5. DISCUSSION

Millet grains generally vary in colour and shape and consist of pericarp, germ and endosperm. The pericarp has three layers, the epicarp, the mesocarp and endocarp. Pericarp has got the lowest protein content and is mainly composed of cellulose and starch. The germ or embryo (approximately 5-10% of the kernel) is surrounded by the soft endosperm and consists of a large scutellum, an embryonic axis, a plumule and a primary root. The embryo is richest in protein and oil. The endosperm generally consists of floury and corneous portions which are surrounded by aleurone layer. The aleurone cells contain protein, minerals, water soluble vitamins, enzymes and oil. They do not contain starch granule (Bewely and Black, 1983).

Millets contain large proportions of husk and bran, require dehusking prior to consumption (Hulse et al., 1980). Millets were decorticated at household level by hand pounding in olden days, but are currently milled using machineries available in the market. The percentage of husk ranged from 15.0 to 25.0 in different small millets. The yield of the milled grains was low in barnyard and kodo millets. Hadimani and Malleshi (1993) reported that the yield of milled grains, bran and husk varied from 63.2 to 90%, 5.0 to 11.0% and 1.5 to 29.3% respectively in pearl millet and small millet.

5.1. Carbohydrate profile

Total carbohydrates in cereal grains include starch, sugars, soluble and/or insoluble fibres. Little, barnyard and proso millets have got lower amounts of total carbohydrates (69.99, 72.2 and 72.96% respectively) (Table 5). Kodo and foxtail millets recorded comparatively higher carbohydrate contents (73.85 and 75.95% respectively). The total carbohydrate and starch contents in small millets were slightly lower when compared to
other cereals viz., sorghum, pearl millet, maize and rice (Subramanian and Jambunathan, 1987). Starch content was low in little millet (62.5) and high in foxtail millet (69.4) confirming the data of Malleshi and Desikachar (1985). Geervani and Eggum (1989) observed that the starch content of small millets varied from 72.07 to 79.32%. The starch content of 4 varieties of proso millet varied from 61.8% - 68.2% (Yanez et al., 1991). With respect to amylopectin the content was high in foxtail millet and low in barnyard millet. Muralikrishna et al. (1982) reported the relative amylopectin (as per cent of starch) content of barnyard (80.3%), little (81.6%) and proso (75.5%) millets.

Proso millet recorded the lowest (17.21%) and barnyard millet the highest absolute amylose content (20.02%). Malleshi and Desikachar (1985) reported the absolute amylose content of fourteen varieties of foxtail millet, two varieties each of proso and barnyard millets, one variety each of little and kodo millets which ranged between 16.8 to 22.0 per cent. The absolute amylose content of seven varieties of proso millet varied from 12.0 to 19.5% (Rakhimbaev, 1968). Wankhede et al. (1979) reported the absolute amylose content of millet starches (finger and foxtail millets) from 15.5 to 17.5%. Muralikrishna et al. (1982) reported the amylose content of small millets from 18.4% to 24.5%. The amylose content of small millets was comparable to that of sorghum and pearl millets (Subramanian and Jambunathan, 1987). These data are in close agreement with the present study.

Soluble starch and soluble amylopectin were higher in foxtail millet and lower in proso millet. Proso millet registered higher soluble amylose content and the kodo millet recorded lowest soluble amylose content. Thayumanavan and Sadasivam (1984) reported soluble starch, soluble amylose and soluble amylopectin of rice varieties. No work has been reported on the soluble starch, soluble amylose and soluble amylopectin in small millets.
Total sugar was low in kodo and foxtail millets and high in barnyard millet. Kodo millet have got higher amount of reducing sugar. Proso, barnyard and little millets recorded comparatively lower reducing sugar content. Foxtail millet was intermediate. Hulse (1980) reported that the proso millet contained 52.1% starch, 0.4% reducing sugar and 0.2% non-reducing sugar.

**Dietary fibre**

Interest on the effect of dietary fibre on the humans has grown in recent years. Researchers have noticed a decrease in dietary fibre in diets of industrialized western populations (Robertson, 1972). A decrease in dietary fibre leads to an increased prevalence of disease such as diabetes mellitus and coronary heart disease (Trowell, 1973), diverticular disease of the colon, gall bladder disease, varicose veins, hiatus hernia (Burkitt, 1975, Burkitt et al., 1974, Findlay, 1974, Painter and Burkitt, 1971) and tumors of the colon (Painter and Burkitt, 1971), compared with population groups with higher fibre consumption. An increase in the fibre content in human diets is being encouraged and advocated.

The fibre content of the whole grain of small millets was higher when compared to other millets (Kamath and Belavady, 1980). Hence the recovery of the decorticated grain was likely to be low. This might be considered as uneconomical by consumers. The fibre content of the dehusked (decorticated) grain was also high. This indicated that millets, even after removal of husk, contained appreciable amount of dietary fibre. Total dietary fibre content was higher in little millet and lower in foxtail millet (Table 6). Barnyard millet contained higher content of soluble dietary fibre among the five small millets studied. The soluble dietary fibre content of decorticated millet was 35.65% - 45.35% of the total dietary fibre, which represents a fairly higher proportion as compared to the
soluble fibre values reported for rice and wheat (Wisker *et al.*, 1985). This indicates that the small millets form an important source of soluble dietary fibre. Ranhotra *et al.* (1990) reported that diet rich in dietary fibre (soluble fibre) was beneficial to diabetics because it has a low energy density. As adult diabetics also often suffer from being overweight, such diet could facilitate weight reduction and thus contribute to normalization of glucose metabolism. Diet rich in dietary fibre also found to reduce serum lipids (Wisker *et al.*, 1985). Hadimani and Malleshi (1993) also reported that the total dietary fibre content of small millets was high when compared to rice and wheat which is in accordance with the present results.

5.2. Changes during germination

One approach to improve the nutritional qualities of the grains is to germinate the seed (Wang and Fields, 1978). The modification that occur during germination are advantageous to produce nutritionally improved meal and to use them in brewing industries. Millets can also be used as a substitute of barley in the preparation of malted drinks. Hence studies were carried out to find out the changes that occur in carbohydrate fractions during germination and the results are discussed.

