

## CHAPTER 3

Objective 2: Determination of the changes in carbohydrate profile and protein content of wheat and bengal gram in response to the process of germination

### Introduction

The sum total of physical and chemical changes which take place during malting is termed as 'modification' which cause transformation of a tough grain into a friable malt (Pomeranz 1972). The process of malting involves steeping followed by germination and kilning of a grain. During the process of malting, modification in a grain is brought about by the activation and elaboration of hydrolytic enzymes, which degrade complex carbohydrate and protein fractions of a grain (Briggs et al 1981, Briggs and MacDonald 1982).

### Seedling weight and malting loss

Kneen et al (1942) had determined the changes in total, green and dry weight of wheat grains germinated over a period of 4 days at 20°C, 6 days at 15°C, 10 days at 10°C and 24 days at 5°C. It was observed that irrespective of the germination temperature, green weight increased and the dry weight decreased with the increase in germination period. After 4 days of germination, at 20°C, the green weight increased by 34.7 g, from 30.0 to 64.7 g and the dry weight decreased by 7.7 g, from 26.7 to 19.0 g. The reduction in germination temperature by 5°C after 4 days of germination led to a relatively smaller increase in

green weight than that observed at 20°C (31.8 Vs 34.7 g). The increase in germination period from 4 to 6 days at 15°C increased the green weight by 59.5 g. But there were no marked changes in dry weight over the germination period of 6 days. At 10°C, the green weight increased by 23.1 g (from 30.0 to 53.1 g) after 4 days, by 30.0 g (from 30.0 to 60.0 g) after 6 days and by 69.2 g (from 30.0 to 99.2 g) after 10 days of germination. As was observed in grains germinated at 15°C, no appreciable changes in dry weight were observed at 10°C. At each germination period the increase in green weight was lower at 5°C than at 10°C. The green weight increased from 30.0 to 49.8, 50.7, 53.5 and 95.5 g after 4, 6, 10 and 24 days of germination, respectively. The dry weight was found to decrease from 26.7 to 22.7 g after 24 days of germination. This decrease of 4.0 g in dry weight after 24 days of germination at 5°C was lower than that (7.7 g) observed at 20°C after 4 days of germination, thereby indicating that regardless of germination period losses in dry weight increased with increase in germination temperature which can be attributed to faster growth rate of seeds at higher temperatures.

Malting loss is the percentage reduction in dry weight occurring in the conversion of a grain to a finished malt. The malting losses are attributed to losses due to leaching during steeping, losses due to the formation of carbon dioxide and water, and losses due to separation of rootlets (Briggs et al 1981).

Malting yields of 3 varieties of wheat at 3, 5 and 7 days of malting were recorded by Sethi and Bains (1978). In one

variety, the malt yields, respectively, were 87.6, 85.7 and 85.5%. In the second variety, the malt yield after 3 days of malting was 85.8%, after 5 days of malting was 84.7% and after 7 days of malting was 82.1%. In the third variety, the malt yield was 92.7% after 3 days of malting which decreased to 89.9% and to 87.8% after 5 and 7 days of malting respectively. In all the 3 varieties of wheat decreases in malt yields with increase in germination time were observed. Singh et al (1983) had observed that the malting yield of Triticum dicocum khapli wheat was 93.5% after 3 days, 91.0% after 5 days and 90.0 after 7 days of malting. The malting yields of wheat ranged from 85.8 to 93.5% after 3 days, from 84.7 to 91.0% after 5 days and from 82.1 to 90.0% after 7 days of malting.

Gupta et al (1985) reported that malting losses in 2 varieties of triticale increased with the duration of germination period from 2 to 6 days and the level of steeping moisture from 38 to 42%. A variety of steeped triticale having 38% moisture exhibited a malting loss of 2.6 to 7.8% when the germination period was increased from 2 to 6 days. But in the same variety of triticale having 48% moisture the malting loss was higher, it was 3.3 to 12.3%. Likewise, in another variety of triticale the malting loss was between 3.3 and 5.8% when the moisture content was 38%, and 6.1 and 10.1% when the moisture content was 42%. Earlier, Wu (1982) had demonstrated a weight loss of 10% in triticale after 3 days of germination which increased to 15 and 23% when the germination period was increased to 6 and 8 days, respectively.

Similar observations have been made <sup>known</sup> in corn (Singh and Bains 1984). The authors had malted 2 varieties of corn and found that malt yield in both the varieties decreased with the increase in germination period. In one variety of corn, the malt yield was 94.8% after 3 days of germination which decreased to 93.6 and 88.6% after 5 and 7 days of germination, respectively. In another variety of corn, the malt yield decreased from 95.0 (3 days of germination) to 92.7% when the germination period was increased to 5 days and to 89.8% when the germination period was further increased by 2 days.

Earlier, malt yields of 2 varieties of barley were recorded by Singh and Bains (1977). In one variety of barley, the malt yield decreased from 87.2 to 80.6% and in the other variety it decreased from 88.8 to 84.8% when the germination period was increased from 3 to 9 days. These data indicated that the increase in the number of germination days increase malting losses. Pathirana et al (1983) have observed that malting losses were directly proportional to the number of days a grain was allowed to germinate. However, the relationship between the steeping period and malting loss was not clear although the results did indicate that longer steeping periods led to faster rates of germination and consequently higher malting losses.

Malleshi and Desikachar (1979) had malted 9 varieties of ragi and found that the malt yields ranged from 62.8 to 70.8% exhibiting malting losses of 29.2 to 37.2%. Malleshi (1983) had demonstrated that the malting losses in millets, maize, sorghum,

rice, wheat and triticale after 48 h of germination ranged from 2.9 to 5.5% and after 96 h of germination from 6.8 to 16.2%.

In 17 varieties of sorghum, Jayatissa et al (1980) had observed that malting losses ranged from 4.2 to 19.4% after 4 days of germination and from 8.4 to 30.3% after 6 days of germination thereby indicating that variety of a grain influenced malting loss.

#### Malting and carbohydrates

As early as in 1934, Acharya had concluded through a series of experiments on sorghum malt that the enzyme diastase was responsible for the hydrolysis of starch and that it had 2 main components, alpha and beta amylases. The alpha amylase acted on the amylopectin and the beta amylase on the amylose fraction of starch. About 8 years later, Hildebrand and Burkert (1942) explained that the alpha amylase ruptured the starch molecule at more or less central linkages producing degradation products of relatively high molecular weight. The hydrolytic products of beta amylase were predominantly of low molecular weight.

Many studies have been conducted on the development of enzymes during germination. MacLeod et al (1964) followed the development of hydrolytic enzymes - notably alpha amylase, endo-beta glucanase and proteinase in germinating barley. It was noticed that the endo-beta-glucanase formation preceded the formation of alpha amylase which, in turn, preceded the formation of protease. They also determined the time of initiation of the

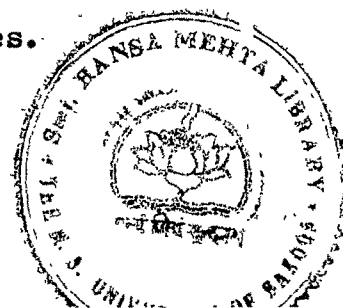
enzymes in the germinating barley grain and found that the enzymes, endo-beta-glucanase, alpha amylase and protease were initiated at 24, 29 and 30 h of germination, respectively. The authors explained that as one of the enzymes, viz., endo-beta-glucanase was mobilised before the initiation of alpha amylase activity, the carbohydrate of the wall was partially degraded before the starch granules in the endosperm were attacked by amylolytic enzymes.

That a new kind of alpha amylase with different electrophoretic pattern than the original amylase develops during germination of wheat was reported by Olered and Jonsson (1970). They observed that during the initial stages of germination, the amylase activity increased owing to the combined action of both the original amylase and the new form.

Kruger (1972) determined the changes in the amylases of hard red spring wheat during germination. He found that alpha amylase appeared after 2 days of germination and steadily increased in amount with the increase in germination time. Two sets of amylases were found, one set of 3 isoenzymes was electrophoretically identical to those found in immature kernels whereas second set of 4 slower isozymes was found only in germinated grain. Amylase formation in germinated seeds was found to occur by de novo synthesis. The electrophoretic identity between the faster set of germinated alpha amylases and the alpha amylases in immature kernels suggested that they might be related. The authors were of the view that the amylases of the immature

kernels were regenerated during germination forming the electrophoretically faster set of germinated alpha amylases, while de novo synthesis was responsible for the electrophoretically slower set of alpha amylases. He also observed that the main beta amylase components of wheat began to disappear at 2 days of germination and totally disappeared after 4 days of germination. Instead, a new electrophoretically slower beta amylase was formed, which increased in activity with time of germination. The author opined that the coincident disappearance of the sound beta amylase with the appearance of the germinated beta amylase might indicate that at least part of the formation of the new amylase is by transformation of one into the other.

Within the various components of the germinated grain, the distribution of amylases has also been investigated. Briggs (1964) determined the origin and distribution of alpha amylase in various parts of germinated and dehusked whole barley. It was observed that in malt about 7% of the alpha amylase was located in the embryo and 93% in the endosperm, of the latter about 6.5% was of embryo origin and the remaining 86.5% originated in the aleurone layer. It was also noticed that during germination, the alpha amylase increased more rapidly in the proximal part of the endosperm than in the distal part. The rapid increase in alpha amylase in the proximal part of the endosperm was attributed to the progressive diffusion of gibberellin from the embryo which stimulated the aleurone layer to release enzymes.



Kneen et al (1942) observed that the appearance of sprouts was preceded by a considerable development of alpha amylase in wheat grain. The authors demonstrated that as growth progressed the enzymic development roughly paralleled sprout elongation. They also found a relationship between temperature and degree of germination. It was observed that particularly in the early stages of seedling development, the lower the germination temperature the higher was the amount of active alpha amylase per millimeter of sprout length. But the time duration required for the production of both sprouts and enzyme at lower temperature was higher than that observed at higher temperature. However, in the later stages of germination the temperature effect was found to be minimum as seedlings with equal sprout lengths had approximately equal alpha amylase activity. Similar effects of germination temperature and time on amylase activity in bajra (Pennisetum typhoidæum) have been shown by Singh and Tauro (1977).

