

CHAPTER 5

DISCUSSION

In this discussion, the findings of this research on pearl millet malting will be compared primarily with those of research into sorghum and barley malting, since these are the major malting cereals. Additionally, an attempt will be made to explain the results in terms of what is known about the biochemistry of germination and malting of these cereals.

In this research, it was observed that the proportion of highly degraded starch granules decreased from the proximal to the distal end in malted pearl millet grain. Modification of horny endosperm was less intense compared to floury endosperm. Similar results, which showed that the degradative enzymes diffuse out from their origin in the scutellum, were found in wheat, rye, oats, maize (Okamoto, Kitano & Akazawa, 1980) and in sorghum (Glennie, Harris & Liebenberg, 1983; Glennie, 1984).

Unlike barley and sorghum, modification of endosperm structure during the germination of pearl millet has received little attention. Much of the information about modification during the process of malting is on barley; probably because barley is the most suitable cereal for malting (Briggs, 1972; Okamoto, Kitano & Akazawa, 1980; Palmer, 1980; MacGregor & Matsuo, 1982; Fretzdorff, Pomeranz & Bechtel, 1982; Briggs & MacDonald, 1983; Fincher & Stone, 1993); and to some extent on sorghum (Hoseney, Varriano-Marston & Dendy, 1981; Aisien, 1982; Glennie, Harris & Liebenberg, 1983; Glennie, 1984). During germination of pearl millet, α -amylase enzyme is known to preferentially attack the spherical granules instead of polygonal granules of the grain and that the starch hydrolysis is more vigorous at the centre of the grain than at the periphery (Hoseney, Varriano-Marston & Dendy, 1981).

In germinating barley, endosperm breakdown begins in the region adjacent to the scutellum and proceeds, roughly parallel to the scutellar epithelium, from the proximal to the distal end of the grain (Brown & Morris, 1890 according to Palmer, 1989; Gibbons, 1981; Ranki, 1990). Briggs & MacDonald (1983) also reported that barley modification begins beneath the scutellum and advances with a "front" roughly parallel to the scutellum "face". These results were taken to indicate that the enzymes that catalyse initial modification of the endosperm come from the scutellum. Later, Duffus (1987) stated that there was also probably sufficient evidence to suggest that in barley, modification begins under the aleurone cells at the acrospire end.

Another noteworthy finding of this study was that the degradation of starch granules appeared to be more intense than degradation of protein bodies. Glennie, Harris & Liebenberg (1983) also reported this for sorghum.

In this present study, it appears that the aleurone layer, cell walls and horny endosperm were not greatly involved in the modification process. This hypothesis is supported by the fact that these anatomical parts of pearl millet grain remained almost intact throughout the course of germination. The findings for pearl millet are contrary to that for barley, where the cell walls are degraded at a relatively early stage (one to two days), but the breakdown of starch granules is not in evidence until 3 to 4 days after modification begin (Palmer & Mackenzie, 1986; Duffus, 1987). The general pattern of modification in germinating barley was established as breakdown of cell walls, protein and starch hydrolysis (Fretzdorff, Pomeranz & Bechtel, 1982). Lewis & Young (1995) also stated that, in barley, the enzymes modify the endosperm cell walls, and the protein and starch granules contained therein, by partially hydrolysing them to release low-molecular weight products that can diffuse and/or be transported back to the scutellum, the cells of which elongate to aid absorption, and hence to the developing embryo. Since the starch and protein is combined within cells, the walls of these cells hydrolyse first. In sorghum,

the endosperm cell wall fraction was found to be more resistant to enzyme attack than the more soluble cell walls of barley; hence, sorghum endosperm cell walls were reported not to be readily degraded during germination (Glennie, 1984). MacGregor & Matsuo (1982) and Briggs (1998) believe that it is the germ which has a dominant role in the modification of barley, not the aleurone layer.

As stated in this study the endosperm cell walls of pearl millet did not show any significant changes due to germination. It is therefore suggested that the mechanisms by which the enzymes impart modification of the grain structure in pearl millet may be similar to that occurring in sorghum, where although the cell walls persist during germination, their chemical composition and solubility patterns change (Glennie, 1984). The fact that the degradation of starch and protein reserves occurred without degradation of cell walls was also observed in sorghum and wheat malts by Glennie, Harris & Liebenberg (1983) and Palmer (1989), respectively. It was suggested that localised "portals" exist in the normal endosperm cell walls of these cereals, through which proteolytic and amylolytic enzymes migrate during malting. This is contrary to what happens in barley where presumably, cell wall disruption as well as protein matrix degradation are required before starch hydrolysis can take place (Slack, Baxter & Wainwright, 1979).

It appears therefore that from all pearl millet grain structure modification observed in this research, the enzymes attack started from the germ, or more precisely from the scutellum, rather than from the aleurone layer. This suggests that pearl millet grain modification is controlled by the scutellum. Thus, it appears that pearl millet is the same as sorghum (Koeher, 1981; Aisien, 1982; Glennie, Harris & Liebenberg, 1983; Dufour, Mélotte & Srebrnik, 1992) in these respects.

In barley, MacGregor & Matsuo (1982) reported, using scanning electron microscopy, that starch degradation starts near the ventral crease and moves along the endosperm-embryo junction to the dorsal edge of the kernel. These observations are at variance with other reported data. However, they were interpreted to mean that the site of initial α -amylase synthesis is not the aleurone layer but the embryo.

The fact that the findings of this research on pearl millet grain structure modification during germination are the same as that of sorghum is consistent with their structural similarity and the fact that these grains are both tropical cereals.

