ENDOGENOUS AND EXOGENOUS FACTORS INVOLVED IN SORGHUM GERMINATION WITH REFERENCE TO MALTING

Janice Dewar

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DEDICATION

I would like to dedicate this thesis to Professor Anne Alexander who died after a tragic accident in 1995. Professor Alexander, or Prof Anne or Auntie Anne as she was affectionately known to her students, was one of the most inspirational figures of my life. She created in me, a passion for science. She was one of the only persons I have ever met who I believe, had truly reached self-actualisation, the top of Maslow's hierarchy of needs. Even although she may not have passed on her genes, she left something of herself in each one of her students.

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ABSTRACT

In Africa, the grain sorghum (*Sorghum bicolor* (L.) Moench), is malted to provide the most important ingredient in brewing, malt, which is used primarily for the production of traditional (opaque) sorghum beer. Malting is the germination of cereal grain in moist air under controlled conditions, the primary objective being to promote the development of hydrolytic enzymes which are not present in the ungerminated grain. The malting process can be physically split into three distinct unit operations (*viz.* steeping, germination and drying). To date, little attention has been given to optimising the conditions of steeping for sorghum. The effects of different steeping variables (time, temperature and aeration) on the quality (in terms of diastatic power (amylase activity), free amino nitrogen and hot water extract) of sorghum malt for brewing were investigated. Malt quality was found to increase with steeping time, over the range 16-40 hours and the optimum steeping temperature was found to be in the range 25 to 30°C. Aeration during steeping appeared to be necessary to maximise the malt quality, particularly when steeping was conducted for long periods at high temperatures. Of particular significance was the observation that final sorghum malt quality was highly significantly correlated ($p<0.01$) with grain moisture content at steep-out (the end of the imbibition period). When steeping conditions based on these findings were used, a germination temperature of 25-30°C was found to be optimal for sorghum malt quality. As with steep-out moisture, green malt (grain after the specified germination time) moisture content was correlated significantly ($p<0.01$) with final sorghum malt quality. The finding that sorghum malt quality is related to steep-out moisture content was given further substance when it was shown that the stimulatory effect on sorghum malt quality of steeping sorghum in a dilute solution

of alkali, actually increases the amount of water taken up during steeping probably because the alkali disrupted the pericarp cell wall structure of the grain.

Barley malting practices have taken advantage of the knowledge that the exogenous application of gibberellic acid can enhance the synthesis of the critically important malt hydrolytic enzyme, α-amylase. To date, literature on the effect of exogenous application of gibberellic acid on sorghum malt quality has been inconclusive; with reports both of no effects, and of positive effects, on amylase activity. To elucidate the possible control mechanisms involved in sorghum germination, a combined HPLC-radioimmunoassay technique was used to determine the levels of selected plant growth regulators from the groups auxin, cytokinins, gibberellins and abscisic acid in sorghum at various stages of germination. Levels of gibberellic acid were low throughout germination. During germination the levels of the other plant growth regulators declined, but a peak in cytokinins followed the first visible signs of root protrusion. The high level of the germination inhibitor and gibberellic acid antagonist, abscisic acid, in the germ (embryo inclusive of scutellum) portion of the mature non-germinated grains was noteworthy. Based on these findings, it was determined that sorghum malt quality could in fact be improved significantly by the application of exogenous gibberellic acid. However, this was effective only if it was administered during the end of steeping or at the beginning of the germination step.

By optimising the conditions of steeping and germination and by steeping in dilute NaOH or in gibberellic acid not only should it be possible to enhance the quality of sorghum malt, it should be possible to reduce the time required to obtain the

specific quality, thereby offering a saving to the sorghum maltster in terms of operation costs and enhancing the total throughput possible from the malting plant.

GLOSSARY OF TERMS

Adjunct	a source of potentially fermentable carbohydrate (often unmalted cereal grain) used in brewing in addition to the malt
Air-resting	a period during steeping where the grain is not immersed in water
Berry	either the unmalted grain or the malt from which the external roots and shoots have been removed
Chit	coleorhiza; root sheath
Chitting	the first sign of germination; the appearance of the root sheath at the basal end of the grain
Diastatic power	joint α- and ß-amylase activity of sorghum malt
Extract	dissolved solids obtained from the malt under specified conditions
Fermentation	alcoholic anaerobic respiration by yeast - the process of converting the sugars in the wort to ethanol and carbon dioxide

Free amino nitrogen	amino acids and small peptides; the products of proteolysis
Green malt	the malt immediately prior to kilning/drying
Germinative energy	the percentage of grains that can be expected to germinate if the grain is malted normally at the time of the test
Grist	the cereal material in the brew
Hopped	where the wort has been boiled with hops
Kilning	drying of the green malt at elevated temperature
Malt	cereal grain which has been germinated for a limited period of time, and then dried
Malting	limited germination of cereal grain in moist air under controlled conditions and drying to produce a shelf-stable product
Malting loss	the decrease in dry matter (%) which occurs during the conversion of grain into malt, as a result primarily of respiration

Mash	a slurry of the brewing grist ingredients
Mashing	the process of converting the malt and cereal adjunct, in the presence of enzymes, into a medium (wort) which can be fermented by yeast into beer
Modification	the enzymatic and physical changes that occur in the grain during malting - renders the malt more easily solubilised during mashing
Pitching	the addition of yeast to wort
Polished malt	malt with the external roots and shoots removed
Steeping	imbibition of water by the grain - the first step in the malting process
Steep-out	at the end of the steeping period
Wort	an aqueous extract of the malt and cereal adjunct (the product of mashing)

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CHAPTER 1: INTRODUCTION

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CHAPTER 1: INTRODUCTION

1 SORGHUM (*SORGHUM BICOLOR* (L.) MOENCH)

Sorghum (*Sorghum bicolor* (L.) Moench) (Fig. 1.1), an indigenous African cereal (DeCandolle, 1886), is a member of the grass family, Poaceae. Cultivated sorghum, first domesticated some 3,000 to 5,000 years ago (De Wet, 1986, reviewed by House, 1995), probably originated in east central Africa, in, or near, Ethiopia or the Sudan (reviewed by Doggett *et al.*, 1970). Irrespective of its exact origin, sorghum is grown today primarily in the semi-arid areas of the world. In addition to being grown over a large part of sub-Saharan Africa, it is also found in India, Pakistan, Thailand, central and northern China, Australia, South America, Mexico, southwestern United States, France and Italy (House, 1995).

Sorghum caryopses, like other cereal grains are composed of three main parts: pericarp, germ (embryo) and endosperm (Fig. 1.2). Although the relative proportions may vary, approximately 6, 10 and 84% of the grain comprise the pericarp, germ and endosperm, respectively (Vietmeyer, 1996). The endosperm can be sub-divided into two component parts; 1) the central soft or floury endosperm which contains mainly starch stored as granules and 2) the outer hard or horny endosperm which is rich in protein, stored in protein bodies (Adams and Novellie, 1975; Aisien and Palmer, 1983).