5.2.1. Changes in the dry matter content

The dry matter loss was relatively high for the first four days of germination and amounted to 50.0 to 23.07% in different small millets studied (Fig.3). After seven days of germination the dry matter loss was higher in barnyard millet than the other millets. Minimum loss was observed in little millet. Bartlett (1917), reported 13.0 and 17.0% loss in the dry matter after six and eight days of germination in oats respectively. According to Wu (1982) the dry matter loss was less during early days of germination in oat and it
increased to 20.0% after eight days of germination. Bhise et al. (1988) and Pathirana et al. (1983) have reported the malting loss in sorghum upto 25.0% after 3 days of germination. The dry matter loss was higher when grains were germinated in the dark (Wu and Wall, 1980). In the present investigation all the small millets were allowed to germinate in the dark at room temperature for seven days and this may be the reason for the higher loss in the dry matter during germination. The losses due to germination can be attributed to the respiratory activity of the grains and the increased losses in the early days may be due to faster rate of respiration (Pathirana et al., 1983). The variation in the decrease/loss of dry matter among millets during germination indicates the variation in the metabolic activity.

5.2.2. Carbohydrates

Total carbohydrate content was decreased during germination in all the millets (Table 7). Morall and Briggs (1978) reported that 18.5% of the carbohydrate was utilised by 6 days old barley seedlings which are originated from aleurone and endosperm cell walls. One of the major constituents of carbohydrate that undergo changes during germination is starch. The starch content was found to decrease continuously and significantly with concomitant increase in the total sugars on progressive malting in all the millets (Table 8). The decrease in the starch content and an increase in the sugars during malting can be attributed due to hydrolysis of starch by endogenous amylases (Pathirana et al., 1983). The reduction in the starch was higher in barnyard and proso millets which indicates that starch degradation varied among millets. Decrease in the starch content was also reported by Subramanian et al. (1992), Parvathy and Sadasivam (1994), Parvathy and Thayumanavan (1995) and Bhise et al. (1988). But the decrease in the starch content was not totally reflected in the total carbohydrate content. This may be due to the partial compensation by simple sugars derived from starch (Briggs et al., 1981). However the decrease in the large amount of starch is undesirable when malting is meant for the
preparation of traditional products such as roti, porridge etc. This would result in the significant loss in the dry matter. An apparent initial increase in the amylose content was noticed in certain small millets viz. proso, foxtail and barnyard millets (Table 9). These changes might be caused by limited $\alpha$-amylolysis of both components of starch by $\alpha$-amylase (Parvathy and Sadasivam, 1982). During germination, amylopectin content decreased significantly in all the millets (Table 10). The per cent decrease varied from 45.43 to 75.61.

In contrast to starch, amylopectin and amylose, the total sugars showed appreciable increase in their quantity during germination (Table 11). The increase in the fermentable sugars during malting due to the action of $\alpha$ and $\beta$-amylases is advantageous to improve sensory properties of traditional food preparations and also for its utilization for brewing (Bhise et al., 1988). Large increase in the total sugars in proso, foxtail, barnyard and kodo millets was noticed which opens way to find out whether these millets can be used as a substitute in brewing industries. Significant increase in the total and reducing sugars during germination of millets was also reported by Parvathy and Sadasivam (1982; 1994). Balasubramanian and Sadasivam (1989) reported that the joint action of $\alpha$ and $\beta$-amylases resulted in a decrease in the starch content and an increase in the total sugars during germination of grain amaranth.

5.3. Changes during seed development

Though considerable work have been done on the carbohydrates during the development of rice, wheat and barley, information on the carbohydrates during development of small millets is scanty. Hence studies were carried out to find out the changes during seed development.
5.3.1. Dry matter

In proso, foxtail and little millets, the dry matter content of the grains increased rapidly during first 25 DAF and thereafter only very little increase was noticed (Fig. 4). In barnyard and kodo millets the increase was faster upto 28 DAF and thereafter slowed down (Fig.5). More than 90% of the dry matter accumulated within that period. Among small millets, proso millet has got larger size and average seed weight (6.1 mg/grain) whereas the weight of the little millet was 2.7 mg/grain which size was the least of all. The grain weights of kodo, foxtail and barnyard millets were 4.9, 3.2 and 3.65 mg/grain respectively. Though the grain weights were different, the dry matter accumulation pattern was similar in all the millets. Macgregor et al. (1971) studied the dry matter accumulation in barley. According to them over 90% of the kernel dry weight was deposited during 25 days period, between 9th and 36th day after ear emergence. The synthesis of kernel dry matter ceased thereafter. In the present study, dry matter accumulation was faster upto 25 DAF.

5.3.2. Carbohydrates

5.3.2.1. Changes in starch content

The starch content increased in all the small millets during maturation and reached maximum on 30 DAF in proso, little and foxtail millets and on 42 DAF in barnyard and kodo millets (Table 12). MacGregor et al. (1971) reported that in barley starch synthesis started about 11 days after ear emergence, continued rapidly for 14 days and then slowed down until day 32 and then stopped. According to them considerable amount of starch was synthesised in the remarkably short period of 17 days ie between 11th and 28th day after ear emergence. This is in agreement with the present study. Merritt and Walker (1969) and Harris and MacWilliam (1958) also reported that a period of 35 to 40 days
was required for the accumulation of starch in barley kernel in western Canada. According to McDonald et al. (1991) the starch content increased steadily and then became constant at 30-35 DAF for the normal barley and the maximum accumulation was between 12-15 days after flowering. Bhatia et al. (1974) reported that the starch accumulation in proso millet started about 11 DAF, continued rapidly for 15 days and then slowed until day 30 which is in agreement with the present study. Perez et al. (1975) reported that the rate of starch accumulation in rice was maximum 11 to 12 days after flowering.