The objectives of steeping are to clean the grain and to hydrate it to the correct extent and to prepare it so that it grows steadily and modifies uniformly during the germination stage (Briggs, 1998). The steeping conditions used in this research were a steeping time of 8 h, with a cycle of 2 h wet, 2 h dry air rest, at four different temperatures, 20 °, 25 °, 30 ° and 35 °C. This steeping time was found to be reasonable and was based on the fact that in the germinability tests, both pearl millet varieties started showing signs of germination after 8 to 10 h. Opoku, Osagie & Ekperigin (1983) showed that pearl millet germinates relatively less rapidly, which was indicated by the appearance of the radicle within the 15-18 h. The difference in the results of this research and that of Opoku, Osagie & Ekperigin (1983) is probably related to the variety differences. Similar results were found by Sheorain & Wagle (1973) who reported that pearl millet varieties began to show evidence of germination (chitting) after 7 or 8 h, while in the case of barley varieties, these authors found that it only occurred after about 24 h. The temperature of steeping was chosen on the basis that it was within the temperature range used by other authors working with tropical cereals: Malleshi & Desikachar (1986c) and Nout & Davies (1982) (finger millet); Gomez, Obilana, Martin, Madzvamuse & Monyo (1997); Muoria & Bechtel (1998) (pearl millet and

sorghum); Morrall, Boyd, Taylor & Van de Walt (1986) and Dewar, Taylor & Berjak (1997b) (sorghum).

In order to germinate, the grain must absorb water during steeping. Hence, the germ becomes active and makes use of the oxygen dissolved in the steeping water. Steeping the pearl millet grain for 8 h resulted in a reasonable percentage of water uptake, compared to sorghum. There was not much difference in the percentage of water uptake between 8 and 10 h steeping time.

Water uptake represents the amount of water taken up by the grain during steeping. This is crucial since during steeping physical and biochemical changes take place. Such changes include swelling of grains and an increase in respiratory activity. The small size of the pearl millet grains may be responsible for the rapid increase in the levels of moisture and water uptake. Small kernels have a proportionally larger surface area than larger ones.

The fact that pearl millet of variety SDM 89004 had a higher water uptake in steeping than SDM 91018 may be related to the texture of the endosperm. Variety SDM 89004 had a softer endosperm than SDM 91018. Softer endosperm texture can be more easily penetrated by water during the steeping process of the grain (Bamforth & Barclay, 1993). Bamforth & Barclay (1993) also stated that water uptake into the starchy endosperm is critical before the food reserves of that tissue can be mobilised through the action of enzymes. Water uptake by grains is complex and is regulated by the porosity of the grain surface layers, the temperature, the osmotic driving force and related properties, the ease of spreading through the grain tissues and the resistance of the grain to swelling (Briggs, 1998). It is widely agreed that

grains which hydrate quickly malt better than those which hydrate more slowly (Bamforth & Barclay, 1993; Briggs, 1998). Similarly, pearl millet of SDMV 89004 variety which had slightly higher water uptake, generally had higher malt quality than SDMV 91018. According to Briggs (1998) some grains take up water more rapidly than others because of a higher osmotic pressure in the hydrated grain and/or a less dense (soft) structure that permits the grain to swell to accommodate the water taken up.

The finding that in both pearl millet varieties the green malt moisture content increased as the amount of water applied in the different watering treatments increased is of crucial importance, since steep-out moisture strongly influences the rate and extent of modification (Lewis & Young, 1995; Dewar, Taylor & Berjak, 1997b). At steep-out the mean moisture content of the grain was about 27% for both varieties. The reason for low steep-out moisture may be related to the size of pearl millet germ and the fact that pearl millet germ is rich in fat. Working with finger millet, Malleshi & Desikashar (1986c) reported steep-out moisture contents of 30-35%. By 5 days of malting the mean moisture contents were 33.2, 48.0 and 60%, for the variety SDMV 89004 and 35.3, 47.3 and 56.3%, for variety SDMV 91018, with the low, medium and high watering treatment, respectively. These results are relatively lower than those of Morrall, Boyd, Taylor & Van der Walt (1986) and Dewar, Taylor & Berjak (1997b) working with sorghum. The reason for this can be the difference in the steeping time used. These authors steeped sorghum with a cycle of 3 h wet, 1 h dry, for 16 and 24 h, respectively. In this research, pearl millet was steeped for 8 h with a cycle of 2 h wet and 2 h dry rest. These conditions were chosen because of the size of the pearl millet grains, and the fact that pearl millet grains started chitting between 8 and 10 h. However, future work should be done to investigate steeping for longer periods of time as was done for sorghum by Dewar (1997).

When malting tropical cereals, the grains are liberally watered during germination and this watering compensates for the relatively low moisture content of the grain after the short steeping time (Novellie, 1962a; Morrall, Boyd, Taylor & Van der Walt, 1986; Dewar, Taylor & Berjak, 1997b). Both pearl millet varieties examined germinated at lower moisture contents compared with barley and sorghum. Bamforth & Barclay (1993) stated that, non-barley grains will germinate below approximately 30% moisture, whereas the most intransigent batches may require a final moisture approaching 50 % to achieve uniform germination and that most barleys require a steeping regime that takes them to 42-46% moisture.

In order to produce malts of good and consistent quality, a pre-requisite is that a good grain for malting must show vigorous and uniform growth and must have a high percentage of germination. To be considered "ready for malting" maltsters would expect sorghum grain to germinate at least 50% by day 2 and 95-100% by day 3 in the Germinative Energy test (Dewar, Taylor & Joustra, 1995). It is also very important that such grain has a good potential for the production of enzymes under the correct malting conditions, particularly in sorghum beer brewing, where the malt generally has to act on at least twice its own weight of starchy adjunct (Novellie, 1966; Dewar, 1997).

Maltsters use the growth of the rootlets and acrospires (shoots) as indicators of the progress of malting. The root and shoot growth reported in this research, 10.4% (of total weight), is higher than that of finger millet, 5.1%, germinated for 5 days at 25 °C, found by Nout & Davies (1982) and lower than that found by Dewar, Taylor & Berjak (1997b), working with sorghum. These latter authors found roots and shoots values around 12 % in 4 days germination at 25 °C. The lower root and shoot growth observed with pearl millet malts in this research compared with that of sorghum, by Dewar, Taylor & Berjak (1997b), may be a reflection of the relatively lower DP levels found in pearl millet, compared to that of sorghum. Dewar, Taylor & Berjak

(1997b) found a DP of 45 SDU/g dry malt, for sorghum; while in this research the highest DP found at 30 °C, high watering, was 34.6 PMDU/g dry malt.

Reports on pearl millet and sorghum root and shoot growth in the literature are presented in the way that they cannot be directly compared with the results of this research, since authors Malleshi & Desikachar (1986c) only reported how the growth of root and shoot influenced the sorghum, finger and pearl millets malting loss. And in some cases the results of root and shoots of sorghum malt are reported in terms of length, as by Okolo & Ezeogu (1996).