Kneen in 1944 confirmed their previous findings that the degree of germination of a grain is determined by increase in alpha amylase content. In various cereals such as barley, wheat, rye (Secale cereale), oats, maize, sorghum and rice, the alpha amylase activity increased during germination of these grains. However, when the alpha amylase activity at a given sprout length of one grain was compared to that of another grain having the same sprout length, it was observed that the amylase production was not proportional to the sprout length. The germinated oat which had the longest sprouts (25 to 35 mm) ranked



fifth in alpha amylase production showing less than one-third the activity of alpha amylase in wheat grains having a sprout length of 20 to 30 mm. Lack of correlation between the degree of germination and alpha amylase activities in wheat and rye has also been reported by Olered and Jonsson (1970).

More recently, Raynes and Briggs (1985) investigated the effect of factors other than temperature on production of alpha amylase during germination of barley grain. The authors observed no relationship between grain nitrogen content and alpha amylase yield. But it was noticed that heavier/larger grains produced more alpha amylase per grain. However, smaller/lighter grains sometimes had larger alpha amylase contents than the larger grains per unit weight of flour. The authors further explained that the endogenous gibberellin levels and the enzyme forming capacities of grain were also important factors in determining the alpha amylase yields.

Earlier, Atanda and Miflin (1970) had shown that the levels of alpha amylase activity differed considerably in 20 different varieties of barley germinated for 3 to 4 days. It was observed that alpha amylase activity ranged from 75 to 337 units/seed. Such variations in amylase activity due to varietal differences have been demonstrated by many investigators in wheat, barley, jowar, bajra, ragi, corn and triticale (Shands et al 1942, Jain and Date 1975, Pal et al 1976, Sethi and Bains 1978, Malleshi and Desikachar 1979, Singh and Bains 1984, Gupta et al 1985).

Studies have been conducted to determine the changes in starch of germinated grains. Greenwood and Thomson (1959) compared the starches of unmalted and malted barley grains. The authors had observed a decrease in the starch content which was attributed to the loss in amylopectin component. The malted barley starch possessed a higher amylose content, smaller granules and needed a higher gelatinisation temperature than that of the original barley. The higher gelatinisation temperature of the malted barley, according to the authors, was related to both its smaller granular size and its higher amylose content.

Lineback and Ponpipom (1977) through scanning electron photomicroscopy observed that the endosperm of ungerminated wheat contained large and small starch granules embedded in a cementing matrix. After 2 days of germination, enzymatic erosion of wheat starch granules in the endosperm became visible. It was also observed that the starch granules near the aleurone layers of the kernel were attacked more severely than starch granules in the inner endosperm. The starch granules isolated from wheat germinated for 4 days showed enzymatic erosion on surfaces and along the equatorial groove. Most of the enzyme attack was confined to the larger granules, which suggested that these were more susceptible to amylolytic degradation than smaller ones.

Earlier, Dronzek et al (1972) had also studied the changes in starch granules during germination of wheat. They found that at an early stage of germination most of the enzyme attack was confined to the large starch granules. However, after 8 days of

germination, both the large and small starch granules were severely eroded. It was also observed that the starch granules near the aleurone layer of the kernel were attacked more severely than those in the inner endosperm which suggested that in the germinated wheat the amylase activity was higher in the aleurone than in the inner endosperm. However, even after 8 days of germination, all the starch granules were not eroded by the amylase as there were still a large number of intact starch granules.

MacGregor and Matsuo (1982) explored the site of starch degradation in wheat kernels during initial stages of germination. It was observed that starch degradation started at the endosperm-embryo junction, usually close to the ventral crease, and moved along the junction to the dorsal edge of the kernel. This degradation was preceded by extensive breakdown of cell wall material and the protein matrix of the endosperm. The authors were of the opinion that the site of initial alpha amylase synthesis in germinating cereal grains was the embryo and not the aleurone layer. They also believed that the aleurone synthesized alpha amylase may not be responsible for starch degradation during malting and that the scutellar epithelium was mainly responsible for the synthesis of alpha amylase during initial stages of germination.

On the other hand working with germinated barley grain, MacLeod and Palmer (1966) had reported that isolated scutellum had a very limited ability to produce alpha amylase and its

powers of enzyme secretion were largely restricted to the peripheral regions where aleurone cells were present. According to the authors the stimulus which initiated enzyme formation in the aleurone was largely derived from the nodal region of the embryo especially from the base of the node where subsidiary rootlets were formed. The translocation of the enzyme inducing hormone from embryo to aleurone, took place through the apical half of the scutellum in which vascular tissue developed. Later, Palmer (1982) supported the results of MacLeod and Palmer (1966). In germinating barley, Palmer (1982) observed that the enzymic modification of the endosperm was under aleurone rather than scutellar control. The author also found out that excised barley embryos could produce alpha amylase because the peripheral areas of the scutellar tissue contained aleurone cells while the aleurone-free tissue of the scutellum was incapable of producing significant quantities of alpha amylase.

Fretzdorff et al (1982) worked with kilned barley malt and observed that the hydrolysis of cell walls, proteins and starch was most extensive in the starch endosperm area adjacent to the scutellar epithelium. It was observed that hydrolysis occurred in areas adjacent to the aleurone layers and that the hydrolysis decreased as distance increased from the embryo to the distal end and from the aleurone layer to the centre of the starchy endosperm. Although no rigid sequence of hydrolysis was observed, generally, hydrolysis of the cell wall was more extensive than protein hydrolysis, and hydrolysis of starch seemed to take place gradually in the later stages of malting and kilning.

Small granules of starch were hydrolysed more extensively than large granules. Earlier, Palmer (1972) had also observed that during malting, amylase had limited action on large sized starch granules of barley endosperm but rapidly degraded the small granules. In contrast, the small granules of starch of wheat endosperm were found to be resistant to enzymic attack. It was also seen that corroded starch granules were mainly found in the proximal (embryo) half of the endosperm where levels of alpha amylase were much higher than at the distal end.

Glennie et al (1983) studied the patterns of modification that occurred in the endosperm during germination of sorghum grain. The authors observed that endosperm modification began at the endosperm scutellar interface and subsequently moved into the endosperm, with slight modification of the peripheral endosperm. The protein matrix was found to disappear first and after it was disrupted, the starch granules and protein bodies were degraded simultaneously. The starch granules were modified by pitting rather than by surface erosion as their interiors had become hollow, while their forms were retained. The cell walls were the only part of the endosperm that remained visually unchanged after germination, they retained their structure even after the endosperm was extensively modified and the cells had lost their contents. It was also observed that the aleurone cells did not appear to be active in enzyme production, rather the scutellum fulfilled this role. In the same year, Aisien and Palmer (1983) reported that the whole body of scutellum was capable of producing alpha amylase in germinating sorghum, while in the

germinating barley, MacLeod and Palmer (1966) and Palmer (1982) had reported that the enzymic modification of the endosperm was under aleurone rather than scutellar control.

Changes in starch and sugars during germination have also been determined. Lineback and Ponpipom (1977) estimated alpha amylase activity in wheat grains before and after soaking and in flours made from wheat germinated for 2 to 14 days and related the effects of enzyme activity to damaged starch and free sugar contents. After soaking, the alpha amylase activity of wheat decreased to about one half of that originally present in the dormant grains which was attributed to leaching of the enzyme during soaking. The activity of alpha amylase in flour of germinated wheat increased and tended to parallel the increase observed in free sugars till 8 days of germination period. Thereafter, until the end of 14 days of germination period, the enzyme activity remained constant. The damaged starch and free sugar contents of flour prepared from germinated wheat increased with the increase in germination time. Earlier, similar results for alpha amylase activity, damaged starch and free sugar contents of 2 to 8 days germinated wheat were observed by Dronzek et al (1972).

Piendl (1971) studied the effect of germination time on the carbohydrates of 4 varieties of barley malt. A considerable increase in hexoses and sucrose was observed during the first 7 days of germination. The increase tended to slow down after 7 days of germination until the experimental period of 10 days.

Varietal differences were observed in the production of these sugars at different germination periods. von Holdt and Brand (1960) observed that the starch content of sorghum decreased while the fructose, glucose, sucrose, maltose and maltose oligosaccharides increased progressively with the increase in germination time from 2 to 7 days. The authors observed that the loss of starch was very much greater than the increases in sugars and lower oligosaccharide contents. Therefore, it was concluded that there was a considerable net loss of carbohydrate in the malting of sorghum. The authors further pointed out that as the breakdown of starch during germination was rapid, the production of sugars probably always exceeded the consumption, so that there was no lag period before the sugar content of the grain started to increase.

Aisien and Ghosh (1978) monitored the changes in starch and soluble carbohydrates in sorghum germinated for 60 h. The authors observed that the starch content decreased from 80 (initial value) to 74% in first 12 h of germination and to 66% after 24 h of germination. Thereafter, the decrease in starch content was relatively low as the starch content of 60 h germinated sorghum was 62%. The contents of soluble carbohydrates increased from zero (initial value) to 2.0% after 12 h of germination and to 3.1% after 24 h of germination. The increase in germination period from 24 to 60 h brought about a relatively smaller increase in soluble carbohydrate contents. It was 4.9% in grains germinated for 60 h.

Similar observations that during germination starch content of the grains decreased and sugar content increased have been made by Chavan et al (1981) and Taur et al (1984a) in different varieties of sorghum germinated for 0 to 120 h. The data presented below indicates that the reduction in starch content

Changes in starch and reducing sugar contents of sorghum

Germination time (h)	Low tannin (Chavan et al 1981)		High tannin (Chavan et al 1981)		Mean values of 5 varieties of sorghum (Taur et al 1984a)	
	Starch (%)	Reducing sugars (mg/g)	Starch (%)	Reducing sugars (mg/g)	Starch (%)	Reducing sugars (mg/g)
0	77.0	0.7	72.1	3.7	75.4	1.1
24	75.0	1.2	69.6	4.4	73.0	1.9
48	65.0	26.0	60.1	26.2	63.4	26.1
72	48.4	97.5	54.6	49.0	47.4	90.4
96	32.0	105.0	48.4	73.5	32.8	98.3
120	22.5	120.0	32.0	80.2	23.8	109.1

and elevation in reducing sugar content was higher in low tannin than in high tannin variety of sorghum. The decrease in starch content in 120 h germinated sorghum as observed by Chavan et al (1981) and Taur et al (1984a) ranged from 56 to 71% and increase in reducing sugar content about 20 to 170 fold.