In South Africa, sorghum cultivars are divided into two groups; 1) those that contain tannins, currently classed by the South Africa Grain Sorghum Board as

Fig. 1.1 Sorghum growing in a field

(Courtesy of the South African Sorghum Board)

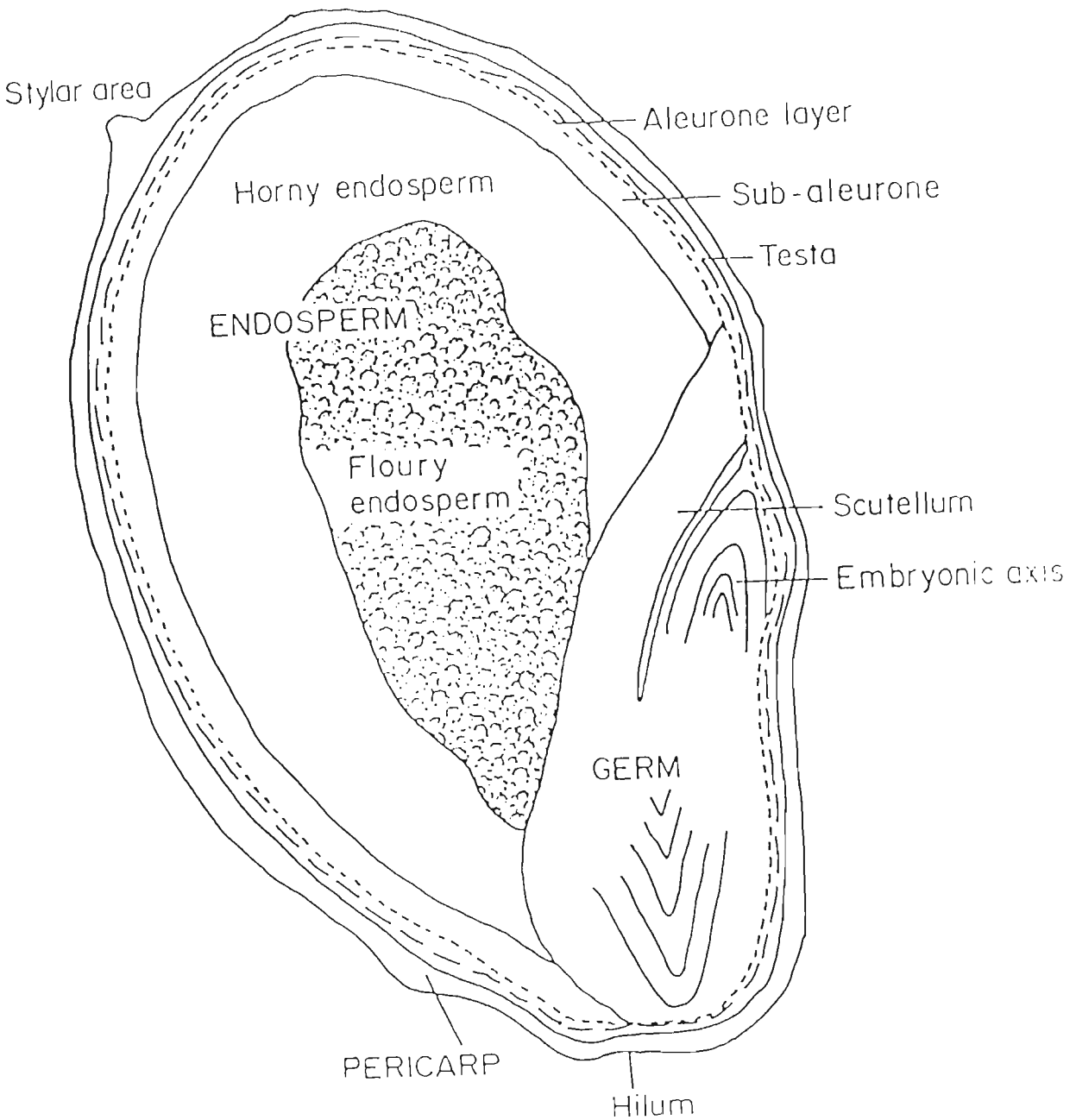

Fig. 1.2 Diagram of a transverse section through a sorghum grain (Courtesy of Professor L.W. Rooney, Texas A&M University, USA)

GH grain, and 2) those that do not contain tannins, classed GM and GL. Classes GH and GM are suitable for malting but GL is a feed-grade sorghum and is not considered suitable for malting purposes. Tannins, located in what is commonly referred to as the testa layer of the pericarp (Fig. 1.2) (Rooney, 1969: Rooney *et al.*, 1980), are condensed polyphenols with a molecular mass of between 500 and 3,000 (reviewed by Gupta and Haslam, 1980). A characteristic of tannins is that they bind with proteins and make them indigestible, thus behaving as nutritional inhibitors (reviewed by Doggett, 1988).

The chemical composition of the sorghum grain (inclusive of pericarp) is similar to that of maize or wheat in that approximately 70% is carbohydrate, 12% protein, 3% lipid, 2% fibre and 1.5% ash (Vietmeyer, 1996).

2 THE IMPORTANCE OF SORGHUM WITH REGARD TO FOOD SECURITY

In terms of production, sorghum is ranked the fifth most important grain, after wheat, rice, maize and barley (reviewed by Doggett, 1988; Dendy, 1995). According to the Food and Agricultural Organisation (FAO, 1962-1993) sorghum and the millets (which are often reported together) account for only 4.7% of the world grain production. Although a minor contribution to the total world cereal production, this figure masks the importance of sorghum and millets as human food. In the semi-arid zones of Africa and Asia these two cereals constitute a major source of energy and protein for millions of people. Indeed, from FAO data (reviewed by Dendy, 1995) it would appear that together they represent around

70% of the total cereals produced in West Africa, 30% in East Africa and 10% in Southern Africa. In fact, sorghum is likely to be of disproportionate importance as a subsistence crop in areas where rainfall is too low for other staples.

As a consequence of the severe droughts of recent years, the world's attention has been attracted to the vital importance of sorghum to the people of the semi-arid tropics (Hulse, 1988). Indeed, in 1983, the Southern Africa Development Community (SADC) set up the (SADC)/ICRISAT (International Crops Research Institute for the Semi-Arid Tropics) Sorghum and Millet Improvement Programme (SMIP) to focus on this very issue.

The world appears to be moving towards a time when its supplies of food will be insufficient for its people (FAO, 1995). The population is projected almost to double in the next generation (FAO, 1995) and it is likely that the challenge of feeding billions of newcomers on reducing choice cropland will probably be an onerous global issue. According to De Wet (1995), it is unlikely that production of the four major cereals can be greatly extended and it is probable that vast amounts of less fertile, marginal lands will have to produce food. In view of the proven versatility of sorghum as a crop in terms of hardiness, dependability, and stability of yield under adverse conditions (House, 1985; Dendy, 1995) it appears to offer great potential for supplementing the world's food resources. Furthermore, with the threat of the global warming, and more recently, widespread manifestation of the phenomena, *el niño*, it is possible that sorghum will be of importance not only in the semi-arid tropics, but could well become the crop of choice over large parts of the world.

3 USAGES

Sorghum has played an important rôle, not only in terms of diet, but also in the development and culture of the peoples of Africa and is often associated with ceremonies such as weddings, funerals and other social gatherings (Novellie and de Schaepdrijver, 1986; Haggblade and Holzapfel, 1989). Sorghum is used to prepare various kinds of porridges, breads and beverages. Rooney and Murty (1982) and Rooney, Kileis and Murty (1986) have described nine major food categories of sorghum: thick porridges, thin porridges, steam-cooked products, fermented breads, unfermented breads, boiled rice-like products, alcoholic beverages, non-alcoholic beverages and snacks (reviewed by Murty and Kumar, 1995).