In barley, rootlets are reported to contain on a dry weight basis non-protein extract 35-40 %, proteinaceous compounds 20-35 %, fatty materials and also vitamins A, B, D and E depending on the malt (Moll & Blauwe, 1991). The roots must be removed since they contribute undesirable substances to the wort (bitterness, too intense coloration of the wort, etc.) (Moll & Blauwe, 1991). However, in sorghum beer brewing roots and shoots are not removed, particularly because they are a good source of FAN in the wort (Dewar, Taylor & Berjak, 1997a).

Unlike malted pearl millet, ungerminated pearl millet grains of both varieties did not exhibited any DP. Diastatic Power, which is a measure of the joint activity of α - and β -amylase, is the single most important indicator of malt quality for sorghum beer brewing (Novellie, 1962a; Dufour, Mélotte & Srebrnik, 1992; Dewar, Taylor & Berjak, 1997b).

The rate of increase in malt DP, which was observed at 25 °C, high watering treatment, declined over longer periods of germination. This negative effect of the high watering treatment on DP of pearl millet malts during the last days of germination observed in this investigation was also observed for sorghum by Novellie (1962a; 1962b); Morrall, Boyd, Taylor & Van der Walt (1986) and Dewar, Taylor & Berjak (1997b). The reason for the decline in the rate of

increase of DP under high watering treatments can be the fact that higher moisture possibly promotes high amylase activity and hence rapid denaturation. This is supported by the fact that contrarily high moisture continued to have a beneficial effect on free α -amino nitrogen (FAN), the products of protease activity (Dewar, Taylor & Berjak, 1997b), late in germination. Dewar, Joustra & Taylor (1993) stated that for sorghum beer brewing a minimum DP of 28 SDU/g is required for sorghum malt. Hence, with respect to DP pearl millet malt, germinated for 4 and 5 days for variety SDMV 89004 and SDMV 91018, respectively, are suitable for sorghum beer brewing. The DP values for pearl millet malt found in this research are similar to those found for sorghum and millets by other authors (Novellie, 1959, 1962a, 1962b, working with sorghum; Morrall, Boyd, Taylor & Van der Walt, 1986, working with sorghum; Gomez, Obilana, Martin & Madzvamuse, 1997, working with sorghum and pearl millet), but lower than those found by Dewar, Taylor & Berjak (1997b) also working with sorghum.

The fact that pearl millet malts of SDMV 89004 variety, germinated with medium watering treatment, gave consistently higher α - (measured by inactivation of β -amylase) and β -amylase activity (measured by inactivation of α -amylase) compared with SDMV 91018 could be related to the former's higher germinability, which is a reflection of higher metabolic activity. Although these α - and β -amylase activities are similar to sorghum malt, they are lower than that of barley malt reported by Taylor & Robbins (1993) in a direct comparative study with sorghum malt.

The optimum temperature for maximum α -amylase activity found in pearl millet, between 25-30 °C, for both varieties is in agreement with the findings of other authors working with sorghum (Novellie, 1959, 1962a, 1962b; Nout & Davies, 1982; Pathirana, Shivayogasundaram & Jayatissa, 1983; Morrall, Boyd, Taylor & Van der Walt, 1986; Nzelibe & Nwasike, 1995; Dewar, Taylor & Berjak, 1997b), with finger millet and sorghum (Nout & Davies, 1982) and

with sorghum, fonio (*Digitaria exilis*) and pearl millets (Nzelibe & Nwasike, 1995). On this basis and that of their own data Muoria & Bechtel (1998) suggested that a germination temperature > 22 °C would be more desirable for sorghum and pearl millet, than barley, in order to obtain higher values of α -amylase. However, the results of this research showed that, in order to produce pearl millet malt of good quality, the temperature of germination should be ≥ 25 °C. Germination temperature of 22 °C is probably slightly too low for pearl millet malting.

A difference between the tropical (sorghum and millets) and temperate climate (barley) cereals is the fact that cereals indigenous to the tropical and subtropical areas of the world have no more than traces of β -amylase (Novellie & De Schaepdrijver, 1986; Dufour, Mélotte & Srebrnik, 1992; Taylor & Robbins, 1993; reviewed by Zeigler, 1999). However, both types of cereals have little α -amylase in the ungerminated grains. Germination of tropical cereals leads to the production of both amylases with α -amylase predominating (Dyer & Novellie, 1966; Novellie & De Schaepdrijver, 1986; Dufour, Mélotte & Srebrnik, 1992; Lewis & Young, 1995; Palmer, 1986). Ungerminated cereals from the more temperate zones have moderate amounts of β -amylase, but little α -amylase (Novellie & De Schaepdrijver, 1986; Dufour, Mélotte & Srebrnik, 1992). On germination, α -amylase is formed and β -amylase is synthesized during temperate cereal (barley) development but is rendered fully active during germination (MacGregor, Gordon, Meredith & Lacroix, 1972; MacGregor & Lenoir, 1987; reviewed by Palmer, 1989; MacGregor, 1996).

Cereal β -amylase plays a crucial role during brewing. As a contributor to the diastatic power of malt, its activity is essential for the generation of maltose, the most abundant fermentable carbohydrate in wort, and other easily fermentable sugars from cereal grain starch in the mashing process to fuel the production of alcohol by yeast (MacGregor, 1996; Zeigler, 1999).

In this research, soluble β -amylase activity (Betamyl assay) of pearl millet malts and the sorghum malt standard showed an effect of the reducing agent (cysteine) used in the assay. These results differ from that for sorghum found by Taylor & Robbins (1993), where there was no reducing agent effect. In the assay used by Taylor & Robbins (1993), the enzyme extraction was carried out using the reducing agent mercaptoethanol. The difference in results may have been due to the fact that the use of cysteine, as a reducing agent in the measurement of both soluble and total β -amylase in cereal grains ensures effective extraction and maximum stability of the extracted enzyme (McCleary & Codd, 1989).