5.3.2.2. Changes in amylose

Increase in the amylose content was noticed throughout the developmental period (Table 13). The increase was high in barnyard and kodo millets. Proso millet contained lower quantity of amylose in the early stages of seed development itself and continued the same trend throughout the developmental period and the matured grain showed 16.8% which was significantly lower than the other millets studied. The percentage contribution of amylose to starch was high in barnyard, little and kodo millets whereas in foxtail and proso millets the contribution of amylose to starch was low. Harris and MacWilliam (1957) and Merritt and Walker (1969) reported the synthesis of individual starch components. The amylose content of the starches increased from a value of 13.8% after 14 days to 22.5% after 30 days and remained constant till maturity. Similar pattern of development have been reported in other cereals as well as in maturing potato tubers. McDonald et al. (1991) reported that the accumulation of amylose in barley starts from 15 DAA (Days after anthesis) and reached maximum on 30 DAA, thereafter very slow increase up to maturity. Boyer et al. (1976) reported the accumulation pattern of amylose in maize which started from 18 days of post pollination and reached maximum at 36 DPP.
5.3.2.3. Changes in amylopectin content

During grain development, amylopectin showed increasing trend in all the small millets (Table 14). Proso millet contained comparatively lower percentage of amylopectin at 10 DAF and continued to increase steadily up to 25 DAF and then slowed down. The initial amylopectin content itself was higher in kodo millet which increased significantly and reached 46.6% at the time of maturity. The contribution of amylopectin to starch was high in kodo millet and low in barnyard millet at the early stages of development. At the time of maturity, the percentage contribution of amylopectin to starch was low in barnyard millet and high in foxtail millet. MacGregor et al. (1971) reported that the amylopectin fraction was synthesised at a relatively faster rate than the amylase during early stages of growth. Similar pattern of development has been reported in other cereals (Geddes et al., 1965).

5.3.2.4. Changes in the total sugars

Total sugar content was decreased during the development in all the small millets (Table 15). During the early stages of development higher quantity of total sugar was found in little millet and lower amount in kodo millet. Similarly at the time of maturity the total sugar content was higher in little millet and lower in kodo millet. MacGregor et al. (1971) reported the total sugar content was maximum at 11 days after flowering and declines thereafter which coincides with the initiation of starch synthesis in barley. According to Singh et al. (1977) the total sugar content was maximum at 9 days after flowering in rice and thereafter declined. The present study is in agreement with the above results.
5.4. *In vitro* hydrolysis of starch by porcine pancreatic α-amylase

It is known that the gelatinisation behaviour of the starch granule is affected by its amylose content. Recently, it was demonstrated that a close correlation exists between the degree of starch gelatinisation and the rate of enzymic hydrolysis both *in vitro* and *in vivo* (Holm *et al.*, 1988). Thus, provided a higher amylose content restricts granule swelling at conditions used during food processing, a reduced enzymic availability would be expected. Hence the present study was carried out to find out whether there is any correlation between the amylose content and the rate of hydrolysis of the starch from small millets.

The amount of reducing sugars released by α-amylase (per cent hydrolysis) in ungelatinised starch was higher in proso millet and lower in barnyard millet (Fig. 6). The foxtail, little and kodo millets were intermediate. The trend was similar in gelatinised starch (Fig. 7). In the case of gelatinised starch, the rate of release of reducing sugars per minute was very high during the initial period and slowed down as the time increased. Amylose and amylopectin are the two important constituents of starch molecules. Porcine pancreatic α-amylase hydrolyses amylose by multiple attack mechanism ie. the enzyme hydrolyse the α-(1-4) linkage randomly, and then a number of linkages in the immediate vicinity of this first point attack are hydrolysed with the liberation of maltose and maltotriose. In the case of amylopectin, not only the branch points themselves resistant to hydrolysis by α-amylases but also confer some degree of resistance to neighbouring α-(1-4) linkages (Wooton and Chaudhry, 1979). Thus in the early stages of digestion, there is rapid release of reducing sugars due to random and non-random (repetitive) attacks. However, when the substrates become smaller and/or the degree of branching increases, amylase will eventually shift towards random attack mechanism only, because of hindrance to repetitive attack by branching and difficulty of hydrolysis of the terminal bonds (Wooton and Chaudhry, 1979).
The amount of reducing sugars released at 60 min for ungelatinised and gelatinised samples were negatively correlated with absolute amylose content ($r=-0.964^{**}$ and $-0.979^{**}$ respectively). The high amylose content increases the probability for complex formation with lipids (Holm et al., 1983). In addition, during gelatinisation at high moisture levels starch may retrograde and the retrograded amylose become totally resistant to amylases (Berry, 1986, Juliano et al., 1990). It has been reported that gelatinised and cooked starches contain higher level of \textit{in vitro} resistant starch because of the more extensive amylose retrogradation in starch gels (Eggum et al., 1993a). Gelatinised barnyard millet might have higher level of resistant starch because of the presence of higher amount of amylose and hence minimum hydrolysis with $\alpha$-amylase was found. Starch-granule-bound-protein may also probably contribute to poor starch digestibility (Bjorck et al., 1986 and Eggum et al., 1993b). The rate of hydrolysis is not correlated with starch, soluble amylose or soluble starch. Thayumanavan (1987) reported that the per cent hydrolysis of amylose and amyllopectin by salivary $\alpha$-amylase was faster in Bhavani rice which contain higher amount of amylose than the other varieties. Azemi and Wotton (1984) reported that the availability of maize starch to $\alpha$-amylase was affected by amylose content. The rate of hydrolysis decreased in the following order: waxy maize (low amylose) > normal maize > high amylose maize. Bjorck et al., (1990) reported that the starch in the waxy variety of barley was highly susceptible to $\alpha$-amylases than glacier normal and glacier high amylose barley.

In the present study, the rate of hydrolysis of gelatinised and ungelatinised starches by $\alpha$-amylase are in the following order: proso millet > foxtail millet > little millet > kodo millet > barnyard millet.
5.5. Scanning electron microscope studies of starch granule

The surface topography of starch granules has received considerable attention from microcopists all over. Due to the very high resolving power, electron microscopes reveal the intrinsic submicroscopic details of the starch granules.