Malleshi (1983) had observed that during the germination period of 96 h the starch content of finger millet (Eleusine coracana) decreased from 65.5 to 53.0 g/100g. On the other hand, the free sugar content increased from 1.1 (ungerminated value) to 5.9 g/100g. Likewise, decreases in the starch content and increases in the free sugar content were observed in 48 h germinated pearl millet (Pennisetum typhoides) and foxtail millet (Setaria italica).

Faparusi (1970) classified sugars into 3 groups in relation to germination period. Those sugars which decreased in concentration on the first day of germination and then showed a rapid increase in concentration on the second day until the third day followed by no further substantial increase were the glucose and sucrose; those which showed a steady increase in their concentrations until the second day, after which there was a relatively sharp increase in the concentration but did not increase after the third day were the fructose, maltose, isomaltose and maltotriose; and sugars which did not show any increase in their concentrations were the raffinose and stachyose. The author explained that the initial fall in the concentrations of some of the sugars could be due to the increase in metabolic activities of the grains on steeping and germination and the utilization of the sugars in the metabolic processes.

Aisien (1982) examined the utilization of soluble carbohydrates during germination and seedling growth of sorghum. A detailed time course study of sucrose, raffinose, glucose and fructose contents of the scutella from germinating intact

sorghum grains showed that the sucrose declined rapidly within 12 h after initial wetting of the grains. Between 12 and 36 h of germination, this rapid decline in sucrose was arrested before re-synthesis from translocated products of endosperm degradation resulted in its recovery. The raffinose content also declined rapidly within 18 h after wetting but was recovered by 24 h. In contrast to the rapid decline in levels of sucrose and raffinose, glucose and fructose levels remained low during the entire germination phase of 24 h. All the sugars accumulated once the 24 h germination period was completed. A similar time course investigation for maltose, maltotriose and glucose levels in the endosperm showed that glucose was the main sugar produced during germination and seedling development although the other 2 sugars (maltose and maltotriose) were present in lower quantities. The determination of invertase activity in the axes of sorghum grain embryos showed that the enzyme was initially present in the ungerminated grain but its activity increased during 24 h of germination which was seen to be associated with low sucrose level.

Similar changes in carbohydrate contents have also been observed in germinated pulses. Azhar et al (1972) examined changes in carbohydrates during germination of bengal gram. They observed that 50% of the dry weight of the seeds was constituted of starch and that this was reduced to about 20% after 7 days of germination. There was a sharp rise in the concentration of soluble sugars mainly hexoses, on the third day

of germination followed by a decrease to make the level slightly lower than that observed on the second day. The pattern of changes in free hexose concentrations was similar to the changes in total soluble sugars. The reducing sugar content declined on the fourth day by about 15% and then began to rise to reach a level comparable to the content observed on the second day. The concentration of keto sugars remained almost constant up to the sixth day and then dropped by nearly 50%.

Jaya and Venkataraman (1980) observed decreases in the starch concentration of bengal gram with progressive germination (see below). The reducing sugar content increased on germination with

Carbohydrate composition of germinated bengal gram

Germination time (h)	Total carbo- hydra- tes	<u>Soluble carbohydrates</u>			Pento- san	Starch	Cellu- lose as glucose
		Total	Redu- cing	Non- redu- cing			
(g/100g)							
0	61.2	14.9	2.2	12.7	4.7	40.5	1.1
48	60.0	18.3	5.7	12.6	5.0	36.0	0.7
96	52.9	21.9	12.5	9.4	5.1	25.2	0.7

a corresponding decrease in non-reducing sugars. The changes in carbohydrate profile were more marked during the latter 48 h than the first 48 h of germination period. This was attributed to the initial lag period before alpha amylase was activated in germinated bengal gram grains. In 96 h germinated bengal gram,

the starch content had decreased by 38% and total sugar content increased by 47%. The reducing sugar content however, in 96 h germinated bengal gram was elevated by 468% over its initial value.

Udayasekhara Rao and Belavady (1978) had determined the changes only in fructose, glucose and sucrose contents of bengal gram after 72 h of germination. It was observed that from the values of ungerminated grains, fructose increased from 0.25 to 0.85 g/100g, glucose from 0.1 to 0.45 g/100g and sucrose from 1.6 to 2.9 g/100g.

Aman (1979) had also monitored the changes in starch and sugar contents over a germination period of 72 h in mung bean and bengal gram. In mung beans, the starch content decreased by 13% from the initial value of 42%. The content of fructose increased to 0.7% from traces in ungerminated seeds, after 48 h of germination and to 0.8% after 72 h of germination. Likewise, the contents of glucose and sucrose increased to 0.7 and 5.1% from their initial (0 h) values of 0.1 and 1.8%, respectively, after 48 h of germination and thereafter decreased to 0.4 and 3.8%, respectively. In bengal gram, a negligible decrease (1%) was observed from the initial starch content of 34.1%. The fructose and glucose contents increased from 0.1 to 0.5% after 24 h of germination, then decreased to 0.1% after 48 h of germination and only traces were observed after 72 h of germination. Likewise, the contents of sucrose increased from 4.3 to 5.3% after 24 h of germination, then decreased to 4.9% after 48 h of

germination and remained constant until 72 h of germination.

El-Shimi et al (1980) investigated the changes in total starch, sucrose and fructose in 8 days germinated broad beans (Vicia faba). The total starch content decreased by 24% from the initial value of 50% after 2 days of germination. Thereafter a progressive decrease to 18.8% was observed up to 8 days of germination. The sucrose content of broad beans increased from 3.7 to 6.5% after 2 days of germination, thereafter it showed a progressive decline to 1.5% after 8 days of germination. Likewise, fructose increased from zero (ungerminated value) to 0.85% after 4 days of germination and then decreased to zero after 8 days of germination.

Variations in sugar content of the great northern beans (Phaseolus vulgaris) with increase in germination period have been monitored by Sathe et al (1983). They have reported that the total sugars (mg/g) decreased from 133.3 to 101.9 in 2 days of germination, and later increased to 118.5 after 5 days of germination period. Sucrose on the other hand, showed a different pattern, it decreased from 52.1 to 47.8 mg/g on the second day of germination, increased to 59.5 mg/g on the third day of germination, again decreased to 27.0 mg/g on the fourth day and increased to 37.6 mg/g on the fifth day of germination.

El-Mahdy and El-Sebaiy (1983) determined the changes in carbohydrates of 96 h germinated fenugreek (Trigonella foenum graecum) seeds, a leguminous herb. It was observed that the reducing sugars increased from 0.15 (ungerminated) to 3.19% in

germinated fenugreek seeds. Likewise, total reducing sugars after acid inversion increased from 2.89 (ungerminated) to 9.52% after 96 h germination in fenugreek seeds. In contrast, the starch content decreased from 20.6 (ungerminated) to 15.6% in 96 h germinated fenugreek seeds. The starch breakdown was attributed to the increases in amylase and phosphorylase activities in respiratory metabolism.

Shiroya (1963) studied the changes in fructose, glucose and sucrose with the increase in sprout length of germinated cotton seeds. The fructose content increased from 0.5 to 2 mg/g to 2 to 5 mg/g when the sprout length increased from zero (ungerminated) to 0.1 cm and remained constant till the sprout length was 5 cm. On the other hand glucose content increased from 0.25 to 0.5 mg/g to 0.5 to 2 mg/g when the sprout length increased from zero to 0.1 cm and to 2 to 5 mg/g when the sprout length increased to one centimetre. Further increase in sprout length to 5 cm brought about no difference in glucose content. In contrast, sucrose content decreased from 2 to 5 mg/g to 0.5 to 2 mg/g when the sprout length was 0.1 cm and then increased to 5 to 10 mg/g when the sprout length became one centimetre and thereafter no change in sucrose content was observed with increase in sprout length to 5 cm.

#### Malting and proteins

Hwang and Bushuk (1973) investigated the effects of 2 days of soaking and of various germination periods on endosperm

proteins of red spring wheat. It was noticed that during sprouting the amount of high molecular weight components gradually decreased while the amounts of smaller molecular weight components increased. Based on solubility fractionation, the authors demonstrated that in flour made from germinated wheat, there was a marked decrease in the amount of insoluble protein component and increase in the number of amino groups which were related to the increase in proteolytic activity. However, 2 days of soaking had no effect on proteolytic activity while 2 days of germination produced a relatively small increase. But the proteolytic activity of the flour increased 7 and 17 fold during 4 and 8 days of germination, respectively. Earlier, Hildebrand and Burkert (1942) had reported that during the process of germination the proteinase activity of wheat increased approximately six times in 4 days and 10 times in 7 days.

Chittenden et al (1978) studied the changes in free amino acids in the aleurone layer endosperm and embryo of the germinated wheat grain. The total free amino acids in the aleurone layer and starchy endosperm increased from the onset of germination up to the fifth or sixth day of germination. Similarly, in the embryo, the levels of free amino acids increased from the onset of germination, and after the second day the rate of increase was dramatic so that by the sixth day the levels were much higher than those observed in the aleurone layer as well as in the starchy endosperm. Regarding individual amino acids, it was observed that glutamine was the prominent

amino acid in both the aleurone layer and starchy endosperm of the ungerminated and the germinated grain. Although proline was present in relatively small amounts in the ungerminated grain it increased to levels approaching those of glutamine by the fourth day of germination. Changes in the proportions of other amino acids were less marked. In the embryo of the germinating grain, glutamine, along with asparagine, alanine and proline was also the major amino acid. In contrast to the changes in the free amino acids in the aleurone layer and starchy endosperm, there was a large increase in the proportion of asparagine in the embryo during germination so that by the fourth day it accounted for 45% of the total free amino acids. By the sixth day there was further increase in asparagine so that it accounted for more than 60% of the total free amino acids.

Taylor (1983) observed that in malted sorghum, the nitrogen of the kernel was transferred to the roots and shoots. Prolamines, the major group of storage proteins, were degraded directly to small peptides and amino acids without the formation of polypeptide intermediates. There was a decline in glutelin proteins whereas some albumin plus globulin proteins increased in quantity. In the roots and shoots there was a considerable increase in both protein nitrogen and non-protein nitrogen as a result of the translocation of the products of storage protein breakdown from the kernel. Several fold increases in all the essential amino acids were observed although the 2 most important free amino acids of sorghum malt were asparagine and glutamine, as in germinated wheat.