Free α -amino nitrogen (FAN) is the second most important indicator of malt quality for sorghum beer brewing, because it is a source of nitrogen for yeast metabolism during fermentation (Daiber & Novellie, 1968; Pickerell, 1986, 1987). Low malt FAN content therefore would result in decreased fermentation, and hence low alcohol beer. During malting, some modification of the proteinaceous matrix is also necessary to make starch more easily degraded in mashing, as well as to form low-molecular weight nitrogenous compounds, especially amino acids (Lewis & Young, 1995). The latter will support the growth of the embryo and also of the yeast during fermentation.

The highest level 199 mg/100 g malt FAN was observed with pearl millet malt of SDMV 91018 variety, germinated at 35 °C for 5 days, medium watering treatment. This may be related to the fact that this variety had the higher percentage of roots and shoots 10.4%, which are a good source of malt FAN. Dewar, Taylor & Berjak (1997b), working with sorghum, reported that although roots and shoots represent only a relatively small proportion of the total weight of sorghum malt, their contribution to the total malt FAN was as high as 62%. During the germination process the increase in the amount of FAN in roots and shoots is a result of translocation of the products of storage protein breakdown from the kernel (Taylor, 1983).

A typical FAN specification for sorghum malt for sorghum beer brewing would be a minimum of 110 mg/100 g malt (Dewar, Joustra & Taylor, 1993). Hence, pearl millet malt is suitable for sorghum beer brewing with respect to FAN.

In cereals, FAN development may vary among varieties, probably because of differences in major enzyme characteristics and rate of protein metabolism during malting, as well as variations in grain protein structure and degradability, amino acid and peptide transport processes (reviewed by Owuama, 1999). It would be expected that the two pearl millet varieties investigated would show differences in malt FAN, since the endosperm texture as well as the protein content and Nitrogen Solubility Index of the two varieties were different. Variety SDMV 89004 had softer endosperm, higher protein content and higher Nitrogen Solubility Index compared to SDMV 91018.

Since the levels of nitrogenous substances in malt are not always consistent with the proteolytic activities, this may suggest the involvement of other factors than proteolysis, which influence protein modification during cereal germination. High solubility of nitrogenous substances may lead to low proteolytic activity or vice-versa (Okolo & Ezeogu, 1996).

Unlike DP, germination at the high watering treatment gave continuously higher malt FAN for both pearl millet varieties investigated. High moisture treatment promotes the protease activity of the germinating grain. The increase in malt FAN with germination time was also found in sorghum (Nout & Davies, 1982; Morrall, Boyd, Taylor & Van der Walt, 1986; Evans & Taylor, 1990; Dewar, Taylor & Berjak, 1997b) and in finger millet (Nout & Davies, 1982). The increase in malt FAN with germination time has to do with the fact that during germination, cereal proteins, mainly insoluble storage proteins in the endosperm, are converted into soluble proteins, peptides and amino acids, by the process of transamination (change of one amino acid into another one), which may have to supply nutrients to the developing embryo,

by the action of proteolytic enzymes (MacGregor, 1996). The FAN content of malt and subsequently of the wort depends on the proteolytic activity of the peptidases and proteinases (proteases) of the malt. The FAN content of wort would also depend on the mashing conditions applied (Taylor & Boyd, 1986).

Pearl millet malts had higher FAN in the grains germinated at high watering treatment, while medium and low watering treatment gave progressively lower malt FAN. In sorghum, Morrall, Boyd, Taylor & Van der Walt (1986) and Dewar, Taylor & Berjak (1997b) also found similar results. This could be due to the fact that high watering treatments favour root and shoot growth, hence the increase in FAN levels.

Agu & Palmer (1996), working with sorghum, also reported that the quantities of nitrogen transferred from endosperm to embryos increase with time and temperature of germination. The fact that DP, α - and β -amylases and FAN increased with an increase of germination time and temperature in malts of both pearl millet varieties investigated suggests that the effect was due to increased amylase and protease activity in the malt.

Malt extract is particularly important in lager beer brewing since it gives an estimation of how much of the malt will solubilise during mashing in the brewing process. In fact, the essence of mashing is to physically and enzymatically solubilise the malt. Hot water extract gives also an indication of the modification of the malt during the malting process. Fermentable sugars dominate the composition of malt wort. The maximum fermentability of wort that can be produced by malt enzymes is 75-78 %. This is mostly made up of maltose and maltotriose, which are result of α - and β -amylase acting together. The non-fermentable fraction of wort is at least 20% of the total extract. Most wort dextrans are branched molecules and contain the α -1-6 link of starch which malt amylases cannot attack (Lewis & Young, 1995).

The highest malt extract of about 70 % was found after 5 days of germination at high watering treatment. The increase in pearl millet malt hot water extract with germination time observed in this research is an indication of the progress of modification (breakdown of the endosperm reserves by amylase and protease activity) of the malt during germination. High watering treatments may have facilitated the solubilisation of the solids of pearl millet malts. An increase in malt extract with germination time was also found, in pearl millet, by Nzelibe & Nwasike (1995); in finger millet, by Malleshi & Desikachar (1986c) and Nout & Davies (1982) and in sorghum, by Morrall, Boyd, Taylor & Van der Walt (1986). These last workers also found that extract increased with watering treatment level.

The difference in the level of malt extract between the two pearl millet varieties investigated as well as with the sorghum and barley standards used are related to the degree of modification of the malts of the cereals used during the malting process, since the action of α - and β -amylase enzymes in the degradation of starch is very important during mashing. Pearl millet malts had higher malt extracts than the sorghum malt standard, but generally lower than the barley malt standard. The difference in malt extract is also related to the degree of solubility in water of the resulting products of the hydrolysed malts.

It would also appear that although it is heat (gelatinisation) that renders starch soluble in mashing to form extract, it is often assumed that α -amylase action helps starch solubilisation. This implies in practical terms that α -amylase is the extract-producing enzyme (Lewis & Young, 1995).