Proso millet contained small spherical, large spherical (few numbers) and large polygonal granules (more numbers) (Plates 3 and 8). Foxtail millet contained small spherical, small polygonal (especially pentagonal) and more number of large pentagonal granules (Plates 4 and 9). Small spherical, large spherical and more number of large polygonal granules are present in barnyard millet (Plates 5 and 10). Kodo millet contained mostly large polygonal granules. Very rarely small spherical and polygonal granules are present (Plates 6 and 11). Little millet contained mostly large polygonal and less number of small spherical and large spherical granules (Plate 7 and 12). The size of the granule was large in barnyard millet and small in proso millet. The ratio of large granules to small granules was high in foxtail millet (40:10) and low in kodo millet (40:1) (Table 16). Several granules had deep indentations caused by protein bodies. Yanez et al. (1991) reported that the proso starch granules have a bimodal distribution with two basic shapes and sizes, small spherical and large polygonal. The sizes of starch granules ranged from 1.8 to 13 μm, varying among cultivars. Dreher et al. (1984) reported that the proso starch granules resemble those of rice, which have a mean granule size ranging from 4 to 6 μm. Malleshi et al. (1986) reported that the finger millet starch contains granules of uneven shape (spherical, polygonal and rhombic) whereas the pearl millet granules were exclusively of spherical shape and contained relatively higher proportion of bigger granules, about 80% of granules being in the range of 8-22 μm. Oat starches were irregular in shape and had several pointed, oval-shaped granules (Reichert, 1913, Matz, 1969), that many of the granules were round on one side and polygonal on
the opposite side indicates growth of the granules in clusters, where they acquired the shape of the neighbouring starch granules. Oat starch granules averaged 3-10µm in size (Reichert, 1913, Winton and Winton, 1932, Matz, 1969, Lineback, 1984, Paton, 1986, Hartunian sowa and White, 1992). Hard endosperm of corn had polygonal starch granules whereas soft endosperm of corn had nearly round starch granule (Robutti et al., 1974). In the present study all the millet starch granules contained more number of polygonal starch granules which indicate the presence of hard endosperm.

Pomeranz (1974) reported that the hard endosperm portion of sorghum grain was characterised by a tightly packed structure with no air spaces. The starch granules were polygonal and appear to be covered with a thin protein matrix.

In the hard endosperm portion of sorghum, starch granule shrinkage of the matrix protein resulting from water loss during maturation, forces the round, relatively soft starch granules together and into their polygonal shape. The relatively small protein bodies were thus forced to the interfacial edges of the starch granules where they were concentrated and become indented at the edges of the polygonal shaped starch granules. Indentations in the starch indicate that the starch was relatively soft at this stage.

SEM of 24 h germinated kodo millet starch showed a size reduction in the starch granules (Plates 13 and 14). The SEM observation showed that during germination the amylases attacked the granules from the surface. As a result of this attack by amylases some pitting or pinholes are seen at some points on malted starches. Earlier studies on the mode of attack of enzymes on barley have indicated that during germination the enzymes preferentially attack the bigger granules and hydrolyse them (Bathgate and Palmer, 1973). This may explain the increased proportion of smaller granules in malted millet starch. Sreenath and West Lafayette (1992) reported the SEM studies of raw corn starch. The
granular shapes varied from round to polygonal with $\alpha$-amylase treatment, the starch granule showed many pits or pores with more or less same size. Starch granules isolated from wheat germinated for 4 days showed enzymatic erosion on surfaces (Lineback and Ponpipom, 1977). This is in agreement with the present study on kodo millet starch which showed enzymatic erosion on surfaces. Most of the enzyme attack was confined to the larger granules, suggesting that they were more susceptible to amylolytic degradation than small ones as reported by Lineback and Ponpipom (1977). There was no evidence of channeling or pitting on the surface of oat starch granules during germination. Starch isolated from oats germinated for 14 days exhibited a mildly corroded rough surface which suggests that oat starch might be less susceptible to attack by $\alpha$-amylase (Lineback and Ponpipom, 1977). Lorenz et al. (1981) also reported that the starch granules were damaged extensively due to increased A-amylase activity during sprouting. The present results are in agreement with the above reports.

SEM of kodo millet starch granule during maturation (28 DAF) showed reduction in size of the starch granule and indicates maximum synthesis of starch was attained during that period (Plate 15).

SEM of barnyard millet starch after one autoclaving cooling cycle showed, disappearance of granular structure and bigger irregularly shaped particles with a continuous spongy like, porous network (Plate 16). In the SEM of barnyard millet starch after five cycles, this porous structure was still evident in some parts but more compact formations predominated (Plate 17).

In RS residues isolated from treated starch, the porous structure was no longer visible and most likely removed by enzymic treatment. In the oven dried residue very compact and dense formations could be observed (Plate 18). The vacuum dried residue
formed an open, fluffy structure (Plate 19). Similar results of SEM on maize starch and RS were reported by Sievert and Pomeranz (1989).

5.6. Brabender visograms

The determination of the pasting behaviour with a Brabender visograph (or amylograph) has long been a standard analytical tool in starch and cereal research. Brabender visograms are usually determined at an arbitrarily fixed starch (or flour) concentration and the resulting peak viscosity (P), break down (BD) and set back (SB) values were used as criteria for starch characterisation (Bhattacharya and Sowbhagya, 1978). Fukuba (1954) and Fukuba and Yamamoto (1954) were the first to study the Brabender visogram of rice flour. They found that indica rice flour showed higher viscosity than japonica rice and wheat starches having higher amylose gave higher cooling curves. Shortly afterwards, Halick and Kelly (1959) made a detailed study of the pasting curves of a large number of varieties. Since then this test has been extensively used by researches of rice quality in various laboratories. A rice flour slurry concentration of 10% (wet basis) has been generally employed.