One of the main uses of the cereal, sorghum, throughout sub-Saharan Africa, apparently since before recorded time, and the aspect with which this thesis is mainly concerned, is that of malting for inclusion in traditional fermented beverages (Schwartz, 1956; Novellie, 1968; Novellie and de Schaepdrijver, 1986; Haggblade and Holzapfel, 1989; Daiber and Taylor, 1995). Today, these beverages remain as popular as ever and during the past fifty years their industrial manufacture has become increasingly common. It is estimated that in Southern Africa, approximately 200,000 tonnes of sorghum are malted annually and some 3,000 million litres of sorghum beer are brewed each year (Taylor, personal communication[1]).

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[1]J.R.N. Taylor, Head of the Department of Food Science, University of Pretoria, South Africa

The alcoholic fermented sorghum beverages go variously under many names, including: sorghum beer (South Africa), opaque beer (Zimbabwe), Chibuku (throughout southern and eastern Africa) and pito (west Africa) (Haggblade and Holzapfel, 1989). Chibuku is a commercial brand name, from the African "mine" language *Fanagalo* and can be translated as "according to the recipe book". In this thesis, the term sorghum beer will be used generically for the southern and eastern African product. Sorghum beer, unlike clear lager-type European beer (Fig. 1.3) is brewed with sorghum malt. It is characterised by being opaque due to the presence of semi-suspended particles of cereal, starch and yeast. The starch gives it a viscous consistency. It is not hopped, but is sour in taste due to the presence of lactic acid, and is consumed in an active state of fermentation (Novellie and de Schaepdrijver, 1986). In contrast, pito is not opaque but clear or only somewhat cloudy and is not substantially sour, but like sorghum beer, is consumed whilst actively fermenting.

Barley is the traditional material of choice for producing clear lager-type beers (Narziss, 1976; Briggs *et al*, 1981), but is, however, suited to temperate conditions and does not grow well in the tropics and sub-tropics. In Africa, the possibility of substituting sorghum malt for barley malt for the brewing of clear lager-type beers has, therefore, received some attention (Skinner, 1976; Okafor and Aniche, 1980; Ajerio, Booer and Proudlove, 1993; Dufour, Mélotte and Srebrnik, 1992) and became particularly significant in Nigeria where the government placed a total ban on the importation of cereals, inclusive of barley malt, in 1988. This forced the clear lager beer brewing industry in Nigeria to utilise locally available sorghum and maize grains and industrial enzymes as a replacement for the previously imported

Fig. 1.3 **Sorghum beer and clear lager-type beer**

barley malt (Koleoso and Olatunji, 1992; Ajerio *et al.*, 1993). It has also focused efforts on the science and technology of brewing with sorghum malt (Palmer, Etokakpan and Igyor, 1989; Ilori, 1991). The events that have occurred in Nigeria since 1988 have created an awareness of the need for systematic research in sorghum production and grain processing to make use of this indigenous cereal effectively.

4 SORGHUM BEER BREWING

Malt (germinated grain) is the most vital ingredient used in brewing. Although there are various different methods of brewing, for both sorghum and clear lager-type beers, generally the aims of the brewing processes are the same; physically and enzymatically to solubilise starch, protein and other constituents of the malt, and often also an unmalted cereal adjunct, and then to ferment this wort using yeast.

The basic sorghum beer process involves the following steps: souring (lactic acid fermentation of sugars by lactobacilli), cooking (starch gelatinisation), mashing (thinning and conversion of gelatinised starches to sugars), straining (grain separation), and alcoholic fermentation (conversion of the sugars to ethanol and carbon dioxide by yeasts). Each of these steps has an effect on the character of the final product. (Novellie and de Schaepdrijver, 1986; Haggblade and Holzapfel, 1989; Daiber and Taylor, 1995).

Traditional home brewing

In the preparation of traditional home-brewed sorghum beer there was very little control over the conditions of malting or brewing. The grain was malted by first soaking it for about one day. This was done either in large, water-filled clay vessels or in woven grass baskets placed in nearby streams. After soaking, the damp grain was allowed to germinate for 2-4 days, depending upon the ambient conditions. Germination was conducted in small baskets or bags which allowed the entry of air. After this, the germinated grain was spread out in a thin layer on the ground and solar dried. Once dry, the malt would be ground, commonly by hand grinding on a stone (reviewed by Novellie and de Schaepdrijver, 1986).

The home-malted sorghum would then be used in the brewing process. A small portion of the ground malt was mixed with hot water, covered and allowed to stand overnight to sour. The soured mash was then diluted with additional water, more starchy material (grain) added and the entire mixture boiled for approximately 2-6 hours, after which it would be allowed to cool, thickening as it did. The following day, a second lot of malt would be added to the now cooled brew. The amylase enzymes in the malt liquefied the brew by converting the starch in the mash to sugars. Depending upon the taste of the brew and the experience of the brewer, the brew might have been recooked, cooled and more malt added. The wild yeasts in the cooled mixture would then, utilising the sugars in the wort, produce ethanol and carbon dioxide, giving a bubbling effervescing liquid. The brew would be allowed to ferment for a few days and when considered to be acceptable, it would be strained by filtering it through braided grass strainers, or by various other

contrived equipment. The strained beer would then be put in clay drinking vessels, and served with great ceremony, to friends and family members (reviewed by Novellie and de Schaepdrijver, 1986). Fig. 1.4 shows a woman serving out beer with a ladle made from a gourd, according to traditional practice in South Africa..

Home brewing of today is done with recipes and procedures passed on from generation to generation. Over the years these have been improved based on the knowledge gained through experience and experimentation. However, the procedures followed are essentially the same as those of ancient times.

Industrialisation of the process of sorghum beer brewing

Industrial sorghum beer brewing first began in the early 1900s at the requirement of municipal governments in South Africa and Zimbabwe (reviewed by Haggblade and Holzapfel, 1989). However, from a very early stage technical difficulties hampered the scaling-up of the traditional craft to factory scale. In 1954, the CSIR (formerly the Council for Scientific and Industrial Research) in South Africa was commissioned to undertake applied research on behalf of the municipal factory sorghum beer brewers.

With scaling-up of sorghum beer production, the first major problem encountered was related to the quality of the malt. Sorghum malt of a high and consistent quality is required when it is used as an ingredient in industrial brewing. At that time very little was know about the physiology of sorghum, let alone of the science

and technology of sorghum malting. Largely through the research efforts of CSIR scientists, by developing a sensitive method to determine the diastatic power (amylase activity) of sorghum malt (Novellie, 1959) and by identifying suitable malting conditions for this cereal (Novellie, 1962), sorghum maltsters of the day were able to produce satisfactory malts.

The spectacular increase in scale from traditional home brewing (approximately 50-200 litre brews) to factory scale brewing (approximately 15,000-27,000 litre brews) has been made possible by the development and institution of carefully specified raw material standards and the use of specialised equipment. The specialised brewing equipment allows both rapid heating and cooling which has enabled the factory brewers to split the key brewing steps into separate unit operations and to conduct them under optimal temperature conditions (reviewed by Novellie, 1983; Novellie and de Schaepdrijver, 1986; Haggblade and Holzapfel, 1989).