Efficient starch conversion does not occur during mashing until the gelatinisation temperatures of the starch granules which are being mashed are reached (Palmer, 1986). Therefore, the temperature (60 °C) at which the hot water extract assay is performed may be another reason for the

differences in hot water extract of the three cereals. Barley starch gelatinises and is completely solubilised in well-made malts during mashing for determination of hot water extract. The temperature of gelatinisation of cereals depends on the source of starch but for barley's is mostly between 65 and 75 °C (Lewis & Young, 1995). However, the starch of pearl millet and sorghum does not begin to gelatinise until substantially higher temperatures are reached (Palmer, 1989; Briggs, 1998). In pearl millet and sorghum, if the temperatures of mashes are raised much over 65 °C, enzyme destruction is rapid and so starch conversion is incomplete and recovered extracts are low (Briggs, 1998). At elevated mash temperatures the fermentability of the wort is low because the β -amylase is rapidly denatured. Beta-amylase works best at 55-60 °C and α -amylase about 10-15 °C higher (Taylor, 1992; Lewis & Young, 1995).

Mashing below 65 °C increases the fermentability of the wort; mashing above 65 °C reduces fermentability and increases molecular sizes of wort dextrins. This alteration to the sugar spectra of wort is understandable because the starch extract-releasing and liquefying enzyme α -amylase is more heat stable than the sugar (maltose) releasing enzyme β -amylase (Palmer, 1989). Beer filtration problems reported in sorghum brewing by Aisien & Muts (1987) may reflect the possibility that during malting localised "breakdown" of the endosperm cell walls may expose portals through which proteases and amylases migrate. This is substantiated by the observation that cell walls do not degrade, like in barley, during malting (reviewed by Palmer, 1989). Incomplete starch conversion is desired for the production of opaque beers. In contrast, total starch conversion is highly desirable for the production of conventional larger beers.

Although the highest hot water extract 69.0 % found is lower than 75-80%; a value considered suitable for conventional beer brewing (Bamforth & Barclay, 1993; Briggs, 1998); the fact that it was, in some cases higher than that of barley malt standard, 59.4 %, and also higher than some reported values for

extracts for barley malt (Briggs, Hough, Stevens & Young, 1981; Bamforth & Barclay, 1993; Briggs, 1998), together with the fact that malting considerably reduced the pearl millet fat content, is *per se* a good indication for the potential use of pearl millet malt in conventional beer brewing. Pearl millet is very rich in fat, which could lead to rancidity problems as well as poor foam head retention during the brewing process, hence affecting negatively the organoleptic properties of the beer produced (Zeurcher, 1971). In this research, the levels to which pearl millet fat content was reduced by malting are almost within the range (2-3 %) as it occurs in barley (Briggs, 1998). Moll & Blauwe (1991) suggested that in order to reduce the lipid content of pearl millet malt in brewing it is recommend to add sulphuric acid during mashing. However, no scientific explanation was given concerning the mechanism by which sulphuric acid reduces the lipid content of pearl millet malts during the mashing process of brewing.

A possible method to improve the extract content was proposed by Nout & Davies (1982) working with finger millet. These authors suggested that a small addition of barley malt could be used. This could simultaneously increase the β -amylase activity in the mash. Later, Taylor & Daiber (1988) suggested that the increased α -amylase stability afforded by calcium conditions, could improve extract in sorghum mashes.

One of the objectives of the malting industry is to operate as economically as possible, using a rapid process with a minimal malting loss (Moll & Blauwe, 1991). The increase in malting loss with the germination time, temperature and watering treatment observed in this research can be attributed to the respiratory activity which takes place on germination. A direct relation between germination time and malting loss was also found in sorghum (Novellie, 1962a; Pathirana, Shivayogasundaram & Jayatissa, 1983; Morrall, Boyd, Taylor & Van de Walt, 1986; reviewed by Owuama, 1999) and in barley (reviewed by Briggs, Hough, Stevens & Young, 1981; reviewed by Bamforth & Barclay, 1993; reviewed by Briggs, 1998).

Potassium bromate has been used to reduce malting loss (Nout & Davies, 1982; Agu & Okeke, 1991; 1992; Agu & Ezeanalue, 1993; Nzelibe & Nswasike, 1995). However, the safety of the use of potassium bromate has not been clarified.

In this investigation, the highest malting loss, around 12 %, was recorded with variety SDMV 91018, at 5 days germination, medium watering treatment. Similar results were reported for sorghum (Novellie, 1962a, 1962b) and finger millet (Nout & Davies, 1982). However, Morrall, Boyd, Taylor & Van de Walt (1986) reported relatively higher losses in sorghum. The high malting losses reported by these authors may be related to the high respiratory activity observed in sorghum and the long steeping time used. These authors steeped sorghum for 16 h, while in this research pearl millet grains were steeped for 8 h only.

In barley, the total malting losses during malting are usually in the range 6-12% of the original dry weight (Briggs, 1998). However, barley malting loss results presented in the literature are not directly comparable to pearl millet results of this investigation since they do not include the roots and shoots.

From now on, in this discussion, changes brought about by germination which have a broader influence on pearl millet's food value than just brewing will be considered. These changes are mainly in nutritional and functional properties.

Carbohydrates are the single most important source of food energy in the world. They comprise some 40 to 80% of total food energy intake, depending on locale, cultural considerations or economic status (FAO/WHO, 1997). The reduction in the total carbohydrate content of the pearl millet varieties, from 78.4 to 53.8% and from 75.3 to 54.5% for the variety SDMV 89004 and SDMV 91018, respectively, and increase in the percentage of the TCES, from 10.6 to 13.5% for the variety SDMV 89004 and from 10.1 to 11.0% for the variety

SDMV 91018, during germination, can be attributed to the fact that some of the endosperm starch is consumed during germination to provide energy.

In this research, pearl millet carbohydrates decreased about 10 % in only 48 h. Opoku, Ohenhen & Ejiofor (1981) found lower decrease in the level of carbohydrates, which decreased about 8% after malting pearl millet at 25 °C for 48 h. Working with finger millet, Malleshi & Desikachar (1986c) reported that germination for 48 h resulted in about 5% loss in starch content and that the continuation of germination up to 96 h resulted in about 10% loss in starch content. Sripriya, Antony & Chandra (1997) found that starch content of finger millet decreased by about 12 % on germination for 24 h at 30 °C. In contrast, the starch content of barley is reduced by only about 10% during malting (Bathgate & Palmer, 1972; Lewis & Young, 1995). The difference between the results of this investigation and that of other authors can be attributed to the differences in the amylase activity of barley, pearl and finger millets varieties used.