As early as in 1929, Bishop had observed that there was an active breakdown of the proteins of barley during germination. The 2 insoluble proteins of the endosperm, hordein and glutelin, were broken down at about the same rate to give salt soluble products. Later, the rate of disappearance of glutelin and hordein fell off and the amount of glutelin increased slightly. The falling off in the rate of disappearance of glutelin and its subsequent increase suggested to the authors that there was a resynthesis of this protein in the embryo. The breakdown of hordein and glutelin gave rise chiefly to the simpler nitrogen compounds comprised in the term 'non protein' nitrogen. In addition, albumin, globulin and proteose were found increased although not markedly in the germinating grain.

Pomeranz (1974) had observed that degradation of protein matrix in response to malting was influenced by the protein content of barley. In the low protein barley, the degradation of protein matrix was extensive while in the high protein barley, much of the protein matrix was found to be largely intact and as a matter of fact some protein was retained in the form of a continuous thick film covering the starch granules.

Chrispeels and Boulter (1975) determined the role of endopeptidase in the control of storage protein metabolism in the cotyledon of germinating ~~mung~~ beans. The autodigestive proteolytic activity of the extracts of cotyledons of mung beans was found to increase 4 to 5 fold during germination. The increase occurred after a lag period of 2 days during the next 2 to 3

days of germination and coincided with the period of rapid storage protein breakdown. Similar results were reported by Harris and Chrispeels (1975), the proteolytic activity was closely paralleled by a 10 fold increase in endopeptidase activity.

Juo and Stotzky (1970) observed changes in protein spectra of red kidney beans (Phaseolus vulgaris) during germination. In ungerminated beans globulins, albumins and basic proteins were found in the ratio of 3:2:1. The globulin fraction constituted a major portion of the reserve proteins and was hydrolysed rapidly during germination. More than 90% of the basic proteins disappeared after 12 days of germination. Although the decrease in total albumin was not as marked as in the other 2 fractions, a number of components of this fraction disappeared during the early stages of germination, but several new components were detected after about 8 days of germination. The apparent synthesis of new globulin components during germination was also observed, but no synthesis of basic proteins was detected.

Krishnamurthy and Venkataraman (1983) observed changes in the reserve proteins of green gram (Phaseolus aureus) during germination. Gel filtration and polyacrylamide gel electrophoresis indicated the degradation of the high molecular weight proteins to low molecular weight proteins. The results also suggested that there was formation of the new proteins during germination consequent to the degradation of stored proteins.

In great northern beans, Sathe et al (1983) observed that during germination, there was a progressive decrease in the

major storage protein subunits. The degradation rate of the major storage protein subunits was slower up to 2 days compared to that at the end of the third, fourth and fifth day of germination. There were increases in total soluble amino acids and total soluble essential amino acids at the end of 5 days of germination.

Beevers and Guernsey (1966) and Beevers (1968) followed the changes in major nitrogenous components during the germination of pea (Pisum sativum) seed. During the period of rapid axis growth, 3 to 8 days following germination, the nitrogen content of cotyledons declined rapidly with an accompanying increase of nitrogen in the developing axis. While the total nitrogen content per seedling remained constant for the first 8 days of germination and then slowly declined which was attributed to the incomplete recovery of the root material of older seedlings. The accumulation of nitrogen in the cotyledons as well as in the axis indicated that the mobilization of nitrogen was facilitated by proteolysis and translocation of the products.

Small apparent increases in protein content of cereals and pulses during the process of germination and malting have been reported. Dalby and Tsai (1976) had demonstrated that the protein content of wheat germinated for 5 days had increased by 15% from its pre-germination value of 13%. Ranhotra et al (1977) had exhibited an increase of 11% (from 13.6 to 15.1%) while Hamad and Fields (1979) had shown that the protein content increased by only 4% (from 10.2 to 10.6%) in 5 days germinated wheat. In cereals other than wheat such as barley, rice, maize,

triticale and oats (Tsai et al 1975, Hamad and Fields 1979, Ram et al 1979, Wu 1982, Wu 1983), also, small increases in protein have been demonstrated.

Palmer et al (1973) have put forward the idea that the nitrogen content of kidney beans, on a dry weight basis, increased with germination probably as a result of utilisation of some of the energy reserves of the seed. It was observed that the nitrogen content of the beans increased from 3.9 (control) to 4.1% after 4 days and to 5.0% after 8 days of germination. On the other hand, Kylen and McCready (1975) have attributed the increase in protein content of the germinated grains of alfalfa (Medicago sativa), lentil (Lens esculenta), mung bean and soya bean, partly to a loss of leachable sugars and seed coats during the sprouting procedure, and partly to the protein synthesis.

Changes in reserve proteins of cowpea (Vigna sinensis), bengal gram and green gram during 72 h of germination were studied by Ganesh Kumar and Venkataraman (1975). The 72 h germinated seeds were also analysed for total nitrogen content. The analysis both by sedimentation and gel electrophoresis indicated breakdown of the proteins during germination although the changes were not drastic. The reduction in total nitrogen was found to be progressive with the increase in germination period. The total nitrogen content of bengal gram as a result of 72 h of germination had decreased from 3.8 to 3.4% and that of cowpea and green gram had decreased from 3.5 to 3.1% and 4.4 to 4.1% respectively.

Later, Ganesh Kumar and Venkataraman (1978) reported similar changes in the proteins of 12 days germinated bengal gram. It was observed that the major cotyledon proteins in ungerminated bengal gram seeds were salt soluble globulins which accounted for 80 to 90% of the total proteins. The globulins comprised of 10S and 7S fractions. On germination, the presence of fractions of lower sedimentation coefficient indicated degradation of these components.

Jaya and Venkataraman (1980) have reported changes in the nitrogenous constituents of bengal gram during germination. In the seedlings, the total nitrogen remained constant whereas the nitrogen in the cotyledons decreased with the increase in germination period. But the protein nitrogen both in the seedlings and in the cotyledons decreased with concomitant increase in the non-protein nitrogen, alpha-amino and amide nitrogen during a germination period of 96 h.

In other legumes such as, navy beans (Phaseolus vulgaris) (Kadake and Evans 1966), yellow beans, faba beans (Vicia faba) and lentils (Hsu et al 1980), increases in protein content have also been observed.

#### Kilning and compositional changes

Kilning is a procedure by which a dry product is produced which is stable during storage. It also adds 'character' to the malt, alters its colour and flavour and reduces its enzyme potential (Briggs et al 1981).

Bathgate (1973) had explained that in kilned malt, changes occurred in 3 phases, viz., a 'germinative' phase, an 'enzymic', phase and finally a 'chemical' phase. The first stage of kilning was regarded as a continuation of the germination of the grain for a limited time while the moisture content was still high and the temperature relatively low (about 50°C). In this phase although the real growth soon stopped but the malt grain retained some germinative power as long as the final curing temperature was not excessive. In the enzymic phase, some of the enzymes were inactivated while the activities of some of these continued, changes in carbohydrate composition and structure (Pomeranz 1972) and proteins and amino acids were observed. In the chemical phase, the reactions predominated the transformations which occur during the final temperature conditioning or 'curing' of malt (usually between 80 and 100°C). These reactions produced most of the coloured matter and aroma substances essential for good malt character. The coloured products of kilned malt have been described as being principally of three types, viz., 'melanoidins', 'caramelized sugars' and 'oxidised polyphenols'.

The literature presented herein highlight the point that during the process of germination high molecular compounds as complex carbohydrates and proteins are broken down into simpler forms due to the enhanced activities of amylases and proteases (Kneen et al 1942, Kneen 1944, Beevers and Guernsey 1966, Dronzek et al 1972, Hwang and Bushuk 1973, Lineback and Ponpipom 1977).

The alpha and beta amylases which are synthesized during germination of a grain are responsible for the hydrolysis of

starch. As a result, progressive reduction in starch and increase in the reducing sugars with increase in germination time of a grain have been reported (Azhar et al 1972, Jaya and Venkataraman 1980, Chavan et al 1981, Aisien 1982, Sathe et al 1983, Taur et al 1984a). Small increases in protein content on germination of grains have also been reported (Palmer et al 1973, Kylen and McCready 1975, Harrison and Vanderstoep 1984).

The present study was planned to monitor the changes in carbohydrate profile and protein content of wheat and bengal gram grains soaked for 12 h and germinated for a period of 24 to 72 h.

#### Materials and methods

##### Malting of grains

One hundred grams of wheat and bengal gram were soaked separately in different bowls of same size and shape, in thrice their volume of distilled water for 12 h. After draining the water, the grains were rubbed lightly on a dry muslin cloth to remove the film of water adhering to the grains. The grains were then wrapped in a moist muslin cloth and kept separately in plastic bowls for germination at room temperature of 27°C (24 to 31°C). The bowls were kept under a wiremesh covered with a wet cloth to minimize moisture evaporation. The muslin cloth containing the grains and covering the wiremesh were wetted every 12 h. The grains were removed after 24, 36, 48, 60 and 72 h of germination. The grains, after germination, were weighed to obtain the green weight. Seeds that had not germinated or with

relatively very small sprouts were discarded and their weight was subtracted from the green weight. The sprout lengths of 20 randomly picked up germinated grains were recorded. After drying for 2 h under the fan, the germinated grains were oven dried at  $70 \pm 5^{\circ}\text{C}$  for 9 to 11 h. The dried grains with rootlets were cooled and weighed to obtain the dry weight. Grains with rootlets were milled and whole flour (100% extraction) was analysed for starch, total and reducing sugars, and protein contents.

#### Analytical procedure

Starch : Invert sugar reduces the copper in Fehlings solution to red, insoluble cuprous oxide. The sugar content in a food sample is estimated by determining the volume of the standard glucose solution required to completely reduce a measured volume of Fehlings solution.

Twenty five millilitres of distilled water was added to 2 g of flour in a plastic centrifuge tube. The contents were stirred and centrifuged at 3000 rpm for 20 min and the supernatant was discarded. The process was repeated twice using 95% ethanol followed by 50% ethanol. The pellet was digested in a boiling water bath for 3 h in 30 ml of 20% hydrochloric acid. The contents were then neutralized with 10 N sodium hydroxide, volume was made up to 60 ml and the contents filtered through a Whatman No. 1 filter paper. An aliquot of 2 ml of the filtrate was used for analysis of reducing sugars by the method of Lane and Eynon (1923) as described by Ranganna (1977).