Today, a number of different industrial brewing processes are used in southern Africa, which have stemmed mainly from differences in the taste preferences of the consumers. However, the most common industrial sorghum beer brewing process conducted, and the one that will be reported on in detail, is the Modified Reef-Type (double cook) Process, illustrated by a flow diagram (Fig. 1.5).

Souring

In South Africa, the lactic acid fermentation referred to as souring, is an essential part of the brewing process. The lactic acid sour lowers the pH of the beer to

Fig. 1.4 **Traditional manufacture of sorghum beer - woman in Northern Province of South Africa serving the beer from clay pots using a gourd ladle**

(Courtesy of Beryl Fabian, CSIR, South Africa)

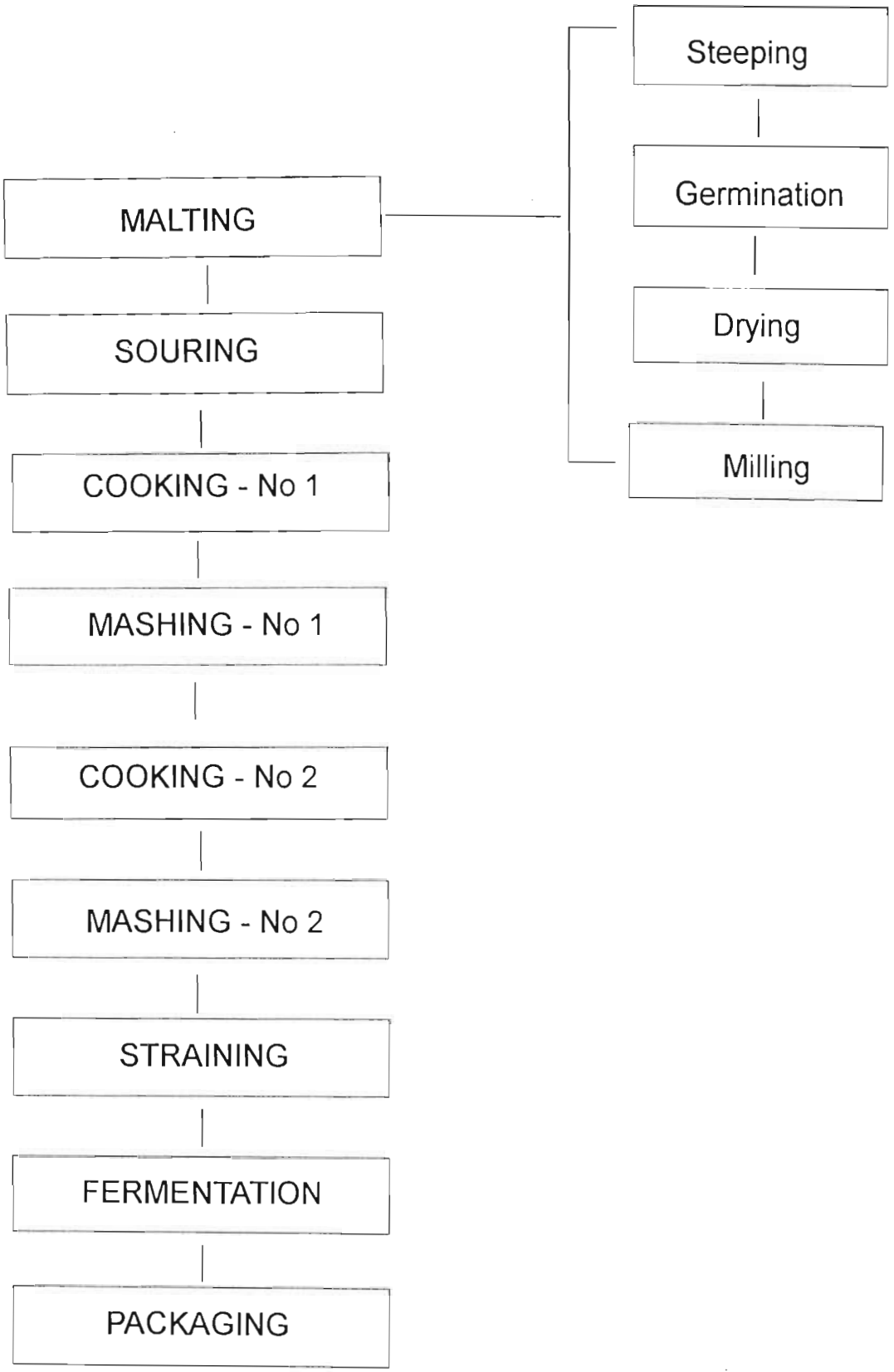

Fig 1.5 A flow diagram of the principal stage of the modified Reef-type sorghum beer brewing process

below 4.0, which helps to prevent the complete hydrolysis of starch into sugars (see below, under mashing), slows down the rate of microbial spoilage and inhibits the growth of pathogenic organisms. The sour also contributes to the characteristic sour flavour of the indigenous beer.

Unlike the home brewing situation, the temperature of souring (in the range of 48-50°C) is strictly controlled under industrial brewing conditions. These conditions favour the growth of thermophilic, homofermentative *Lactobacillus* spp., notably *L. delbruekii*, otherwise known as *L. leichmannii*. Not only does this species produce solely lactic acid, which gives the beer a characteristic clean, sharp taste, unlike the mesophilic lactic bacteria, it produces only the laevorotatory isomer which may be nutritionally preferable (reviewed by Novellie and de Schaepdrijver, 1986; Haggblade and Holzapfel, 1989; Daiber and Taylor, 1995).

Cooking

After fermenting for lactic acid, the sour is diluted with water, after which brewers' grits (refined maize or sorghum meal), referred to as starchy adjunct, is added and the mixture is boiled for approximately 1-2 hours under atmospheric conditions, or for rather less time under pressurised conditions. The purpose of cooking is to gelatinise the grain starch and render it more easily hydrolysed by the malt amylase enzymes later during mashing. After cooking, the boiled wort is cooled rapidly to approximately 60°C. As a consequence of starch gelatinisation, the mash is relatively viscous at the end of this step (reviewed by Novellie and de Schaepdrijver, 1986; Haggblade and Holzapfel, 1989; Daiber and Taylor, 1995; Harris, 1996).

Mashing

Once the mash has been cooled to approximately 60°C, additional malt (i.e. "conversion" malt) is added. As a consequence of the malt α-amylases converting the gelatinised starch to dextrins, the mash is rapidly liquefied. The acidity of the mash (about pH 4.0), because it is below the optimum for sorghum α- and ß-amylase activity (Botes, Joubert and Novellie, 1967a,b), limits the starch hydrolysis, preventing the production of too thin a beer (Novellie, 1966). During mashing, proteolytic enzymes from the malt hydrolyse some of the proteins, producing additional free α-amino nitrogen (FAN) which is a valuable source of yeast nutrients and, therefore, necessary for alcoholic fermentation. Under industrial conditions, mashing is carried out for about two hours at constant temperature, between 50 and 65°C (Novellie, 1968; reviewed by Novellie and de Schaepdrijver, 1986; Haggblade and Holzapfel, 1989; Daiber and Taylor, 1995; Harris, 1996).