The fact that the reduction in carbohydrate content was higher in variety SDMV 89004 than SDMV 91018 could be related to its higher germinability. Variety SDMV 89004 had higher DP and higher carbohydrate content. This also indicates that the carbohydrate content of pearl millet was affected by the grain variety and as well as by the respiratory activity of the grains.

The reduction of carbohydrate during germination observed in this research would contribute to a decrease in the energy value of food products prepared from germinated flours. In adults, it is important that the amount of energy ingested be matched to the amount of energy expended. However, this decrease is compensated by the fact that carbohydrates of malted pearl millet are more soluble than that of non-germinated grains. In infants the amount of energy ingested is more than they expend since they use the rest of the energy to build up their bodies (Mosha, 1985). Carbohydrates exert a protein-

sparing effect. When carbohydrates are depleted in the animal body and the animal needs additional energy, it gets this energy by oxidizing fats and proteins (Potter & Hotchkiss, 1995). However, if carbohydrates are supplied, the body oxidizes them for energy in preference to protein and, thus, the protein is spared. It is crucial that the reduction of the level of carbohydrates (starch) should not be very high if the malts are meant for the preparation of traditional southern African food products, such as opaque beers, porridges, and traditional unleavened pancakes, called *makati* in Mozambique, as well as weaning foods for infants called *nthlatu* in the south of Mozambique, where minimum carbohydrate reduction may be advantageous.

The increase in the TCES with germination time observed in this investigation was also found by Khetarpaul & Chauhan (1990b). These authors found that the *in vitro* starch digestibility increased by more than three-fold when pearl millet grains were germinated for 24 h. Improvements in starch digestibility of pearl millet through germination has also been reported by Khetarpaul & Chauhan (1990a); Chaturvedi & Sarojini (1996) and Pawar & Pawar (1997).

The changes in the susceptibility of starch to enzyme attack which took place during the germination of pearl millet are advantageous in respect of producing a product with improved nutritional quality, which can be used as an ingredient in various food products, particularly weaning foods for infants.

The non-significant difference between the percentage of the TCES of the two pearl millet varieties before they were germinated can be explained by the fact that the ratio of amylose/amylopectin in both varieties was also not significantly different. A difference may have been expected if the ratio had been different. Amylopectin is a highly branched chain biopolymer which is more susceptible to enzyme attack than amylose. Its branched chains give water the ability to penetrate the structure more easily than that of amylose and to gelatinise the starch (Rebar, Fishbach, Apostolopoulos & Kokini, 1984).

The lower phytic acid content in pearl millet of variety SDMV 89004 may be the reason for the higher percentage of the TCES observed in the pearl millet malts of variety SDMV 89004 compared to SDMV 91018. Phytic acid may decrease starch digestibility by binding with calcium which is known to be necessary for α -amylase activity (Yoon, Thompson & Jenkins, 1983).

The Water Absorption Index (WAI), which is a reflection of the amount of pearl millet starch dispersed in excess water, was not significantly different between the two pearl millet varieties. This may be attributed to the fact that both pearl millet varieties had similar starch amylose contents. The decrease in the Water Absorption Index and the increase in the Water Solubility Index (WSI) with germination time for both varieties may have to do with the fact that during the germination process the carbohydrate content decreased as a result of hydrolysis by the amylase enzymes. The increase in WSI with germination is of significance since it gives an indication that germination can be used to increase the amount of soluble materials, such as starch and amino acids, which can be easily digestible.

Fat content was reduced from 6.4% to 3.4% and to 3.1% for the varieties SDMV 89004 and SDMV 91018, respectively, after only 5 days of germination. During germination, lipids are not metabolised as fast as other food reserves like carbohydrates and proteins (Opoku, Ohenhen & Ejiofor, 1981). Other authors also reported that there is a reduction in lipid (fat) content in finger and pearl millets during germination (Opoku, Osagie & Ekperigin, 1983; Mtebe, Ndabikunze, Bangu & Mwenezi, 1993; Pawar & Pawar, 1997). Since fat provides twice as much energy as carbohydrates, the reduction in fat content observed during germination implies a reduction in the energy value of pearl millet malt compared to grain. However, in the case of pearl millet, this reduction may bring an increase in palatability of pearl millet food products. The development of fatty acids, which occur mainly due to the action of lipase, cause bitterness and can make pearl millet meals unacceptable (Lai & Varriano-Marston, 1980a). Generally, pearl millet

varieties are characterised by high lipid and high protein contents compared to other species of millets and sorghum (Hoseney, Varriano-Marston & Dendy, 1981). This is due to a high ratio of germ to endosperm of the pearl millet grain, which is responsible not only for the high protein content but also the high lipid content of the grain.

In both pearl millet varieties, malts had lower protein content than the grains. The slight decrease in protein content of pearl millet germinated grains with germination time can be attributed to the loss of low molecular weight nitrogenous compounds during the steeping process and rinsing of the grains during germination.

The results of this research are similar to those of Bhise, Chavan & Kadam (1988), who found that the crude protein of pearl millet decreased from 11.6 to 11.2% after 72 h of germination at 30 °C. Mtebe, Ndabikunze, Bangu & Mwenezi (1993) revealed that germination did not induce significant variation in protein content of pearl millet and other cereal grains investigated. However, Opoku, Ohenhen & Ejiofor (1981) found an increase in protein content of pearl millet, from 8.6% to 11.8 %, after germination for 3 days at 25 °C. The increase in protein content during germination was also observed in wheat, triticale, barley and rye by Dalby & Tsai (1976). In sorghum, Subramanian, Sambasiva, Rao, Jambunathan, Murty & Reddy (1995) reported that, malts show lower protein than non-malted grains. The difference of the results of this research and that of other workers can be explained by the fact that the reported increase in protein content during germination, which is attributed to loss in dry weight, particularly carbohydrates, through respiration, is not true, but apparent, since the absolute amount of protein per kernel does not change significantly during germination.