Reducing sugars : Fifty millilitres of distilled water was added to 2 g of flour in a conical flask. The contents were kept overnight in a refrigerator, the volume was made up to 60 ml and the contents were filtered through Whatman No. 1 filter paper. An aliquot of 10 ml of the filtrate was analysed for reducing sugars by the method of Lane and Eynon (1923).

Total sugars : To 25 ml of the filtered extract (used for the estimation of reducing sugars), 10 ml of 50% hydrochloric acid was added. The contents were allowed to stand at room temperature for 24 h. The solution was neutralized with 10 N sodium hydroxide and the volume was made up to 40 ml. An aliquot of 10 ml of the filtrate was taken for determination of total sugars by the method of Lane and Eynon (1923).

The sample aliquot was taken in a conical flask containing 5 ml each of commercially obtained Fehlings solutions A and B and approximately 10 ml of distilled water with 3 drops of one percent methylene blue indicator. The conical flask was heated on a burner until the contents came to a boil. Standard glucose solution (2 mg/ml) was added from the burette drop by drop until the contents of the flask turned brick red. A blank with 5 ml each of Fehlings solutions A and B and 15 ml of distilled water was titrated against the standard glucose solution. The titre value of the sample was subtracted from the blank titre value.

### Calculations :

$$\text{Reducing sugars (g/100 g)} = \text{BT-ST} \times C \times \frac{V}{V_1} \times \frac{100 \text{ g}}{S} \times \frac{1}{1000}$$

$$\text{Total sugars (g/100 g)} = \text{BT-ST} \times C \times \frac{VI}{V_1} \times \frac{V}{VI_1} \times \frac{100 \text{ g}}{S} \times \frac{1}{1000} \times 0.95$$

$$\text{Starch (g/100 g)} = \text{BT-ST} \times C \times \frac{V}{V_1} \times \frac{100 \text{ g}}{S} \times \frac{1}{1000} \times 0.90$$

where :

- BT = blank titre (ml)
- ST = sample titre (ml)
- C = concentration of the standard glucose solution (mg)
- V = total volume after extraction (ml)
- $V_1$  = aliquot taken for titration (ml)
- $VI_1$  = volume of the extract taken for inversion (ml)
- VI = total volume after inversion (ml)
- S = weight of the sample taken for analysis (g)
- 0.95 = ratio of the formula weight of anhydroglucose unit of sucrose to the molecular weight of glucose
- 0.90 = ratio of the formula weight of anhydroglucose unit of starch to the molecular weight of glucose

**Protein :** The various nitrogenous compounds are converted into ammonium sulphate by boiling with concentrated sulphuric acid. The ammonium sulphate formed is decomposed with an alkali (sodium hydroxide), and the ammonia liberated is absorbed in excess neutral boric acid solution and then titrated with standard acid. The method followed was as given in Ranganna (1977).

Approximately 1 g of flour was digested with 25 ml of concentrated sulphuric acid and a pinch of catalyst (potassium

sulphate, copper sulphate and selenium dioxide in the ratio of 100:20:2.5 g). After digestion the contents were transferred to a volumetric flask and the volume was made up to 100 ml with distilled water. Ten millilitres of 40% sodium hydroxide was added to the Kjeldahl trap to which one millilitre of the digested sample was added. The contents were vigorously boiled and the released ammonia was collected in 10 ml of 4% boric acid solution containing a mixed indicator (0.1% bromocresol green and 0.1% methyl red in 95% alcohol in the ratio of 5:1). The contents were titrated against 0.01 N hydrochloric acid. A blank with 25 ml of concentrated sulphuric acid and the catalyst was carried through the entire procedure.

#### Calculation :

$$\text{Protein (g/100 g)} = \text{ST-BT} \times \text{N} \times \frac{\text{V}}{\text{V}_1} \times \frac{100 \text{ g}}{\text{S}} \times 6.25 \times \frac{1}{1000} \times \frac{0.14 \text{ mg}}{\text{nitrogen}}$$

where : ST = sample titre

BT = blank titre

N = normality of hydrochloric acid (0.01 N)

V = total volume after digestion (ml)

V<sub>1</sub> = aliquot taken for distillation (ml)

S = weight of the sample taken for analysis (g)

1 ml of 0.01 N HCl = 0.14 mg nitrogen

Protein = nitrogen x 6.25

#### Statistical analysis

Differences among the means were tested at 5% of significance level by student's 't' test (Snedecor and Cochran 1968).

## Results and discussion

Malting losses, changes in carbohydrate profile and protein contents were determined in 12 h soaked wheat and bengal gram grains over a germination period of 72 h.

### Seedling weight and sprout length

Wheat : Table 5 displays changes in weight and sprout length of wheat at different stages of malting after various germination periods. The green weight of wheat increased from 100 g of the initial value to about 145 g after 24 h of germination period. But as the germination period progressed by 12 h, at each point of time until 72 h, the green weight increased but at a rate markedly slower than that observed after 24 h of the germination period. However, after 48 h of germination the green weight of wheat increased to about 150 g as against that of 145 g observed after 24 h of germination (Table 5). With a further prolongation in germination time to 72 h, the green weight increased to 153 g. The changes observed in green weight of wheat over a germination period of 72 h were in accordance to that observed by Kneen et al (1942) although the actual values were smaller than those reported by them. In their experiment, the increase in green weight was progressive up to 60 h of germination period and the weights ranged from 164 g to 190 g although after 60 h of germination the green weight decreased from 190 g to 184 g. In the present study it was progressive up to 72 h and the weights ranged from 145 to 153 g (Table 5). The differences observed in the actual green weights between the present study and that of

Table 5. Changes in weight and sprout length of wheat grains during different stages of malting at different germination periods

	Germination period (h)				
	24	36	48	60	72
Weight of raw grains (g)	100	100	100	100	100
Weight of germinated grains, green weight (g)	144.8	148.5	150.3	150.8	153.0
Weight of malted grains, dry weight (g)	94.6	94.6	94.2	92.5	92.4
Malting loss (g)	5.4	5.4	5.8	7.5	7.6
Sprout length (cm) (Mean $\pm$ SE)	0.50 $\pm 0.029$	1.49 $\pm 0.136$	1.62 $\pm 0.127$	1.94 $\pm 0.187$	2.04 $\pm 0.241$

Kneen et al (1942) might have been due to varietal variations in the wheat grains used which might have resulted in the variations in water uptake and seedling growth. In addition, germination temperature might have also affected the green weights as Kneen et al (1942) had germinated their grains at 20°C while in the present study the germination temperature was 27°C (24 to 31°C).

On drying, the loss in weight from the green weight of 24 h germinated wheat was 35% (Table 5). It increased progressively from 35 to 40% with the increase in germination period from 24 to 72 h. Earlier, Kneen et al (1942) had demonstrated a progressive increase in weight loss from 45 to 54% on drying of wheat germinated for 0 to 72 h. They had observed a 45% loss in green weight of wheat grains germinated for 24 h in comparison to 35% observed in the present study. This greater loss in weight observed by Kneen et al (1942) could be due to higher green weight of the wheat grains as compared to that observed in the present study (164 Vs 145 g).

Table 5 shows that the malt yield was between 92.4 and 94.6% in wheat grains germinated for 24 to 72 h. In 3 varieties of wheat, Sethi and Bains (1978) had obtained malt yields of 85.8, 87.6 and 92.7% after 72 h of malting. In the present study, the malt yield after 72 h of germination was 92.4% which was comparable with the value of 92.7% reported by Sethi and Bains (1978) and that of 93.5% reported by Singh et al (1983).

Table 5 also includes the values for malting loss (weight loss from raw weight). Comparing the weights of raw and malted

wheat grains it was observed that on drying of wheat germinated for 24 to 72 h, there was a 5 to 8% loss in weight. Somewhat smaller losses (3 to 5%) in 3 varieties of wheat germinated for 24 and 96 h have been reported by Nielson et al (1978). Malleshi (1983) had demonstrated that malting loss in wheat germinated for 48 h was 4.8%. Earlier, Wu (1982) had observed a 10% loss in weight of triticale germinated for 72 h. In 1957, McConnell had germinated  $C^{14}$  labelled wheat and observed 17% carbon loss after 5 to 7 days of germination. The losses in dry weights have been ascribed to the oxidation of substances and leakage of materials from the seed (Dalby and Tsai 1976, Lorenz 1980).

The mean value for sprout length of wheat grains consistently increased with the increase in germination time (Table 5). After 24 h of germination the sprout length was 0.50 cm which exhibited a 3 fold increase by the end of 36 h of germination. Thereafter, the increase in sprout length was of a smaller magnitude. It remained less than 0.5 cm with every 12 h increase in germination period. By the end of 72 h of germination, the sprout length had increased to 2.04 cm from 0.50 cm, observed after 24 h of germination. Earlier, Inamdar (1980) had reported that the sprout lengths of wheat germinated for 24 or 48 h was 1.10 cm and 1.94 cm, respectively. In 24 h germinated wheat the sprout length of 0.50 cm observed in the present study was lower by 0.6 cm than that observed by Inamdar but the values observed for 48 h germinated wheat were comparable (1.62 Vs 1.94 cm). Kneen et al (1942) had germinated wheat for 24 to 72 h and noted that

the sprout length increased by one millimetre after every 12 h from 24 to 48 h of germination period, thereafter, a higher increase of 3 mm during 48 to 60 h germination and of 2 mm during 60 to 72 h germination was recorded. In another study, conducted after 2 years, Kneen (1944) had reported that the wheat grains germinated for 48 h acquired a sprout length of 75 mm. The sprout length of wheat germinated for 48 h reported by Kneen in 1944 was almost twice (7.5 mm Vs 3 to 4 mm) of that reported by Kneen et al in 1942. The difference in the results appeared to be due to the temperature at which wheat grains were germinated as in 1942 wheat was germinated at 20°C while in 1944 it was germinated at 30°C. In the present study, the sprout length of wheat grains germinated at 27°C (24 to 31°C) for 48 h was 1.62 cm which was higher than the values reported by Kneen et al (1942) and Kneen (1944), the variations in sprout length could be due to varietal differences as suggested by Ram et al (1979).

**Bengal gram :** The green weight of bengal gram germinated for 24 to 72 h increased from 100 g of ungerminated weight to 197.6 to 203.5 g (Table 6). The increase in green weight of bengal gram grains was almost twice as compared to that observed in wheat grains (Table 5) germinated for the same length of time (98 to 104% Vs 45 to 53%).