Second cooking and mashing

The gelatinisation temperature of sorghum starch is between 68 and 72°C (Taylor, 1989; 1992). Therefore, under the conditions of conversion at approximately 60°C (the previous step), the sorghum malt starch is not gelatinised. In the modified Reef-Type brewing process the mash is re-cooked (80-100°C for approximately 20 minutes) at the end of mashing. This second cooking stage pasteurises the mash and also gelatinises the sorghum malt starch, leaving virtually no ungelatinised starch. Thereafter, the mash is cooled to 60°C and a second short mashing period (approximately 20 minutes) allowed, either with a small amount of conversion malt or commercially-produced amylase. The objective of this

second conversion (mashing) stage is to reduce the viscosity in the mash to the desired level by the thinning action of the amylase enzymes. The second cooking and mashing steps effectively increase the amount of soluble solids in the mash and improve the efficiency of the process (reviewed by Novellie and de Schaepdrijver, 1986; Daiber and Taylor, 1995; Harris, 1996).

Straining

The objective of straining is to remove the coarse cereal particles from the wort. In industrial brewing in South Africa, straining is carried out directly after mashing and prior to fermentation. Before straining there are approximately 14% solids in the wort and after staining approximately 11-12% solids remain (Joustra, personal communication[2]).

Fermentation

Under industrial brewing conditions the strained wort is cooled rapidly. The wort is transferred to stainless steel vessels and alcoholic fermentation is brought about by pitching with commercially produced, active dried yeast (0.3 g/litre), rather than relying on the growth of wild yeasts in the wort as is the case in traditional home brewing. The mixture is then allowed to ferment for 8-24 hours (reviewed by Novellie and de Schaepdrijver, 1986; Daiber and Taylor, 1995; Harris, 1996).

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[2] S.M. Joustra, Brewing Engineer, CSIR, Pretoria, South Africa

Packaging

The beer is then packaged in large bulk containers (100-500 litre) for sale in beer halls. Alternatively, the beer is packaged in vented milk-type cartons or plastic containers (500 ml-25 litre) for sale in the retail market (reviewed by Daiber and Taylor, 1995).

Unlike clear lager-type beer, sorghum beer is not filtered. Indeed, not only is the yeast not removed, the beer is actually sold and consumed in a state of active fermentation. The beer, when ready for consumption, has an alcohol content of approximately 3% by weight and the shelf-life of the product is approximately 5-10 days after straining (Haggblade and Holzapfel, 1989).

5 MALTING

Malting involves the germination of the cereal grain in moist air under controlled conditions. The objectives of malting are to mobilise the endogenous hydrolytic enzymes of the grain and by means of these enzymes to modify the structure of the grain so that is readily solubilised during the brewing process to produce a fermentable wort. In sorghum beer brewing, the malt enzymes are also required to solubilise and hydrolyse a cooked starchy adjunct during the mashing stage of brewing (see above). When the degradation of the endosperm, which naturally sustains the development of the growing embryo or germ during germination, has progressed to only a limited extent, the maltster terminates both its degradation and the growth of the germ to produce a shelf-stable product, by drying the grain (Briggs *et al.*, 1981; Taylor and Dewar, 1992; Dewar, Taylor and Joustra, 1994).

Most of what is known about malting for brewing is based on research conducted on barley for conventional beer brewing. As highlighted in the review by Briggs *et al.* (1981), not all grains are suitable for malting. The selection of suitable grain is based on, amongst other things, 1) rapid and even germination of the grains, 2) even hydrolysis of the endosperm, and 3) an adequate complement of enzymes after drying/kilning.

It is recommended that barley for malting should be grown from pure seed of a variety that maltsters have found to be suitable, and which meets the farmer's needs with respects to yields and disease-resistance (Briggs *et al.*, 1981). In South Africa, malting-class sorghums are identified by the Agricultural Research Council (ARC) which conducts annual cultivar evaluation trials. A sorghum is listed as suitable for malting purposes, when it has been grown under various climatic conditions for three consecutive years, produced acceptable yields and, under standard laboratory malting conditions, has produced malt of acceptable quality (Pretorius, personal communication[3]). (The breeding, production and identification of suitable malting class sorghums does not fall within the ambit of this thesis, which is specifically concerned with optimising the process of sorghum malting).

The malting process can be physically split into three unit operations; steeping, germination and drying. Steeping involves immersing the selected cereal grain in water until it has imbibed a suitable amount to initiate the metabolic processes

[3] A. Pretorius, Manager of Breeding/Seed Quality, ARC-Grains Crop Institute, Potchefstroom, South Africa

of germination. During the germination phase, the moist grain is allowed to grow in a humid atmosphere under controlled conditions. When the biochemical and physical changes (modification) in the grain have proceeded far enough the "green malt" is kilned/dried in a flow of hot dry air (Briggs *et al.*, 1981).

In South Africa, sorghum is malted in one of two different processes (*viz.* Floor or Pneumatic malting). Under floor malting conditions, the grain is malted outdoors in the traditional way in relatively thin layers. In this process the conditions of germination are not easily controlled, and indeed, are largely dependent upon the prevailing weather conditions. Consequently the quality of the malt produced tends to be low and inconsistent (Taylor and Dewar, 1992). Sorghum malt of high and consistent quality is required when it is used as an ingredient in industrial brewing. Today, most of the sorghum malt used in factory brewing is malted indoors in modern pneumatic industrial installations (Fig. 1.6), which offer more control over the conditions of temperature, moisture and aeration. The resultant malt is referred to as "conversion" or "industrial" malt (Taylor and Dewar, 1992).

To date, several noticeable differences have emerged between barley and sorghum malting. At the end of steeping, the chosen moisture content for barley is within the range 42-48%, on a fresh mass basis (reviewed by Briggs *et al.*, 1981; Bamforth and Barclay, 1993). Sorghum, however, has been found to take up water slowly during steeping (Hofmeyer, 1970) and indeed, although steeping is widely acknowledged as the most critical stage of the malting process (Briggs *et al.*, 1981; French and McRuer, 1990), it has been considered to be relatively unimportant for sorghum (Novellie, 1962). This assumption is reflected in

Fig. 1.6 Industrial pneumatic malting of sorghum

(Courtesy of the South African Sorghum Board)

commercial sorghum malting practice in South Africa, where there is no standard prescribed steeping procedure. Sorghum is steeped commercially from anything between approximately 4-6 hours to a maximum of 24 hours under non-controlled temperature conditions and only in a very few cases does the moisture content at the end of steeping reach 33% on a fresh mass basis (personal observation). Commercial barley steeping procedures have changed somewhat over the years. Traditionally, barley was not aerated during steeping. Subsequently, in view of the requirement for oxygen by the respiring, metabolically active grain, and the need to remove the by-products of respiration, heat and carbon dioxide, many tanks are now equipped with aeration devices (Briggs *et al.*, 1981; Cantrell, Anderson and Martin, 1981; French and McRuer, 1990). Probably related to the assumption that steeping is relatively unimportant for sorghum, relatively few commercial steeping tanks in South Africa are fitted with aeration facilities. Even in those that are, steeping is very often conducted without aeration (personal observation).