Nitrogen Solubility Index or Modification Index, or Kolbach index, as is known by brewers (Lewis & Young, 1995; Nzelibe & Nwasike, 1995), gives an indication of the amount of water-soluble nitrogen expected in the wort. The increase in Nitrogen Solubility Index and soluble nitrogen with germination time for pearl millet of SDMV 89004 variety can be due to gradual degradation of reserve protein into amino acids and short peptides caused by rising the levels of protease enzymes during germination. Working with pearl millet, Nzelibe & Nwasike (1995) also found that soluble nitrogen of wort and Modification Index increased with germination time. The fact that in variety SDMV 91018, ungerminated pearl millet grains had higher Nitrogen Solubility Index and soluble nitrogen than germinated samples may have been due to the loss of amino acids through leaching during the steeping process and during the watering treatments. Nitrogenous substances are lost by leaching during steeping, but there are no gains or appreciable losses from the whole grain during the other stages (Briggs, 1998). This decrease was unexpected since, as from the malt FAN results, the two pearl millet varieties used apparently had similar grain protein structure and degradability. Additionally, variety SDMV 89004, which had softer endosperm, had lower NSI and soluble nitrogen than SDMV 91018.

Nitrogen Solubility Index is important for food use since soluble nitrogen is regarded as digestible nitrogen. Hence, the increase in Nitrogen Solubility Index due to malting is a compliment of the increase in *in vitro* protein digestibility observed in both varieties of pearl millet malts investigated.

The increase in *in vitro* protein digestibility with time of germination, from 69% to 95% and from 58% to 94% for the varieties SDMV 89004 and SDMV 91018, respectively, can be attributed to an increase in soluble proteins, due to partial hydrolysis of storage proteins by endogenous proteases produced during the germination process. Such partially hydrolysed storage proteins may be more easily available for pepsin attack (Wu & Wall, 1980; Bhise,

Chavan & Kadam, 1988). Pepsin is used to hydrolyse the insoluble protein into soluble amino acids and peptides in the *in vitro* protein digestibility assay.

The decrease in antinutrients (phytic acid) may have also contributed to the high levels of protein digestibility observed. In this research, ungerminated pearl millets had 0.09% and 0.11% of total polyphenols in the variety SDMV 89004 and SDMV 91018, respectively. These values are similar to that reported in pearl millet by other authors (reviewed by Serna-Saldivar & Rooney, 1995). Antinutrients, such as tannin-polyphenols and phytic acid can bind to proteins including enzymes, and are therefore likely to inactivate enzymes involved in hydrolysis of endosperm materials (Chavan, Kadam & Salunkhe, 1981).

Other authors have also reported a significant increase in *in vitro* protein digestibility when finger and pearl millets are germinated (Bhise, Chavan & Kadam, 1988; Khetarpaul & Chauhan, 1990b; Pawar & Pawar, 1997). The increase in *in vitro* protein digestibility due to malting observed in pearl millet is of great nutritional significance for people living in the Semi-Arid Tropics (SAT) of the world since it will mean better utilisation of the protein of pearl millet.

Protein quality is critically important in developing countries where human diets consist mainly of cereals. This is especially valid for weanling children and infants who have a high essential amino acid requirement per kilogram of body weight (Serna-Saldivar & Rooney, 1995). However, a diet of cereals (with 8-10% protein content) is able to meet the protein requirements of adults, provided enough is eaten to supply the energy requirements (Cheftel, Cuq & Lorient, 1985).

Amino acid composition of food is important in evaluating the nutritive value of the protein, while protein digestibility is a primary determinant of the availability of its amino acids (FAO/WHO, 1990). A complete protein is one

that contains all the essential amino acids in the amounts and proportions to maintain life and support growth when used as the sole source of protein. Such a protein is said to have high biological value (Potter & Hotchkiss, 1995). In general, the levels of essential amino acids showed little changes with germination time. Although a change in the amino acid profile was observed due to germination, the total amino acid content remained the same since the protein content did not change.

Like in other tropical cereals, the most limiting amino acid in pearl millet is lysine. Lysine content of the variety SDMV 89004 showed a non-significant decrease. However, the lysine content of the pearl millet of the variety SDMV 91018 increased throughout germination. The difference in lysine content between the two varieties could be due to slight differences in the proportion of germ in both pearl millet varieties investigated. The increase in the lysine content of the protein of germinated pearl millet of variety SDMV 91018 is related to the transamination (change of one amino acid into another one), which may have occurred during germination affecting the amino acid profile of pearl millet. This transamination was also reported in sorghum by Taylor (1983).

In this research, the level of leucine in pearl millet malts was generally higher than the FAO Scoring Pattern (Serna-Saldivar, McDonough & Rooney, 1990; Hosene, 1994). Generally, the essential amino acid contents found in this investigation are between the range published by other authors (reviewed by Lásztity, 1984; Chung & Pomeranz, 1985; Ejeta, Hassan & Mertz, 1987; Serna-Saldivar, McDonough & Rooney, 1990; reviewed by Serna-Saldivar & Rooney, 1995) for pearl millet grains. With the exception of the leucine, they were lower than that of the FAO Scoring Pattern. However, the lysine content of the malt of one of the pearl millet variety investigated was about 75% of the FAO Scoring Pattern for lysine. Taylor (1983) found an increase of nearly 4-fold in lysine content of sorghum during germination. Almeida-Dominguez, Serna-Saldivar, Gomez-Machado & Rooney (1993) reported an

increase in lysine from 2.2 to 3.2 and 3.0 to 7.8 g/100 g of protein when normal and high lysine-sorghums were germinated. The difference between the results of pearl millet with that of sorghum may be related to the fact that in pearl millet grains, lysine content is already relatively high compared to sorghum.

The malting process drastically reduced the pasting peak viscosity of pearl

The nutritional and health significance of high leucine content cereals has been a controversial subject. Reports from Bender (1983) and Magboul & Bender (1983) indicate that diets in leucine can precipitate niacin deficiency. Niacin deficiency disease, pellagra, is endemic, and signs of it are sometimes referred as the four successive "D": dermatitis, diarrhea, dementia and death (Hulse, Laing & Pearson, 1980). However, more recent studies showed that leucine is not involved in the etiology of pellagra (Cook & Carpenter, 1987; Young & Fukagawa, 1988).

malting process, have also been reported for other cereals.