The higher increases in green weight of bengal gram in relation to those of wheat could be due to the greater water imbibition by the bengal gram grains in comparison to that by



Table 6. Changes in weight and sprout length of bengal gram during different stages of malting at different germination periods

	Germination period (h)				
	24	36	48	60	72
Weight of raw grains (g)	100	100	100	100	100
Weight of germinated grains, green weight (g)	197.6	198.5	198.6	202.4	203.5
Weight of malted grains, dry weight (g)	92.0	91.3	90.2	90.2	89.7
Malting loss (g)	8.0	8.7	9.8	9.8	10.3
Sprout length (cm) (Mean $\pm$ SE)	1.14 $\pm 0.099$	2.22 $\pm 0.017$	3.34 $\pm 0.110$	3.69 $\pm 0.134$	4.94 $\pm 0.185$

the wheat grains as was reflected by their moisture contents after 12 h of soaking, 54 versus 35% (Tables 1, 3).

On drying, the decrease in green weight of bengal gram was higher than that observed in wheat grains, 8.0 to 10.3% versus 5.4 to 7.6% (Tables 5, 6). The greater loss of weight in bengal gram versus wheat was considered to be the consequence of greater water uptake during soaking period and its loss on drying. The bengal gram grains after malting weighed 8 to 10% less than their initial raw weights (Table 6). The malting loss increased with the increase in germination time. It was 8% in 24 h germinated grains and 10% in 72 h germinated grains. It was also noticed that malting loss did not vary markedly between bengal gram and wheat grains after 72 h of germination (10 Vs 8%). The malting loss might have been due to respiration losses during germination as suggested by Dalby and Tsai (1976) and Lorenz (1980).

The mean value for sprout length of bengal gram was 1.14 cm after 24 h of germination period (Table 6). Thereafter, an increase of one centimetre was observed for every 12 h enhancement of germination period until 48 h. Inamdar (1980) had reported that the sprout lengths of bengal gram germinated for 24 and 48 h at 29°C were 2.12 cm and 3.16 cm, respectively. The sprout length of 1.14 cm in bengal gram observed in the present study was 0.98 cm lower than that observed by Inamdar (1980) after 24 h of germination period. It may be worth repeating here that a difference in sprout lengths of wheat grains germinated for

24 h was also observed between the results of Inamdar's study (1980) and the present study. However, after 48 h of germination period the difference in sprout lengths between her study and the present study narrowed both in wheat (1.94 Vs 1.62 cm) and in bengal gram (3.16 Vs 3.34 cm) grains.

Changes in starch, total sugar and reducing sugar contents of grains in response to germination for various periods

#### Wheat

Starch : Table 7 exhibits changes in carbohydrate profile of wheat soaked for 12 h and germinated for various periods. The starch content of wheat grains decreased significantly (Table 8) from its original value of 60.9 g/100g after 24 h of germination. Thereafter, with the increase in germination period to 72 h, no appreciable decreases in starch content were observed. A decrease of smaller magnitude in malted barley starch during the initial stages of the germination period, has been reported by Greenwood and Thomson (1959).

Many investigators have monitored changes in starch content in response to germination of grains. Opoku et al (1983) monitored the changes in starch content of millet (Pennisetum americanum) over a germination period of 96 h. They reported that the starch content of millet decreased by 15% (from 48 to 41 g/100g) after the first 24 h of germination and by 27% (from 48 to 35 g/100g) after 36 h of germination period. Thereafter, the decrease in starch content was not so marked at each 12 h germination interval.

Table 7. Changes on malting in carbohydrate profile of wheat grains, soaked for 12 h and germinated for different periods (Mean  $\pm$  SE)

Germination period (h)	Starch	Total sugars (g/100g)	Reducing sugars
0	60.9 $\pm$ 0.101	3.63 $\pm$ 0.102	1.28 $\pm$ 0.038
24	50.3 $\pm$ 0.670	5.23 $\pm$ 0.536	3.19 $\pm$ 0.083
36	50.1 $\pm$ 1.755	5.87 $\pm$ 0.547	3.86 $\pm$ 0.054
48	49.8 $\pm$ 0.684	7.52 $\pm$ 0.090	4.56 $\pm$ 0.005
60	49.4 $\pm$ 0.517	5.08 $\pm$ 0.133	4.32 $\pm$ 0.116
72	49.0 $\pm$ 0.332	5.06 $\pm$ 0.302	4.43 $\pm$ 0.123

Table 8. 't' values for the variables of Table 7

Comparisons	Starch	Total sugars	Reducing sugars
0 Vs 24	15.634***	2.930*	20.989***
24 Vs 36	0.106 NS	0.836 NS	6.768**
36 Vs 48	0.159 NS	2.978*	12.500***
48 Vs 60	0.467 NS	15.155***	2.051 NS
60 Vs 72	0.651 NS	0.061 NS	0.651 NS
0 Vs 36	6.143**	4.029*	39.091***
0 Vs 48	16.064***	28.603***	82.000***
0 Vs 60	21.822***	8.631***	24.918***
0 Vs 72	34.294***	4.483*	24.609***

NS = Non Significant

\* = Difference between means significant at 5% level of significance

\*\* = Difference between means significant at 1% level of significance

\*\*\* = Difference between means significant at 0.1% level of significance

During the entire germination period of 96 h a reduction of about 46% (from 48 to 26 g) was observed in the starch content of millet (values taken from graph). In the present study after an initial decrease of 17% (from 60.9 to 50.3 g/100g) in the starch content of wheat grains during the first 24 h of germination (Table 7), no further marked decreases were observed at each subsequent 12 h germination periods. But the decrease in starch content over a germination period of 72 h observed in the present study for wheat grains was lower than that observed by Opoku et al (1983) in millet grains (20 Vs 38%).

Somewhat similar results were observed by Chavan et al (1981) and Taur et al (1984a). They reported that the starch content of sorghum decreased by 3% over 24 h of germination and by 16% over 48 h and by 38% over 72 h of germination. Comparing the reduction in starch content of 24 h germinated millet with that of 24 h germinated sorghum it seemed that the reduction in the former was about 5 times higher than that observed in the latter. However, the decrease in starch content of 48 h germinated sorghum was quite comparable with that of wheat germinated for the same length of time (present study). But after 72 h of germination the decrease in sorghum starch was almost twice that observed in wheat starch (38 Vs 20%). Earlier, Aisien and Ghosh (1978) had reported that the starch content of sorghum decreased by 11% in the first 12 h of germination and by 18% in 24 h of germination. Thereafter, the decrease in starch content was slow so that in the next 36 h the decrease was only 6%. These results support those of the present

(Raynes and Briggs 1985), grain variety (Shands et al 1942, Atanda and Miflin 1970), starch composition and physical nature of the starch granules (MacGregor and Matsuo 1982). All these factors listed above could have contributed to the variations

the increase in damaged starch content with subsequent germination suggested that starch was gradually degraded as germination proceeded. Likewise, in the present study, progressive decrease in starch content was observed with the increase in germination period from 0 to 72 h. During the entire germination period the starch content decreased by 20% from 60.9 to 49.0 g/100g. The decrease in starch content was attributed to the increase in alpha amylase activity as opined by Dronzek et al (1972) and Lineback and Ponpipom (1977).

**Total sugars :** The total sugar content of 24 h germinated wheat was higher by 44% over the initial value of 3.6 g/100g (Table 7). Increasing the germination period from 24 to 48 h, increased the total sugar content of wheat by another 44% (from 5.2 to 7.5 g/100g). But total sugar content had significantly (Table 8) increased after 24 h of germination. Further increase in germination time to 60 h caused no increase in sugar content. As a matter of fact, the sugar content of 60 h germinated wheat had decreased significantly (Table 8). The total sugar content of 72 h germinated wheat was comparable to that of 24 h germinated wheat. Recently, Opoku et al (1983) had reported progressive increases in soluble carbohydrates of millet with increase in germination time. But the increase was rapid up to 48 h of germination. Likewise in the present study the increase in total reducing sugar content of wheat was found only up to 48 h of germination.

**Reducing sugars :** The reducing sugar content of 24 h germinated

wheat increased significantly on malting (Tables 7, 8). The increase was 149% over its initial value of 1.28 g/100g. The increase in germination time from 24 to 36 and 48 h increased the reducing sugar contents from 3.19 to 3.86 and 4.56 g/100g, respectively (Table 7). By the end of the 48 h of germination period, the reducing sugar content had increased by 256% (1.28 to 4.56 g/100g) over its original value. Further increase in germination period to 60 and 72 h brought about no appreciable changes in reducing sugar contents of wheat, although the values tended to be lower than that observed after 48 h of germination. Earlier, Dronzek et al (1972) had reported that the free sugar content of flour made from germinated wheat increased with the increase in germination period from 2 to 8 days. The free sugar content increased by 29% (from 0.31 to 0.40 g/100g) after 48 h and by 174% (from 0.31 to 0.85 g/100g) after 96 h of germination period. It was 2.16 g/100g after 8 days of germination. Lineback and Ponpipom (1977) had recorded the increases in free sugar content of wheat germinated up to 14 days and reported that the free sugar content increased by 48% (from 0.52 to 0.77 g/100g) after 48 h and by 333% (from 0.52 to 2.25 g/100g) after 96 h of germination period. The free sugar content of 14 days germinated wheat was 6.89 g/100g. In wheat germinated for 48 h, Lineback and Ponpipom (1977) had reported a higher increase (48 Vs 29%) in reducing sugars in comparison to that reported by Dronzek et al (1972). In the present study (Table 7) the increase in reducing sugars of 48 h germinated wheat was 256% as against 29 and 48% reported by Lineback and Ponpipom (1977) and Dronzek et al (1972),



respectively. The variations in reducing sugar contents could be due to varietal differences in the wheat grains used in the studies. Piendl (1971) had demonstrated that 4 varieties of barley differed in production of sugars at different germination periods. In addition, this greater increase in reducing sugar content could be due to higher germination temperature (27 Vs 20 and 15°C) used in the present study in comparison to those used by Dronzek et al (1972) and Lineback and Ponpipom (1977). Moreover, Kneen et al (1942) had demonstrated that the alpha amylase activity increased with the increase in germination temperature. This higher increase in amylase activity at high germination temperature might have caused relatively greater increase in reducing sugars observed in the present study.

von Holdt and Brand (1960) had reported increases in fructose, glucose, sucrose and maltose contents of sorghum with increase in germination period to 7 days. Likewise, Malleshi (1983) had observed increases in free sugars of finger millet with increase in germination period to 4 days. Chavan et al (1981) and Taur et al (1984a) had also observed progressive increases in reducing sugar contents of sorghum grains with increase in germination period.