Gelatinisation temperature is the measure of loss of birefringence and depends upon the rigidity of granules. Small millet starches exhibited comparatively higher gelatinisation temperature than rice and other cereals. Higher gelatinisation temperature for millet starches may be due to the presence of high content of amylose (Malleshi, 1984). Gelatinisation temperature was high in barnyard and low in proso millet (Table 17) (Fig. 1 and 8). Foxtail, Kodo and little millets have got intermediate gelatinisation temperature (Fig. 9, 10 and 11 respectively). Regression analysis showed, among small millets, significant positive correlation between amylose and the gelatinisation temperature (r=+0.937**). Juliano et al. (1964a) also reported similar correlation between amylose
and gelatinisation temperature in rice which ranged from 56.5°C to 71°C. Hadimani and Malleshi (1993) reported that the gelatinisation temperature of small millets starch were around 73-77°C except for little millet (80°C). The starch was not isolated from the grain by Hadimani and Malleshi (1993).

The peak viscosity of proso millet was high which was followed by foxtail, little, kodo and barnyard millets. Hadimani and Malleshi (1993) reported that the peak viscosity was highest for proso millet and lowest for foxtail millet. Negative correlation was seen between the amylose content and the peak viscosity in the present study \((r=-0.943 **)\). Correlation between peak viscosity and amylose content was also observed by Halick and Kelly (1959). Generally sticky rice show higher peak viscosity and long grain varieties (less sticky rice) show lower peak viscosity. The drop in starch paste viscosity on cooking at 95°C, relative to peak viscosity is a measure of the degree of disintegration of gelatinised granules (Mazurs et al., 1957). The final viscosity at 95°C ie. hot paste viscosity was low in barnyard millet and high in proso millet. Kodo, little and foxtail millets showed the intermediate hot paste viscosity.

The viscosity of the cooked starch pastes on cooling to 50°C reflects the degree of reassociation (retrogradation) of amylose (Mazurs et al., 1957). Barnyard millet showed the highest cold paste viscosity which differ significantly from other small millets and this was followed by kodo, little, foxtail and proso millets. Positive correlation was obtained between amylose and cold paste viscosity \((r=+0.932 **)\). Generally, the viscosity of the cooked rice pastes when cooled to 50°C was positively correlated with amylose content (Juliano et al., 1964a). High cold paste viscosity indicates high degree of retrogradation of amylose and formation of RS. In the present study, barnyard millet starch showed the highest cold paste viscosity which will favour the formation of higher amount of resistant starch than the other small millets.
Breakdown (BD), the drop in viscosity on cooking at 95°C, is a measure of fragility of the starch. Proso millet starch showed highest BD value followed by foxtail, little and kodo millets. Barnyard millet showed lowest BD value which was significantly different from other small millets. Regression analysis showed negative correlation between the amylose content and the breakdown value ($r=-0.994^{**}$). The breakdown is presumably an indication of the ease with which the swollen starch granule can be disintegrated i.e. of the degree of its organisation. It is therefore significant that the BD data bore an excellent inverse relation to the amylose content of the sample (Bhattacharya and Sowbhagya, 1979). Juliano et al. (1964a) also reported the negative correlation between amylose content and break down value in rice. Low break down value indicates less susceptibility by the enzyme attack.

The present study indicated, the break down value of barnyard millet starch was low when compared to other small millets which also showed less susceptibility to porcine pancreatic $\alpha$-amylase. Regression analysis showed positive correlation between the break down value and the rate of hydrolysis of starch by porcine pancreatic $\alpha$-amylase in vitro ($r=+0.985^{**}$).

Set back value is a measure of starch retrogradation (Bhattacharya and Sowbhagya, 1979). ie. the rise in viscosity on cooling with reference to the peak viscosity. Barnyard and kodo millets have got higher set back value and differed significantly from other small millets. Proso and foxtail millets showed lower set back value. Little millet showed intermediate set back value. Set back value of small millets was positively correlated with amylose content ($r=+0.938^{**}$). Set back values of rice pastes have been shown to be positively correlated with amylose content (Beachell and Stansel, 1963, Halick and Kelly, 1959 and Juliano et al., 1964a). High amylose content is associated with high set back.
Total set back is the total or entire rise in viscosity occurring during cooling from the point where the cooking ended. Total set back value was low for proso millet and high for barnyard millet. Regression analysis showed that positive correlation exist between amylose content and total set back value \((r=+0.936^{**})\).

Relative break down (BD/SBt) ie. BD\(_r\) expresses relative magnitude of break down as a function of the total set back (Bhattacharya and Sowbhagya, 1978). Horiuchi (1967) observed that the BD\(_r\) was inversely related to the amylose content. BD\(_r\) value of the small millets starch was low for barnyard, kodo and little millets. Proso and foxtail millets recorded the high BD\(_r\) value. BD\(_r\) was negatively correlated with amylose content \((r=-0.974^{**})\). This is in agreement with reports by Bhattacharya and Sowbhagya (1979). Mazurs et al. (1957) reported the BD\(_r\) value of corn and wheat as 0.42 and 1.6 respectively. Low BD\(_r\) value indicates the low susceptibility by enzyme attack. BD\(_r\) value of barnyard millet starch was lower than the other millets which also indicates the low susceptibility by enzyme attack. The per cent hydrolysis of barnyard millet starch by porcine pancreatic \(\alpha\)-amylase \textit{in vitro} (Fig. 6 and 7) was low which confirms the above data. Positive correlation was noticed between the relative break down value and the per cent hydrolysis of starch by pancreatic \(\alpha\)-amylase \textit{in vitro} \((r=+0.975)\).

The cooking time for small millets ranged from 3-6 minutes and for pearl millet it was 9 minutes. Quick hydration and softening property of millets may be advantageous for the development of flaked products and quick cooking cereals. The low break down viscosity during continuous heating phase at 95°C indicates the stability of millets for use in porridges (Hadimani and Malleshi, 1993).