During the germination phase, no further water is supplied to barley, which takes up all it requires during steeping (Briggs *et al.*, 1981). It is necessary, however, to supply further water to sorghum during the germination period (Novellie, 1962). Another difference is that barley is germinated under cool conditions. Traditionally, this was 12-15°C, but with the pressure for malt production as rapidly as possible, and since the rate at which a grain germinates, within limits, is raised with increasing temperatures, barley is now often malted at 18-20°C (reviewed by Briggs *et al.*, 1981). Morrall *et al.* (1986) investigated the effect of a range of different germination temperatures (i.e. 24, 28, 32 and 35-38°C) on sorghum malt

quality and discovered that sorghum malts best at 24 to 28°C. That is, in a significantly higher temperature range than has been used for barley.

The main purposes of kilning/drying are to arrest the growth and enzymic activities of the green malt at the end of the germination process and to reduce the moisture content so that a shelf-stable product can be produced. In barley, the malt is kilned (rather than only dried), in that it is not only dehydrated, but partly cooked. This procedure partially or wholly destroys some of the hydrolytic enzymes developed during malting. It also develops colour and flavour in the final product (Briggs *et al.*, 1981). In sorghum, however, because the hydrolytic activity of the malt (particularly the ß-amylase activity) is inherently lower than that of barley (Novellie, 1960; Jayatissa, Pathirana and Sivayogasunderam, 1980; Aniche and Palmer, 1990), the malt is dried at a relatively low temperature (50°C) as opposed to being kilned, as higher drying temperature significantly reduces the already-low amylase activity (Novellie, 1962; Okon and Uwaifo, 1985). The main aim of sorghum malt drying is to conserve as much of the enzyme activity of the malt as possible whilst producing a shelf-stable product.

In barley malting practice, certain special techniques have been used to achieve various ends. For example, hypochlorites, alkalis and formaldehyde have been used to reduce microbial proliferation during malting; soluble polyphenol content of malt has been reduced by formaldehyde [also used in sorghum malting to prevent the tannins in the GH type grains from combining with the essential malt enzymes; Daiber (1975)]; malting losses have been reduced by many different chemical and physical treatments; and gibberellic acid (GA_3) has been used to

accelerate grain modification (Palmer, 1974; review by Briggs *et al.*, 1981). Application of GA_3, cultured on an industrial scale from the fungus *Gibberella fujikuroi* (syn. *Fusarium moniliforme*), is the most widely used additive in the barley malting industry.

The knowledge that GA_3 can be used to accelerate barley malting came about by a systematic study of the biochemistry of the germinating grain (see below). The barley malting industry, economically the most important malting industry, has taken advantage of this knowledge. In practice, the maltster applies relatively low levels of GA_3 (0.05 to 0.25 mg/kg barley) to malting barley usually during the first day of malting (Briggs *et al.*, 1981; Palmer, 1990). This supplements the endogenous gibberellin (GA) content of the grain, releases it from dormancy, enhances metabolism and growth of the embryo, but more importantly for malting, it induces a relatively greater increase in the hydrolytic enzymes (notably α-amylase, but also other hydrolytic enzymes; endo-ß-glucanase, pentosanases and proteases) which bring about modification during this process (reviewed by Briggs, 1963; Briggs *et al.*, 1981; Palmer, 1974; 1989). For GA-supplemented barley, maximal extract has been found to be obtainable 1-3 days sooner than would otherwise be the case (reviewed by Briggs *et al.*, 1981), thereby reducing the operational costs and increasing the malt production capacity.

In addition to GA_3, other hormones are thought to play a rôle in barley germination (see below). With regard to sorghum germination however, the underlying mechanisms involved in controlling germination are not understood and the rôle

played by plant hormone/ plant growth regulators (PGRs) is almost completely unknown.

Sorghum malt quality aspects

Germinability

For the production of malt of a good and consistent quality, it is a pre-requisite that a high proportion of the grain must germinate (Merdith, Anderson and Hudson, 1962; Moll, 1979; Briggs *et al.,* 1981, Palmer, 1989; 1990; Bamforth and Barclay, 1993). For sorghum, the Germinative Energy (GE), "a measure of the percentage of grains which can be expected to germinate if the grain is malted normally at the time of the test" (European Brewery Convention, 1987), is recommended to be >90% after 72 hours of germination (Dewar, Taylor and Joustra, 1995). This germination test is an accepted method of sorghum analysis and is generally used by the sorghum malting industry to ascertain the suitability of the grain for malting.

Amylase activity

Diastatic power (DP) is a measure of the joint α- and ß-amylase enzymatic activity of sorghum malt and is measured in Sorghum Diastatic Units (SDU) per gram of malt. This is probably the single most important indicator of malt quality for sorghum beer brewing (Novellie, 1962), where the malt generally has to act on at least twice its own weight of starchy adjunct during brewing (Novellie, 1966; 1968). At the biochemical level, the combined action of the α- and ß-amylases, which develop during malting (Daiber and Novellie, 1968; reviewed by Palmer, 1989; Dufour *et al.,* 1992), is responsible for the breakdown of starch to fermentable

sugars during the malting process and also later for hydrolysis of the starchy adjunct, during the sorghum beer brewing process. Alpha-amylase attacks α-(1→4) glucosidic bonds within starch molecules to produce dextrins (short chains of glucose molecules) and a variety of sugars including maltotriose and maltose, and glucose. Alpha-amylase is an endoenzyme, and as such, rapidly solubilises starch to yield the smaller fragments. This is extremely useful during the mashing stage of brewing, as it reduces the viscosity of the starch solution (reviewed by Palmer, 1989; Bamforth and Barclay, 1993; Daiber and Taylor, 1995; Lewis and Young, 1995). In contrast, ß-amylase is an exoenzyme. This enzyme releases maltose by hydrolysing the penultimate α-(1→4) glucosidic bond from the non-reducing ends of the dextrins produced by the action of α-amylase (reviewed by Palmer, 1989; Bamforth and Barclay, 1993; Daiber and Taylor, 1995; Lewis and Young, 1995).

Free amino nitrogen

The other major sorghum malt quality criterion is free amino nitrogen (FAN), which consists of free amino acids and small peptides, produced by proteinase and peptidase activity in the malt; FAN is a major source of nitrogen for yeast during brewing (Daiber and Novellie, 1968; Baxter, 1981; Pickerell, 1986). Adequate FAN levels in wort ensure the rapid utilisation of sugars and maximal ethanol production during fermentation (Pickerell, 1986). As sorghum malt comprises only a relatively small proportion of the cereal grist (Novellie, 1966; Taylor, 1989), production of malt FAN is of considerably importance. Pre-formed FAN in the sorghum malt (developed during malting; Morrall, *et al.*, 1986; Taylor, 1991) is a major source of FAN, comprising up to 70% in the wort (Taylor and Boyd, 1986).

Extract

Hot water extract (HWE) is a measure of the soluble solids in solution and gives an estimate of how much of the malt will solubilise during the brewing process (Briggs *et al.*, 1981; Bamforth and Barclay, 1993). Also, HWE should give an indication of the modification of the malt during malting (i.e. the breakdown of the endosperm reserves by amylase and protease activity). Extract is particularly important in lager beer brewing where, in some cases, an all-malt grist is used (Narziss, 1984; Palmer, 1989) rather than an approximately 30% malt grist which is generally the situation in opaque sorghum beer brewing.