But decreases in sugar contents during the initial period of germination have also been reported. Faparusi (1970) had demonstrated that in sorghum grains the glucose and sucrose concentrations decreased during the first 24 h of germination and then increased with increase in germination period to 72 h.

The authors attributed this decrease in sugars to the absence of detectable amylase activity in the first 24 h of germination and the utilization of sugars for the metabolic activity of the grain. In pearl millet, Lineback and Ponpipom (1977) had shown that the free sugar content decreased from 0.59 (control) to 0.46 and 0.43 g/100g after 48 and 96 h of germination respectively although it showed an increase to 0.66 and 1.09 g/100g after 144 and 192 h of germination, respectively. Aisien and Ghosh (1978) had observed increases in soluble carbohydrates until 48 h of germination in sorghum grains. There was no change in soluble carbohydrates during 48 and 54 h of germination although an upward trend in their concentrations was observed after 54 h.

In malted sorghum, von Holdt and Brand (1960) had observed that the loss of starch was very much greater than the increase in the content of sugars and concluded that there was a net loss of carbohydrates. The authors had further pointed out that the breakdown of starch during germination of sorghum was rapid, the production of sugars probably always exceeded the consumption so that there was no lag period before the sugar concentration of the grain started to increase.

In the present study, there was a rapid breakdown of starch in the first 24 h of germination which would have resulted in an increase in the sugar contents up to 48 h. As further breakdown of starch was slow and the metabolic activity of the grains would have increased due to germination, the consumption of sugars might have outstripped the production of sugars from starch thereby

decreasing their concentration. From Table 7, it can be observed that the total sugars after acid inversion had decreased significantly while the reducing sugars were not affected. It could be possible that the germinating wheat grain utilized sucrose for respiration and synthesis (MacLeod et al 1953).

#### Bengal gram

**Starch :** Changes observed in carbohydrate profile of bengal gram germinated for 24 to 72 h are exhibited in Table 9. The starch content of bengal gram grains, decreased progressively from the initial value of 43.1 to 37.9 g/100g with the increase in germination period from 0 to 60 h although the decrease was significant (Table 10) only after 24 h of germination (from 43.1 to 42.1 g/100g). Thereafter no decreases in starch content were recorded with the increase in germination period. These results were in accordance with those reported by Azhar et al (1972), Ganesh Kumar and Venkataraman (1976) and Jaya and Venkataraman (1980). Azhar et al (1972) had monitored the changes in starch content of bengal gram over a germination period of 168 h. They had observed that the starch content of 24 h germinated bengal gram seeds decreased by 6%, from 53.0 to 50.0 g/100g. After 24 h of germination the decrease was 5% for every additional 24 h of germination until 72 h of germination period. In the present study with the increase in germination period from 24 to 48 h, the starch content decreased by 4% until 48 h of germination and by additional 6% between 48 and 72 h of germination periods. Over 72 h of germination period, the decrease in starch content in

Table 9. Changes on malting in carbohydrate profile of bengal gram grains, soaked for 12 h and germinated for different periods (Mean  $\pm$  SE)

Germination period (h)	Starch	Total sugars (g/100g)	Reducing sugars
0	43.1 $\pm$ 0.058	3.30 $\pm$ 0.293	0.48 $\pm$ 0.021
24	42.1 $\pm$ 0.334	3.96 $\pm$ 0.074	0.94 $\pm$ 0.039
36	41.6 $\pm$ 1.014	4.02 $\pm$ 0.124	1.30 $\pm$ 0.108
48	40.3 $\pm$ 0.029	4.17 $\pm$ 0.084	1.47 $\pm$ 0.030
60	37.9 $\pm$ 0.567	1.28 $\pm$ 0.154	1.13 $\pm$ 0.018
72	37.8 $\pm$ 0.498	1.24 $\pm$ 0.030	1.19 $\pm$ 0.047

Table 10. 't' values for the variables of Table 8

Comparisons	Starch	Total sugars	Reducing sugars
0 Vs 24	2.950*	2.185 NS	10.454**
24 Vs 36	0.468 NS	0.417 NS	3.130*
36 Vs 48	1.282 NS	1.000 NS	1.518 NS
48 Vs 60	4.225*	16.514***	9.714***
60 Vs 72	0.132 NS	0.255 NS	1.200 NS
0 Vs 36	1.476 NS	2.264 NS	7.454**
0 Vs 48	43.077***	2.852*	27.500***
0 Vs 60	9.123***	6.103**	25.000***
0 Vs 72	10.579***	7.007**	13.922***

See Table 8 for foot note

response to germination was 12% in the present study and 15% in the study of Azhar et al (1972). The small differences in the decrease in starch content of bengal gram observed in present study and that reported by Azhar et al (1972) might be due to the differences in amylase content and/or amylase activity of the bengal grams used in these studies. It has been shown earlier that varietal differences in a grain influence amylase production (Shands et al 1942, Atanda and Miflin 1970).

Ganesh Kumar and Venkataraman (1976) and Jaya and Venkataraman (1980) had also monitored changes in starch content of bengal gram over a germination period of 96 h. Ganesh Kumar and Venkataraman (1976) observed no change in starch content of bengal gram germinated for 24 h but after 72 h of germination, the starch content had decreased by 28% from the initial starch content of 40.5%. Jaya and Venkataraman (1980) had reported decreases of 11 and 38% in the starch content of bengal gram from its original value of 40.5% after 48 and 96 h of germination, respectively. The decreases in starch content after 48 (11 Vs 6%) and 72 h (28 Vs 12%) of germination as reported by Jaya and Venkataraman (1980) and Ganesh Kumar and Venkataraman (1976) were almost twice of those observed in the present study. Again, the variations might be due to varietal differences of the grains used which probably affected the amylase contents of the grains.

In contrast to the results observed in the present study and those by Azhar et al (1972) and Jaya and Venkataraman (1980), Aman (1979) had observed only a decrease of 1% in the starch

content of bengal gram over the germination period of 72 h.

In green gram, Jaya and Venkataraman (1980) had observed a much higher decrease in starch content as compared to that observed in bengal gram after 48 h of germination period (30 Vs 6%). These findings lend support to the fact that the hydrolysis of starch in response to germination differs with grain type.

In mung beans, Aman (1979) had observed a decrease of 10% in the starch content over its initial value of 42% during the germination period of 48 h. El-Shimi et al (1980) had observed a decrease of 24% in the starch content of broad beans from the initial value of 50% after 48 h of germination while El-Mahdy and El-Sebaiy (1983) had analysed 96 h germinated fenugreek seeds and found a decrease of 24% in their starch content from their initial value of 20.6%. In the present study, the decrease in starch over 72 h of germination period was 12%.

**Total sugars :** Table 9 shows that the total sugar content of bengal gram increased by 20% from its original value of 3.30 g/100g after 24 h of germination. Thereafter, the rate of increase in total sugar content was relatively slower and by the end of 48 h of germination the total sugar content of bengal gram was 4.17 g/100g which was significantly (Table 10) higher than the original value of 3.30 g/100g. After 48 h of germination, a significant decrease from 4.17 to 1.28 g/100g in total sugar content was observed. But no change in its content was observed between 60 and 72 h of germination. Earlier, Azhar et al (1972)

Changes in the  
had monitored the soluble sugar content of bengal gram in relation to various germination periods. The authors observed a sharp rise in the concentration of soluble sugars up to 72 h of germination which was followed by a decrease so that the level of soluble sugars was slightly lower than that observed after 48 h of germination. In the present study, a similar pattern in total sugar content that it became lower than the initial value was observed but the decrease was observed after 60 h of germination.

On the other hand, Jaya and Venkataraman (1980) had reported progressive increase in total soluble carbohydrates in bengal gram over a germination period of 96 h. They observed increases of about 23 and 47% after 48 and 96 h of germination. The magnitude of increases after 48 h of germination observed in the present study and that by Jaya and Venkataraman (1980) were comparable (26 Vs 23%). However, Jaya and Venkataraman (1980) had observed progressive increases in total soluble carbohydrates over a germination period of 96 h, but Azhar et al (1972) noticed a decrease in soluble sugars after 72 h of germination while in the present study a decrease in total sugars was observed after 48 h of germination.

**Reducing sugars :** The reducing sugar content of bengal gram increased progressively with the increase in germination period (Table 9) up to 48 h. The increase was significant only after 24 h germination period (Table 10). Thereafter the rate of increase became smaller as the germination period progressed from





24 to 36 to 48 h. After 48 h of germination period, a significant (Table 10) decrease in reducing sugars (from 1.47 to 1.13 g/100g) was observed. Earlier Azhar et al (1972) had reported a decline in the reducing sugar content of bengal gram by about 15% after 96 h of germination but it began to rise again to reach a level comparable to that observed after 48 h of germination.