The viscosity indices have been observed to correlate significantly with eating and other quality parameters of starch and also with amylose content. Halick and Kelly (1959)
noted that long-grain varieties ie. those with higher amylose, usually gave a positive set back and that they had generally lower peak viscosity. Kurasawa et al. (1962, 1969) observed that sticky rice (low in amylose) preferred by Japanese, generally showed a higher peak viscosity and break down and lower set back as compared to less sticky rice (high in amylose). In the present study, proso millet showed a higher peak viscosity and break down and lower set back as compared to barnyard millet (high in amylose) which is in agreement with the above results. Tani et al. (1969) and Chikubu (1967) in Japan, and Hampel (1965, 1967) in Germany made similar observations. Such correlations have also been made by Beachell and Stansel (1963), Juliano et al. (1964a, 1964b), Juliano and Perdon (1975), Lorenz et al. (1976) and Juliano and Pascual (1980).

In countries such as Malaya, the important cooking quality requirements are flakiness and high volume expansion when cooked (Van, 1960). The preferred varieties have high amylose content, which is consistent with the observations that high amylose rices are flaky or less sticky than low amylose samples (Williams et al., 1958). Amylose content is now well recognized as the most important determinant of rice quality (Bhattacharya et al., 1982). In the philippines and in Indonesia atleast two characteristically different types of rice quality are preferred. Most persons want a rice that remains soft after cooking, even when cold. Other persons prefer a rice that has high volume expansion and is flaky when cooked. Cooked rice of high amylose varieties hardens on cooling as a result of amylose retrogradation. This is reflected in high set back values. Cooked rice of high amylose varieties hardens on cooling whereas low amylose varieties provide softer cooked rice (Juliano et al., 1964a). Cooked barnyard millet hardens on cooling whereas proso millet provide softer cooked consistency. In the present study, barnyard millet showed high gelatinisation temperature. According to several reports the gelatinisation temperature of starch was generally increased at a higher amylose content (Colonna and Mercier, 1985, Eliasson et al., 1988). A higher amylose
content restricts granule swelling at conditions used during food processing, a reduced enzymic availability would be expected, which was confirmed in the present study (Fig. 5 and 6).

Panlasigui et al. (1991) reported rice with high gelatinisation temperature showed low glycemic index value and rice with low gelatinisation temperature showed high glycemic index. Barnyard millet starch showed high gelatinisation temperature and low glycemic index whereas proso millet showed low gelatinisation temperature and high glycemic index.

Juliano and Goddard (1986) also reported that low amylose rices had low gelatinisation temperature which is in agreement with the present result ie. proso millet starch with low amylose content registered low gelatinisation temperature.

The peak viscosity of barnyard millet was lower when compared to other small millets which indicates, the stickiness of this small millet is low.

The cold paste viscosity of barnyard millet starch was higher than the other small millets starch which indicates high degree of retrogradation of amylose and the formation of higher amount of resistant starch, than the other small millets.

Set back value is an indication of amylose content and also measure of starch retrogradation. Barnyard millet starch showed the highest set back value which may be due to the high content of amylose. Barnyard millet starch showed minimum breakdown (BD) and relative break down (BDr) value than the other small millets which suggested less susceptibility of the starch to $\alpha$-amylase.
5.7. Chain length of amylose and amylopectin

Starch is a major reserve polysaccharide in plants. It consists mainly of two glucose polymers; amylose and amylopectin. In amylose, which is essentially linear, the glucose units are alpha-(1-4) linked while only very few alpha-(1-6) linkages are present. The molecules, with a degree of polymerisation (DP) in the range of 500-6000 glucose residues, are divided into one to 20 chains (Hizukuri et al., 1981). Amylopectin, on the other hand, is highly branched. Apart from alpha-(1-4) linkages, alpha-(1-6) branch points are present (4-5% of the glycosidic bonds).

The chain length of amylose from proso millet was significantly higher than other millets (Table 18). Barnyard millet recorded lower chain length in amylose (275 glucose units). Amylose from kodo, little and foxtail millets showed the chain length of 285, 300 and 310 glucose units respectively.

Amylopectin from barnyard millet recorded the highest chain length (25.0) whereas amylopectin from proso millet recorded the lowest chain length (18 glucose units).

Takeda et al. (1986a) reported that the average chain length of amylose from rice, varied from 250-370 glucose units. The amyloses from amylomaize had lower chain length values than normal maize (chain length 295-335) and those of other sources (270-670) (Hizukuri et al., 1981, Takeda et al., 1984, 1986a, 1986b, 1987). Amylose from barnyard millet (high amylose) showed lower chain length which is in agreement with the above results.

Hizukuri (1985) determined the chain length of amylopectins from 20 species. The average chain length of the amylopectins was in the range of 23-44 glucose units. Banks
and Muir (1980) observed an average chain length of about 21 and 25 for isolated amylopectin from normal and high amylose barley respectively. In the present study isolated amylopectin from barnyard millet (high amylose) showed higher chain length which was in agreement with the above results. Thayumanavan (1987) reported the chain length of 21-26 glucose units for amylopectin from rice. Jane and Chen (1992) reported the average chain length for amylopectins of high amylose corn (30.9), waxy maize (18.6) and normal rice (17.8). Biliaderis et al. (1981) reported the average chain length for most of the amylopectins ranged from 20 to 26 glucose units. The present results are in agreement with these results.

5.8. Formation of resistant starch

Resistant starch is a fraction of starch not digested in the small intestine. It may, however be (partially) fermented in the large bowel by the microflora, depending upon the source (Englyst and Macfarlane, 1986; Gee et al., 1992a). Since RS is not digested in the small intestine, it lowers the caloric density of foods. As a result of fermentation of RS in the large intestine by the microflora, an increase in short chain fatty acids excretion was observed. RS may protect against colon cancer by providing faecal bulk and stabilizing colonic cell proliferation (Gee et al., 1992b). Foods that are high in RS can be recommended for patients with chronic disease such as diabetes, hyperlipidemia and obesity (Panlasigui et al., 1990).

RS was comparatively very low both in native and treated starches of rice when compared to the five small millets (Table 19). Among the small millets, RS in native starch was markedly lower in proso millet and foxtail millet as compared to barnyard millet; kodo millet and little millet were intermediate. Among treated starch, barnyard millet registered higher content of RS and differed significantly from the rice.
RS in native and treated starch in small millets were positively correlated with absolute amylose content ($r=+0.992^{**}$, $r=+0.918^{**}$ respectively). Though the amylose content of rice was high, the RS was comparatively very low when compared to all the small millets which may be due to their physicochemical properties (Panlasigui et al., 1991).

