeds (16.80), seed length (2.20cm), seed breadth (1.55cm), seed thickness(0.69cm) and seed weight (1.31g) indicating that cluster V, which is represented by a single genotype (PRB 1) contained all the desirable characters, which could be directly selected for utilization in the breeding programmes. Hybridization between the genotypes of different clusters was necessary for the development of desirable genotypes, which accommodates genes from the two parents. The crosses involving cluster II and cluster
V may generate a material where negative correlation between the pod length and pod weight is broken down (Ram et al., 2002).

The cluster mean values on biochemical parameters showed that different clusters showed marked differences in all the 15 biochemical parameters studied (Table 4.4.18). Cluster 1 exhibited higher mean values for acidity (0.65%) and bound phenols (1.69mg/g) whereas Cluster 2 has higher mean values for Starch (21.37mg/g), globulin (14.76%) and prolamin (2.52%). Cluster 3 exhibits maximum values on total phenols (5.62mg/g) and total protein (29.82%) while cluster 4 exhibits higher mean values on tannin (23.21mg/g) and free amino acids (52.82mg/g). The higher cluster mean values on vitamin C (56.76mg/100g), orthodihydric phenols (2.31mg/g), total soluble sugar (57.46mg/g), albumin (9.32%) and glutelin (3.02%) were observed in cluster 5. Moreover, the biochemical characters like Vitamin C, TSS and albumins are the indicators of nutritive values in legumes, the genotypes in cluster V may be used for utilization in the quality breeding programmes. Genetic alteration of specific protein fractions provides a means for increasing total protein content, raising limiting essential amino acid concentration by differentially regulating fractions with different amino acid composition. Such alternative should however be taken into account for the proportion of the different seed parts and their composition in protein fractions (Baudoin and Maquat, 1993).

5.4.8. Canonical Analysis: Following the analysis of canonical vectors and canonical roots, it was possible to represent a two dimensional graph with Z1 and Z2 co-ordinates (Figure 4.4.8. and 4.4.9). In the 2D graph of the morphological characters (Figure 4.4.8), the clusters of the cultivars were distinctly delineated to their respective positions. From the canonical graph, it was revealed that the cultivars PRB 1 of cluster V and PRB 24 of cluster III are situated at the farthest distance indicating considerable divergence between them. However, in case of biochemical parameters, PRB 4, PRB 5 and PRB 10 are situated at places that show considerable distances among themselves (Figure 4.4.9).

Yield of a crop is the result of interaction of a number of inter-related characters. Therefore, selection should be based on these component characters after assessing their correlation with yield. As the cost of Parkia is based on the size of the pod, pod breadth and pod length are two important parameters for taking a decision regarding the nature of selection to be followed for improvement of this crop. In the present investigation, pod breadth seemed to be an important parameter over pod length as it is positively and highly correlated with pod thickness, pod weight, seed length, seed breadth and seed weight at both genotypic and
phenotypic level. Stalk diameter also played an important role by showing highly positively correlated with yield parameters like pod breadth, pod thickness, pod weight, seed length and seed breadth at both genotypic and phenotypic level. Therefore, these characters should be considered while making selection for yield improvement in *Parkia roxburghii*.

The Northeast Hill region is considered to be the primary and secondary source of origin of *Parkia roxburghii* (Singh *et al.*, 2000). A wide range of diversity was observed in *Parkia roxburghii* in Manipur because of the climatic and altitudinal variations and such other favourable factors (Singh *et al.*, 2000). The region had remained isolated for a long time. Even today the accessibility is rather poor in many parts of the region. This undisturbed condition combined with suitable environmental conditions and varying altitudes are the major reasons for genetic diversity (Borthakur, 1992). The wide variability in the *Parkia roxburghii* genotypes of Manipur is a major source of improving productivity, enhancing quality, resistance to biotic and abiotic stresses. The germplasms collected were diverse in terms of quality traits and biochemical constituents due to genetic factors. Thus, selection of superior *P. roxburghii* cultivars require careful evaluation and characterization for yield, disease resistance and quality traits. The diversity of *Parkia roxburghii* is of great importance not only to improve the quality of the plants and to break the yield barriers through incorporation of desirable genes from a wide genetic base but also utilize them for studying the origin of the plants, their idiotypes etc. as well as to study the development and mobilization of land races (Singh *et al.*, 2000).