6 FACTORS THAT AFFECT GERMINATION

Moisture, time, temperature and aeration

Since malting relies on initiating germination suitably to mobilise the endogenous enzymes and thus modify the endosperm, certain factors that have been found to be important for seed germination need to be considered, noting that presently this does not represent a comprehensive account of all the intricacies of the germination process. When a viable, quiescent, seed is imbibed, water is taken up and, a sequence of metabolic activities begins, resulting in mobilisation of food reserves to the embryo (the living part of the seed), elongation of the embryonic axis and then cell division. After a certain time, part of the embryo (generally the distal hypocotyl/radicle tip) emerges from the seed. This is what is referred to as germination (Bewley and Black, 1978; 1994).

For a seed to germinate (provided it is not dormant or the dormancy has been broken) certain requirements must be met; an adequate supply of water to initiate and sustain germination, sufficient oxygen to allow aerobic respiration, a suitable temperature to allow the processes to advance at an adequate rate and in some cases, the light intensity, are all important (the effect of light intensity on germination will not be discussed further). Although the specific conditions may vary among seeds, dependent largely upon the species and variety (reviewed by Bewley and Black, 1978; 1994; Mayer and Poljakoff-Mayber, 1982), a critical minimal level of hydration must occur before the seed is responsive to changes in external conditions (Vertucci and Leopold, 1986; Vertucci, Vertucci and Leopold, 1987).

Imbibition of water is the first process which occurs during germination (and indeed, during the steeping period of malting). Generally, the seed water content increases during imbibition until the level of hydration is adequate to support the processes of germination (Bewley and Black, 1994; Bradford, 1995). The amount of imbibition that will occur is related to (*i.a.*) the composition of the seed, the permeability of the seed coat and the availability of water (reviewed by Mayer and Poljakoff-Mayber, 1982; 1989; Bradford, 1995). Initially, water enters the seed rapidly, largely as a consequence of the large water potential gradient between the dry seed (generally in the range -350 to -50 MPa; Roberts and Ellis, 1989) and the environment (0 to approximately -2 MPa; Bradford, 1995). This initial uptake is purely physical in character and occurs equally well in dead and living tissues and is independent of the metabolic activity of the seed (reviewed by Bewley and Black, 1978; Mayer and Poljakoff-Mayber, 1982; 1989). Water generally enters

the seed through the micropylar end where the seed coverings are invariably thinnest. The water taken up is not necessarily evenly distributed between tissues within the seed (Reynolds and MacWilliam, 1966; McDonald, Vertucci and Ross, 1988; McDonald, Sullivan and Laver, 1994; Bradford, 1995) or even between different organelles (Connelly, *et al.*, 1987). This, in turn, may lead to differences in rate of hydration of the various proteins in the different parts of the seed and may affect the sequence of the processes triggered by the various levels of hydration (Vertucci and Leopold, 1986).

After this initial rapid uptake, and once the gradient for water uptake decreases, the water content approaches a plateau (reviewed by Bradford, 1995). The length of this plateau phase is determined by factors that influence the timing of germination (reviewed by Hofmeyer, 1970; Bradford, 1995). During this phase, should water uptake occur, it does so, slowly, being controlled by the metabolic activity, particularly the rate of respiration of the seed (Dewez, 1964; Hofmeyer, 1970). Thereafter, the water uptake increases sharply in association with growth of the emerging embryo (reviewed by Hofmeyer, 1970; Bradford, 1995).

An increase in the rate of respiration is one of the first noticeable metabolic changes that occurs in the seed; well before root protrusion (reviewed by Mayer and Poljakoff, 1982). Indeed, integrated metabolism (respiration) can occur at water potentials (as low as -15 MPa; Vertucci and Roos, 1990; Vertucci and Farrant, 1995), that would preclude radicle growth (reviewed by Bradford, 1995). The rate of respiration has been found to be dependent on time, temperature, grain moisture and concentration of oxygen and carbon dioxide in the steeping

water (Enari, Linnahalme and Linko, 1961; Reynolds and MacWilliam, 1966; Abdul-Baki, 1969; Bewley and Black, 1978; Mayer and Poljakoff-Mayber, 1982; 1989). The germinating seed is greatly affected by the composition of the ambient atmosphere because, as an active process, germination requires an expenditure of energy. Although a small number of species can germinated under anoxic conditions (Corbineau and Côme, 1995), most seeds require oxygen to germinate (Al-Ani *et al.*, 1985). The energy required for germination is usually met by the utilisation of adenosine triphosphate (ATP), formed via aerobic oxidation processes (or occasionally, as a result of glycolysis only) (Hourmant and Pradet, 1981; review by Mayer and Poljakoff-Mayber, 1989). The temperature is also important. At extremes of temperatures, low and high, germination is prevented. There is usually a range of temperatures within which seeds of particular species will germinate, and within this range, there will usually be an optimal temperature for germination. The optimal temperature may, in fact, be related to the length of the germination period (review by Mayer and Poljakoff-Mayber, 1982;1989) or the previous pre-hydrated history of the seed (Bradford and Haigh, 1994; Dahal and Bradford, 1994; Bradford, 1995). Further, as highlighted by Reynolds and Thompson (1971) (cited in Mayer and Poljakoff-Mayber, 1989), the range of temperatures at which germination can occur can be altered by exogenous compounds such as plant growth regulators. Thus it would appear that the optimal temperature for germination may not be absolute but rather, a function of the interaction of various factors.

Seeds generally contain relatively large amounts of food reserves which support growth and development of the seedling until it can produce its own food via

photosynthesis, and consequently establish itself as an autotrophic plant. The second metabolic change that occurs during germination, initiated by the hydration of enzymes proteins, is the breakdown of the reserve materials in the seed. In general, as germination proceeds various enzymes, which are either present in the dry, quiescent, seed, become active as the seed hydrates, or are synthesised *de novo* at this stage, break down starch, proteins, hemicellulose, polyphosphates, lipids and other storage materials (reviewed by Mayer and Poljakoff-Mayber, 1989). In terms of sorghum malting, we are specifically interested in the hydrolytic enzymes which break down starch and proteins (Novellie, 1959; Evans and Taylor, 1990).

In addition to the hydrolysis of the storage materials, breakdown products are transported, from the storage areas to the growing embryo or axis (in cereals, mainly from the endosperm to the embryo) and new materials are synthesised from the breakdown products. For example, in maize, dry weight and total nitrogen was recorded as decreasing during the initial five days of germination (Ingle, Beevers and Hagemann, 1964; Ingle and Hageman, 1965). The drop, however, was primarily in the endosperm and the embryo showed an increase in dry weight and total nitrogen. Similar results have, for example, been obtained for rice (Palminano and Juliano, 1972) and Douglas fir (Ching, 1966). During the initial phase of germination, prior to the new plantlet being capable of photosynthesising, only water and oxygen are taken into the seed. As a consequence of aerobic respiration, carbon dioxide and water are lost. Thus during germination there is an overall loss of dry matter.

Plant growth hormones/regulators and germination

There is a great deal of evidence to suggest that plant hormones, or plant growth regulators (PGRs; gibberellins, cytokinins, ethylene, auxins and abscisic acid), are important in controlling germination (reviewed by Bewley and Black, 1978; Mayer and Poljakoff-Mayber, 1989; Fincher and Stone, 1993). Most of the substances included in the group of PGRs either stimulate or inhibit germination.