A positive correlation has been reported between amylose content of the starch and RS formation in wheat which is in agreement with the present study (Berry, 1986, Sievert and Pomeranz, 1989). Yield of up to 8.45% RS was reported when native starch of wheat was subjected to five repeated cycles of autoclaving and cooling (Ranhotra et al., 1991). Bjorck et al. (1987) have reported changes in RS formation due to processing parameters such as autoclaving, cooling cycles and freeze drying. Autoclaving and cooling cycles favour RS formation more than autoclaving temperature (above 100°C) or the freeze drying step. They have reported the formation of 8 per cent RS from wheat starch, a value similar to that reported by Sievert and Pomeranz (1989). Eggum et al. (1993a) reported a high correlation between in vitro resistant starch values and values for apparent amylose content of the milled rices. A similar observation was also made by Russell et al. (1989). The present study is in agreement with the above results.

The starch in cooked foods, e.g. bread, breakfast cereals and potato is known to retrograde on storage. Retrogradation, the formation of crystallites (predominantly small aggregates of highly structured hydrogen-bonded amylose) results in a fraction of the starch becoming resistant to hydrolysis by $\alpha$-amylase both in vitro (Kerr, 1950) and in vivo (Bjorck et al., 1986). Other starch complexes such as starch lipid may also be present, but these have been shown to be digested by $\alpha$-amylase (Holm et al., 1983). Starch fractions resistant to $\alpha$-amylases are collectively called resistant starch (Englyst et al., 1982) and manipulation of variables during processing of high starch foods may
alter the levels of RS occurring in the product (Berry, 1986). Starch that remains undigested in the small intestine, constitutes an easily available substrate for the colonic microflora where they are (partly) fermented depending upon the source. Active fermentation in the colon is conducive to good health (Englyst and Macfarlane, 1986) which has preventive role in the genesis of colonic diseases e.g. adenoma (Thorne et al., 1985) carcinoma (Anon., 1988) and ulcerative colitis.

5.9. Comparative study of treated starch from small millets on body weight, intestinal responses, blood glucose, serum cholesterol and triacylglycerol in rats

Till recently starch was thought to be completely hydrolysed, and absorbed from the small intestine because of the presence of excess quantities of salivary and pancreatic $\alpha$-amylase (Englyst and Cummings, 1987). But, it is now generally accepted that important amounts of starch in ordinary food items escapes digestion and absorption in the human small intestine, (Englyst and Cummings, 1985).

The microflora of the large intestine however is able to ferment partly and the extent of fermentation depends upon the source. RS has important physiological effects similar to dietary fibre. ie. easily fermented types of dietary fibre have poor bulking capacity and dietary fibre that resists bacterial degradation has good bulking capacity. Hence, the present study was carried out to find out the effect of RS on faecal bulking capacity and the blood glucose, serum cholesterol and triacylglycerol in rats. Diet A, B and C from rice fed rats showed significant increase in gain in body weight than the rats which consumed test diets (Table 20). Rats fed with diet A and B from barnyard and kodo millet showed minimum weight gain which may be due to the slower rate of digestion and due to the presence of higher amount of resistant starch.
Rats fed with control diet from rice showed significant decrease in faecal wet weight than the rats fed with test diets (Table 20). Rats fed with diet B and C from control and test showed about 6 and 18 fold increase in faecal wet weight respectively than diet A from control and test. Faecal wet weight was significantly high in rats which received diet from barnyard and kodo millets which may be due to the higher content of resistant starch and soluble dietary fibre that resists hydrolysis by α-amylase. Faecal wet weight was positively correlated with in vitro resistant starch content of diet A (r=+0.999**) diet B (r=+0.965**) and diet C (r=+0.836**) which indicates that the higher faecal wet weight was due to the higher content of resistant starch in barnyard millet both in native and treated starch. Rats which received diet C from control and test showed significant increase in faecal dry weight (Table 20). Among the small millets rats received diet A, B and C from proso millet registered the minimum faecal dry weight which may be due to the lower content of resistant starch. Positive correlation was found between the faecal dry weight and in vitro resistant starch content of diet A (r=+0.992**), diet B (r=+0.958**) and diet C (r=+0.940**) which proves that the higher faecal dry weight of barnyard millet was due to higher content of resistant starch in NS and TS.

Rats which received diet B and C (from control and test) showed significant increase in faecal volume when compared to rats which received diet A from control and test diet (Table 20). Highest faecal volume was recorded by the rats which received diet A and B from barnyard millet. Faecal volume was correlated significantly with in vitro resistant starch content of diet A (r=+0.991**), diet B (r=+0.90**) and diet C (r=+0.942**).

Human epidemiological studies show lower incidence of colorectal cancer in population groups that consume diets high in fibre (Trowell, 1985). Various hypotheses
were put forward to explain this, one postulate is that a higher faecal bulk would result in dilution of potential carcinogens in the intestinal lumen.

Faecal bulking capacity of fibre sources varies. Sources that are high in insoluble fibre such as wheat bran provide more faecal bulk than those that are high in soluble fibre, a fraction highly fermentable in the colon (Nymann and Asp, 1982). For all the varieties, treated starch in the diet increase the faecal wet weight over 6 fold in comparison with native starch (diet B vs A). The increase in faecal dry weight was of a similar magnitude when the hindgut bacterial population was effectively, if not completely, suppressed by antibiotics the increase in faecal wet weight was even greater, nearly 18 fold (diet C vs A). Thus faecal weight and volume measurements suggested that the faecal bulking capacity of RS was substantial. Among the five small millets, barnyard millet has the maximum faecal bulking capacity followed by kodo, little, foxtail and proso millets.

**Digestibility of resistant starch**

Rats which received diet A and B from barnyard millet showed minimum digestibility (Table 20). Diet A and B from proso millet showed maximum digestibility which may be due to the presence of lower content of resistant starch, which paves the way for maximum hydrolysis.