The fact that phytic acid content was reduced from 0.24% and 0.27% to 0.024% and 0.037% for the varieties SDMV 89004 and SDMV 91018, respectively, with germination time is presumably due to phytase activity. Phytase is an enzyme which can hydrolyse phytic acid to inositol and free orthophosphate (Thompson & Serraino, 1985). Like other cereals, pearl millet contains a considerable amount of phytic acid, representing more than 70% of the total phosphorus in the grain (Chauhan, Suneja & Bhat, 1986). Opoku, Ohenhen & Ejiofor (1981) also found a decrease in pearl millet phytic acid from 0.26 % in grain to 0.04 % in malt. Hulse, Laing & Pearson (1980) reported phytic acid values from 0.21 to 0.25 % in finger millet, whereas phytate content values for whole meal grain of proso millet ranged from 0.17 to 0.47%.

malting process, have also been reported for other cereals.

As stated, phytic acid interacts with minerals and other nutrients such as proteins making them unavailable to the organism (Kumar & Kapoor, 1984). The decrease in pearl millet phytic acid observed in this research will improve the nutritional quality of pearl millet malt food products by increasing the

bioavailability of proteins and minerals. Pearl millet is known as a good source of the essential minerals, calcium, iron, zinc, copper and manganese (Kumar & Kapoor, 1984). However, the bioavailability of these minerals may be affected by the presence of antinutrients such as phytic acid and polyphenols.

The malting process drastically reduced the pasting peak viscosity of pearl millet malts in both varieties investigated. Malleshi & Desikachar (1986b) and Mbithi-Mwikya, Van Camp, Yiru & Huyghebaert (2000) working with finger millet, have also reported this. The reduction in peak viscosity may be attributed to the high α -amylase activity of malts.

Alpha-amylase is known to rapidly solubilise starch into dextrans, thereby reducing the viscosity of starch solutions (Bamforth & Barclay, 1993). Additionally, the decrease in starch content observed in both pearl millet varieties investigated may have also contributed somewhat to the reduction in flour paste viscosity. When α -amylase acts on starch molecules a few cleavages of α (1 \rightarrow 4) glucosidic bonds by this enzyme causes rapid decrease in the size of starch molecules, and there is an accompanying dramatic decrease in the viscosity of a starch paste. For this reason, the enzyme is commonly called the liquefying enzyme (Lewis & Young, 1995).

Germination of pearl millet for more than 1 day produced malts of free-flowing slurry with minimum viscosity. The rate of lowering of viscosity in pearl millet malt flours was greatest up to 3 days germination, beyond which the changes were slight, in fact could not be any further reduction since it was like water. This observation agrees with the findings of Mtebe, Ndabikunze, Bangu & Mwemezi (1993), working with finger and pearl millets.

The reduction of viscosity of the variety SDMV 89004, which had higher α -amylase activity than SDMV 91018, was greater than in SDMV 91018. This clearly shows the role of α -amylase in malt in reducing the flour viscosity. The peak viscosity of samples germinated for longer periods was lower than that

of samples germinated for shorter periods, presumably due to the partial hydrolysis of starch by α -amylase during the germination process and the higher level of α -amylase in the longer germinated malts.

In the SADC region and most other African countries where porridge is a staple food, the pasting properties (i.e. viscosity of the flour) is an important parameter. Porridges of high viscosity are more preferable for adults, because porridge is generally eaten with the fingers. Low viscosity porridges are suitable for consumption by infants as weaning foods due to their limited stomach capacity and the ability to chew (Pelembé, Erasmus & Taylor, in press). The period of a human being's development from the neonatal stage to the preschool stage is critical to growth. During this period, adequate food intake (including that of weaning foods) is vital to good nutritional status (Mosha & Lorri, 1987). The low viscosities observed after germinating both pearl millet varieties is a good indication that malted pearl millet is suitable as a diastatic adjunct to reduce the viscosity of cereal-based weaning porridges.

Mosha & Lorri (1987) found that three times as much germinated flour could be used, while maintaining the same consistency of the gruel using equal values of porridge. The addition of 5% germinated low-tannin sorghum flour (enzyme rich) to thick ungerminated sorghum and maize gruels reduced the viscosity to acceptable weaning food consistency. Food intake by preschool children 12 - 48 months of age was found to be significantly higher for bulk-reduced, low viscosity gruel with 20 % solids, than with ungerminated gruel (Mosha & Lorri, 1987). In ungerminated gruel, the viscosity of 1000 - 3000 cP (spoonable) is only obtained with gruels containing about 10 % flour (Mosha & Svanberg, 1983). Hence, the low viscosity malts found in this research could be suitable as bulk-reduced weaning foods of high nutrient density, which could eventually improve the nutritional status of young children and raise the nutrient intake of the infirm.

The fact that germination successfully reduced the mousy odour of pearl millets is important for the rural communities of Africa and India, where pearl millet is a staple food, since it will increase the palatability and could increase the consumption of pearl millet food products.

Since the exact compound, which causes the mousy odour, as well as the mechanism in which the mousy odour is promoted, are not known for certain, one could speculate that the phenolic pigments, which are responsible for the mousy odour, may have been leached out during the germination process. In fact the reduction of the C-glycosylflavone (Reddy, Faubion & Hosney, 1986), the major flavone known to be present in pearl millet grains and mainly concentrated in the germ, could be noticed by the changes in the colour from the natural grey colour characteristic of pearl millet grain to the light brown (tan) of the pearl millet malts. The decrease in mousy odour observed during the pearl millet malting process can also be attributed to the decrease in pH due to the growth of lactic acid bacteria. The colour of phenolic pigments can be changed as a function of pH (Davídek, Velíšek & Pororný, 1990).

FAN, and moderate malting loss are 25-27°C and 1.5 days germination, medium watering treatment. However, if the malting process is to be conducted in a short period of time, i.e. 1-3 days, the germination temperature and watering regime should be higher, 30-35°C, high watering regime. The levels of DP, FAN, α -amylase activity and malting loss of pearl millet malt, which are similar to sorghum malts, represent an excellent potential for utilization of pearl millet for opaque beer brewing purposes. Additionally, pearl millet malt could be better than sorghum as an alternative for lager beer brewing due to the fact that it has higher β -glucanase activity.

The high level of malt extract found in pearl millet malt, around 70%, together with the fact that malting considerably reduces the fat content to the levels which occur in barley, is an indication that pearl millet malt can be used in lager beer brewing at least as a barley malt extender. However, brewing studies are recommended to find out to what extent pearl millet malt can