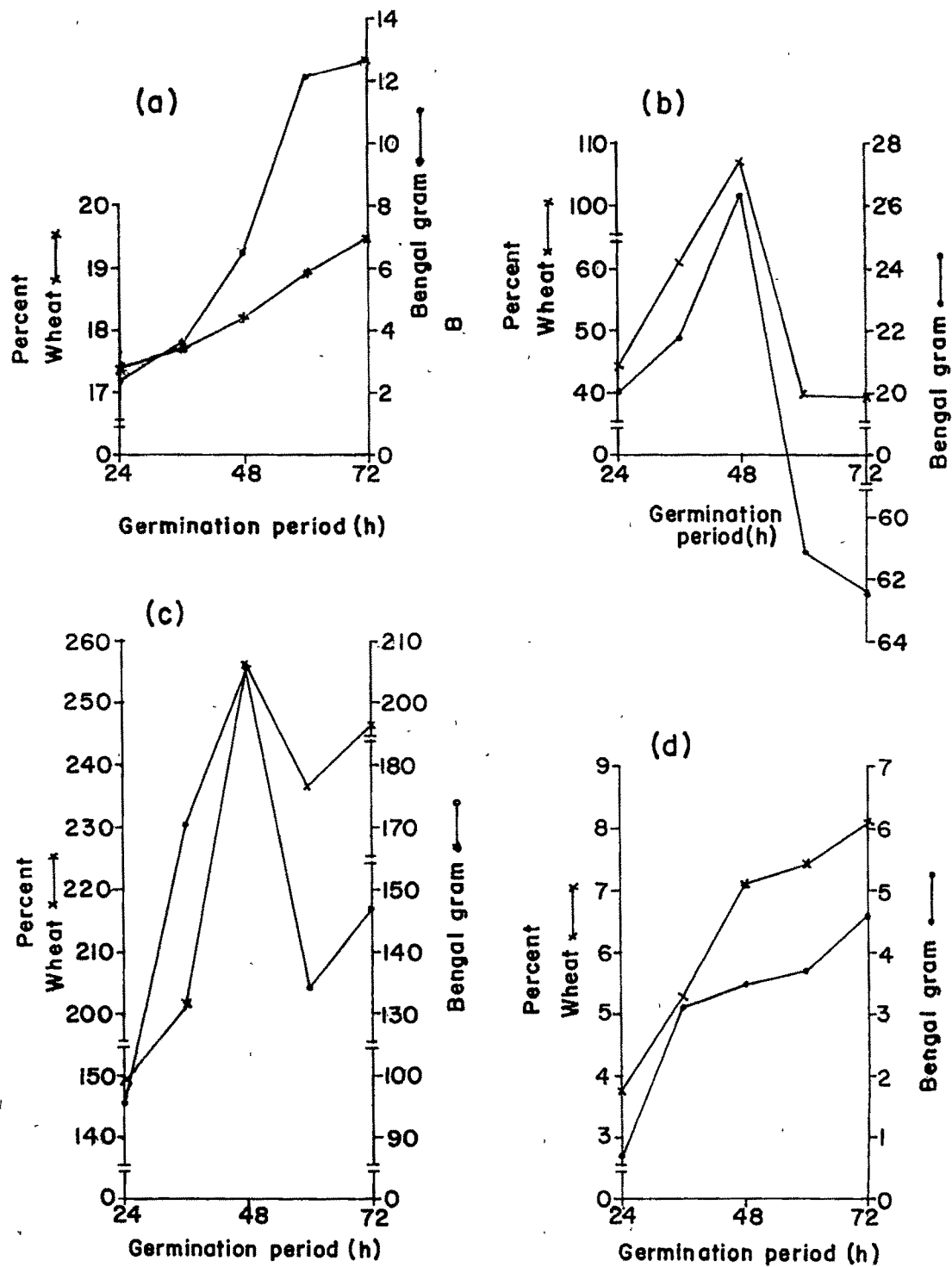
Aman (1979) had also observed decreases in the contents of fructose, glucose and sucrose<sup>of bengal gram</sup> after 24 h of germination period. The contents of fructose and glucose increased from 0.1 to 0.5% up to 24 h of germination, then decreased to 0.1% after 48 h of germination and only traces were observed after 72 h of germination. Likewise, sucrose increased from 4.3 to 5.3% after 24 h of germination and then decreased to 4.9% after 48 h of germination and remained constant until 72 h of germination.

Jyothi and Reddy (1981) had subjected ungerminated and germinated bengal gram to alpha amylolysis. The authors observed that the yield of maltose increased progressively from 39.3 (ungerminated) to 48.0 mg/100 mg legume up to 36 h of germination and thereafter tended to decline to 47.7 mg/100 mg legume after 48 h of germination. Because a decrease in maltose yield was observed after 48 h of germination by Jyothi and Reddy (1981) which reflected a decrease in amylase activity, it is possible that in the present study a similar decrease in amylase activity occurred in the bengal gram grains resulting in a decrease in total and reducing sugars. Jaya and Venkataraman (1980) also observed a progressive increase in reducing sugars with the

increase in germination time. They observed the increases of about 159 and 468% in 48 and 96 h germinated seeds. Comparing the results of the present study with those of Jaya and Venkataraman (1980) it appeared that the increase in reducing sugar content observed in the present study was higher than that reported by Jaya and Venkataraman (206 Vs 159%) after 48 h of germination.

Figure 6 presents the changes in carbohydrate profile of wheat and bengal gram. The decrease in wheat starch was markedly higher after 24 h of germination than that of bengal gram starch because the decrease was 17% in wheat starch and 2% in bengal gram starch (Fig 6a). With the increase in germination period from 24 to 72 h the decrease in wheat starch increased from 17 to 20% while that of bengal gram starch from 2 to 12%. The increase in total sugars was higher in wheat than in bengal gram. The increase in total sugars (Fig 6b) was to the tune of 44% in 24 h and 107% in 48 h germinated wheat but thereafter up to 72 h no increases were observed. Unlike bengal gram grains, the total sugar content of wheat after 72 h of germination remained higher than the original content of 3.30 g/100g (Table 9). The increase in total sugars of bengal gram was 20% after 24 h of germination which increased to 26% after 48 h of germination and thereafter showed a drastic decline so that the values after 60 and 72 h of germination were lower than that of the original value (Table 9). The increases in reducing sugars in wheat grains were also higher than that observed in bengal gram grains (Fig 6c). In wheat

Fig 6. Percent changes in (a) starch (b) total sugar (c) reducing sugar and (d) protein contents of wheat and bengal gram grains over a germination period of 72 h



grains, the increase in reducing sugar was 149% after 24 h of germination, 256% after 48 h of germination and 246% after 72 h of germination; corresponding values for bengal gram were 96, 206 and 148%. Although the pattern in changes of starch, total sugars and reducing sugars in wheat and bengal gram was more or less the same, higher decreases in starch and higher increases in sugars were observed in wheat in comparison to those of bengal gram.

Effect of malting on the protein content of grains soaked for 12 h and germinated for different periods

Wheat : The protein content (Table 11) of wheat grains germinated for different periods increased with the increase in germination period from 24 to 72 h. This apparent increase was probably due to loss in dry matter (Dalby and Tsai 1976). However, a significant increase in protein content over its initial value occurred only after 48 h of germination period (Table 12). By the end of 48 and 72 h of germination periods the increases of about 7 (from 14.03 to 15.02 g/100g) and 8% respectively (from 14.03 to 15.17 g/100g) in the protein content were observed.

These findings that the protein content increased with increase in germination period, were in accordance with those reported by Ranhotra et al (1977). The authors had reported an increase of about 5% from 13.58 to 14.28% in the protein content of 72 h germinated wheat. In the present study, however a higher increase (8 Vs 5%) in the protein content of 72 h germinated wheat grains was observed. An even higher increase of 10% (from 14.1 to 15.5%)

Table 11. Changes on malting in protein content of wheat and bengal gram soaked for 12 h and germinated for different periods (Mean  $\pm$  SE)

Germination period (h)	Wheat (N x 5.83) (g/100g)	Bengal gram (N x 6.25)
0	14.03 $\pm$ 0.232	15.28 $\pm$ 0.087
24	14.57 $\pm$ 0.050	15.39 $\pm$ 0.240
36	14.78 $\pm$ 0.212	15.75 $\pm$ 0.037
48	15.02 $\pm$ 0.249	15.81 $\pm$ 0.153
60	15.07 $\pm$ 0.009	15.85 $\pm$ 0.129
72	15.17 $\pm$ 0.147	15.98 $\pm$ 0.260

Table 12. 't' values for the variables of Table 11

Comparisons	Wheat	Bengal gram
0 Vs 24	2.278 NS	0.431 NS
24 Vs 36	0.963 NS	1.481 NS
36 Vs 48	0.734 NS	0.382 NS
48 Vs 60	0.201 NS	0.200 NS
60 Vs 72	0.680 NS	0.448 NS
0 Vs 36	2.389 NS	4.947**
0 Vs 48	2.912*	3.011*
0 Vs 60	4.483*	3.654*
0 Vs 72	4.145*	2.555 NS

See Table 8 for foot note

in protein content of Triticum dicoccum wheat after the same period of germination (72 h) has been observed by Singh et al (1983). Hamad and Fields (1979) reported that when the wheat grains were germinated for 120 h, the protein content of the flour increased apparently by only 4% from 10.23 to 10.64%. But a much higher increase of 15% (from 13 to 15%) in crude protein of 5 days germinated wheat (values taken from graph) has also been reported (Dalby and Tsai 1976).

Wu (1983) monitored the changes in protein content of oats germinated over a period of 192 h. He reported that the protein content initially decreased by 21% (from 17.8 to 14.1 g/100g) after 24 h of germination, and later increased by 19% (from 14.7 to 17.5 g/100g) after 48 h of germination thus the value became comparable (17.5 Vs 17.8 g/100g) to that of the raw oats. Beyond 48 h of germination, the protein content had increased by 6% (17.5 to 18.6 g/100g) and 14% (from 17.5 to 19.9 g/100g) after 72 h and 192 h of germination periods, respectively. The increase of 6% observed in the protein content of oats after 72 h of germination is comparable to the 8% increase observed in the present study in wheat grains. In millet, Opoku et al (1983) had reported an increase of 20% (from 10 to 12%) after 24 h of germination and of 40% after 36 h (10 to 14%) of germination period. Beyond this period (36 h) the protein content of the millet remained constant. The findings reported by others and those of the present study show that during the process of malting the protein content of a grain increases apparently and the increase relates to the grain type and the germination period.

Bengal gram : Table 11, also includes values for protein content of 12 h soaked bengal gram grains germinated for various periods. Similar to that observed in wheat, the protein content of bengal gram increased steadily from its initial value as the germination progressed. By the end of 48 and 72 h of germination period, increases of 3% (from 15.28 to 15.81 g/100g) and 5% (from 15.28 to 15.98 g/100g) were observed. The increase in protein content of bengal gram from its original value was significant after 36 h of germination period (Table 12). Azhar et al (1972) determined the protein content of bengal gram seeds over a germination period of 168 h. They had reported that within 120 h, the seedlings showed an increase of 30% in their net protein content. Likewise Jaya and Venkataraman (1980) had observed that the protein content of 72 h germinated bengal gram increased by 3% after 24 h of germination, and by 8 and 10% after 48 and 72 h of germination, respectively, from its original protein content of 20.2 g/100g seeds. In the present study, the magnitude of increase in protein content of bengal gram in response to 72 h of germination was lower, 5% versus 10%, as compared to that reported by Jaya and Venkataraman (1980). This might be due to the difference in activities of proteolytic enzymes present in bengal grams.

Figure 6d displays the changes in protein content of wheat and bengal gram grains germinated over a period of 72 h. It was observed that in both the grains the increase in protein content was steady. The percent increase in wheat was 4% after 24 h of