RS is viewed by Berry (1986) and Bjorck et al. (1987) as component like soluble fibre that was easily fermented by bacteria. It was however measured as essentially all insoluble fibre and expected to ferment less readily than soluble fibre. According to Ranhotra et al. (1991), in the group of rats fed with treated starch without antibiotics, about one third of RS consumed was fermented. In the present study, when hindgut
fermentation was suppressed by antibiotics, only 11.64% - 15.13% of the RS consumed was digested. This suggests that RS is highly resistant to mammalian amylolytic enzymes.

Ranhotra et al. (1991) reported that the rats fed with treated starch of wheat (no antibiotics) digested 37.1% RS; those fed treated starch of wheat with antibiotics digested only 14.3% RS. RS thus appears to be highly resistant to mammalian enzymes and may be classified as a component of fibre. Faulks et al. (1989) reported that, in the rats, resistant maize starch appears to be partially digested and the remainder being almost totally fermented after an initial adaptive period. In contrast, resistant pea starch appears both less available and more resistant to fermentation. They have concluded that substantial differences in utilisation in vivo may exist between RS from various food, which is in agreement with the present study.

Tomlin and Read (1990) reported that RS in its various forms contribute more substrate to the colonic bacteria than the other polysaccharides. RS may, therefore, be very important in maintaining normal colon function.

Muir et al. (1993) reported that the amount of starch reaching the colon was greatly influenced by the nature of the diet (ie. quantity and botanical sources of the starch) and the ways in which food has been processed. They have also reported that the high RS meal resulted in an increase in the dry weight of material escaping digestion in the small intestine which increases the faecal bulking and changes in faecal pH both of which may also have important implications for bowel disorders such as constipation, diverticulitis, hemorrhoids as well as bowel cancer. Englyst and Macfarlane (1986) reported that an active fermentation in the colon was conducive to good health which may be achieved by an increased consumption of RS. Feeding of foods containing non-starch polysaccharides (fibre) leads to increased faecal bulk in humans and animals. Products
such as wheat bran increase stool mass in humans (Jenkins et al., 1987a). Feeding of raw starch or retrograded amylose (prepared by gelatinisation of high amylose corn starch) leads to increased faecal mass (Mazur et al., 1990).

**Effect of treated starch on blood constituents**

Significant reduction in blood glucose was noticed in rats which received diet A, B and C from barnyard millet (Table 21). Rats which received diet B and C from control showed significantly higher value of serum cholesterol than the rats fed with diet B and C prepared from millets (Table 22). Gee et al. (1991) reported that there was no significant effects of dietary resistant starch on serum cholesterol in experimental rat. Toshinao et al. (1994) also reported, there was no significant difference in serum cholesterol in rats fed with high amylose diet.

Rats which received starch from small millets (test) showed significantly lower level of serum triacylglycerol than the rats which received starch from rice (control). Among the small millets, the percentage reduction in serum triacylglycerol was minimum in proso millet and maximum for barnyard millet (Table 23). Toshinao et al. (1994) reported that the serum triacylglycerol level in the rats fed with the high amylose diet was significantly lower than that of the control group. From these studies it was concluded that the RS from barnyard millet may be considered as an effective faecal bulking, hypoglycemic and hypolipidemic agent which can be recommended for patients with diabetes, cardiovascular disease and hyperlipidemia.
5.10. Glycemic index

5.10.1. Glycemic index in normal human beings fed with native and treated starch of rice and small millets

Normal human beings fed with native starch of rice and small millets recorded higher glycemic index when compared to treated starch of rice and small millets (Fig. 14 and 17). Normal human beings who received native starch of barnyard millet showed significantly lower glycemic index when compared to rice based diet which may be due to the higher content of resistant starch. Treated starch of rice showed significantly higher glycemic index when compared to treated starch of small millets. Among small millets, treated starch of proso and foxtail millets showed higher glycemic index than the treated starch of other small millets. Groups fed with treated starch of barnyard millet showed significantly lower glycemic index as in the case of native starch.

5.10.2. Glycemic index in non-insulin dependent diabetes mellitus (NIDDM) patients fed with native and treated starch of rice and small millets

NIDDM patients, fed with native starch of rice and small millets showed significantly higher glycemic index, when compared to treated starch of rice and small millets (Fig 20 and 23). NIDDM patients who received treated starch of rice showed significantly higher glycemic index when compared to treated starch of small millets. There was no significant difference in the glycemic index between native starch of kodo and little millets. NIDDM patients who received native starch of barnyard and kodo millets showed significantly lower glycemic index which may be due to the higher content of resistant starch.

Among the groups fed with treated starch, NIDDM patients fed with control diets (treated starch of rice) showed significantly higher glycemic index when compared to the
test diets (treated starch from small millets) (Fig. 23). NIDDM patients who received treated starch of proso millet showed higher glycemic index than the other small millets. Treated starch of barnyard millet showed significantly lower glycemic index. Regression analysis showed, negative correlation between glycemic index and \textit{in vitro} resistant starch content in normal human beings ($r = -0.963^{**}$ for NS, $r = -0.915^{**}$ for TS) and in NIDDM patients ($r = -0.921^{**}$ for NS and $r = -0.892$ for TS). Eggum \textit{et al.} (1993a) reported negative correlation between glycemic index and \textit{in vitro} resistant starch content in rice ($r = -0.75$), which is in agreement with the present study.

The resistant starch contents of both native and treated starch of the barnyard millet was significantly higher when compared to other small millets. Both in the case of normal human beings and NIDDM patients, barnyard millet showed lower glycemic index which may be due to the higher content of resistant starch which is in agreement with the results reported by Eggum \textit{et al.} (1993a).

Barnyard millet possessed higher soluble dietary fibre, recorded higher gelatinization temperature and low amylograph viscosity when compared to other small millets studied. These factors may also contribute to the lower glycemic index recorded by barnyard millet. This is also in agreement with the \textit{in vitro} hydrolysis experiment conducted on the isolated starch from barnyard millet by porcine pancreatic $\alpha$-amylase which showed slower rate of hydrolysis.