Gibberellins (GAs), one of the groups of plant hormones involved in plant growth and development, either endogenous or exogenously applied are considered to be essential factors in inducing germination (Groot and Karssens, 1987). Thomas (1992a), in a review of endogenous hormones and light-mediated seed dormancy, concluded that gibberellins are involved in post-dormancy metabolic processes leading to embryo growth and radicle emergence, such as food reserve mobilisation and endosperm softening.

In cereal grains, GAs induce the synthesis of α-amylase, a critically important enzyme with regards to malting, which leads to the breakdown of the storage reserves of the endosperm and the transport of the substrate(s) for liberation of energy to the metabolising embryo (Bewley and Black, 1978; Briggs *et al.*, 1981; Palmer, 1989; Mayer and Poljakoff-Mayber, 1989; Fincher and Stone, 1993).

In barley, the rise in the level of α-amylase in the endosperm during germination was found to be dependent on the presence of the embryo, removal of which resulted in a decrease in amylase activity (Kirsop and Pollock, 1957; cited in

Mayer and Poljakoff-Mayber, 1989). The barley embryo was shown to be capable of producing gibberellins (MacLeod, Duffus and Johnston, 1964; MacLeod and Palmer, 1966; 1967), which appear to migrate via vascular tissue preferentially to the aleurone cells on the dorsal side of the kernel (Palmer, 1972). Gibberellic acid (GA_3), one of the gibberellins released from the barley embryo, is thought to be primarily responsible for the production of several hydrolytic enzymes in the aleurone layers. The level of GA_3 was found to increase in barley during the early stages of germination (Brookes and Martin, 1975) and its production to be at its peak in the germinating embryo after the first two days (Cohen and Paleg, 1967), indicating that this is a critical period for barley malting practice. The aleurone of cereal grain has been used as a model system for studying GA-action (Fincher, 1989). Detached barley aleurone cells greatly increase their secretion of α-amylase [apparently largely through a stimulation of α-amylase gene transcription (Jacobsen and Beach, 1985; Zwar and Hooley, 1986)] in response to exogenously applied GA_3 (Paleg, 1960; Yomo, 1960; Chrispeels and Varner, 1967; Stuart, Loi and Fincher, 1986). Synthesis of the proteinase enzymes in barley is also thought to be controlled by GA_3 (Jacobsen and Varner, 1967).

Unlike the situation with barley, it is not clear whether application of GA_3 to germinating sorghum grain increases amylase activity. In fact, it has been generally believed for many years that GA_3 does not stimulate amylase activity in sorghum (Daiber and Novellie, 1968; Aisien and Palmer, 1983; Aisien, Palmer and Stark, 1983). However, recently Agu *et al.* (1993) and Nzelibe and Nwashike (1995) have indicated that application of GA_3 can improve the amylase activity of sorghum malt. The stimulatory effect, however, appears to be somewhat

inconsistent and may be variety-dependent. Indeed, it has been reported that in some sorghum varieties, GA_3 actually reduced the amylase activity and inhibited the proteolytic activity of sorghum (Nzelibe and Nwashike, 1995). Irrespective of the recent findings, sorghum malting practice does not utilise exogenous GA_3.

The mode of endosperm modification in the cereals, barley and sorghum, also appears to differ. 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 and Morris, 1890; Gibbons, 1981; Briggs and MacDonald, 1983; Ranki, 1990; reviewed by Fincher and Stone, 1993). It is believed that enzymes secreted from the scutellum initially degrade the endosperm adjacent to the scutellum and as germination proceeds, the aleurone tissue becomes the major source of hydrolytic enzymes (Gibbons, 1981; McFadden *et al.*, 1988). With respect to α-amylase, there has been a great deal of debate in the literature as to the relative importance of the scutellar epithelial and the aleurone cells in synthesising and secreting the endosperm-degrading enzyme into the endosperm (see Palmer, 1989). However, it is now generally accepted that the aleurone layer is the tissue that is principally responsible for its synthesis (Ranki and Sopanen, 1984; Ranki, 1990). Indeed, biochemical studies indicate that even with some aleurone contamination, the scutellum can account for less than 10% of the α-amylase found in the endosperm of barley malt (reviewed by Palmer, 1989). The scutellum does, however, appear to synthesise, particularly during the early stages of endosperm mobilisation (McFadden *et al.*, 1988), relatively high levels of another hydrolytic enzyme (*viz.* ß-(1→3), (1→4)-glucanase) (Stuart *et al.*, 1986). The relative

contribution of the scutellum and the aleurone to the total hydrolytic activity of enzymes secreted into the starchy endosperm, thus varies according to the particular enzyme and to the time after the initiation of germination.

In sorghum, however, α-amylase does not appear to be synthesised in the aleurone layer. In this grain, the evidence suggests that amylases are synthesised in the scutellum and then diffuse directly to the endosperm (Daiber and Novellie, 1968; Daiber, Malherbe and Novellie, 1973, Aisien and Palmer, 1983; Aisien *et al.*, 1983). Thus, even in the details of their mode of endosperm modification, sorghum and barley seem somewhat different.

Cytokinins are generally considered to be involved in cell division and cell enlargement (Letham and Bollard, 1961; Fosket, Volk and Goldsmith, 1977) and to be primary factors in the initiation of radicle growth (Haber and Luippold, 1960; Pinfield and Stobart, 1972) and seedling development (Smith and van Staden, 1978; van Staden, 1981a,b). They have also been found to be involved in breaking dormancy (van Staden, Webb and Wareing, 1972; Brown and van Staden, 1973; van Staden, Davey and Brown, 1982), countering the effects of germination inhibitors (Khan, 1971; 1975) and enhancing the activities of GAs (Thomas, 1992b). There is also evidence that implicates cytokinins in nutrient mobilisation during germination (Tavener and Laidman, 1972; Hocart, Letham and Parker, 1990) and that embryos of germinating seeds of e.g. lupin (Nandi *et al.*, 1988) and maize (Hocart and Letham, 1990), are capable of cytokinin biosynthesis.

MacLeod and Palmer (1966) suggested that during germination, auxin (indole-3-acetic acid; IAA) may be involved in extension growth of the coleoptile and, possibly in deposition of lignin in the vascular system of the scutellum. Verbeek-Wyndale (1973; cited in MacLeod, 1979) has provided evidence that coleoptile extension is related to α-amylase formation in the intact grain and indicated that IAA may act synergistically with GA in α-amylase formation. Since these early studies there seems to have been very little evidence of a direct rôle for IAA during germination of cereals. Tillberg (1977) observed a sharp increase in the level of IAA during imbibition, particularly during the early hours, in seeds of French bean, maize and Scots pine. The three different types of seed, however, showed wide divergence in the levels of IAA. According to Mayer and Poljakoff-Mayber (1989), such wide difference would seem to suggest a liberation of IAA from a bound form. There is evidence that IAA occurs as esters in seeds of at least two species; maize and rice (Epstein, Cohen and Bandurski, 1980; Nowacki and Bandurski, 1980) and perhaps the mode of action in the plant is actually via the esterified IAA and not its free form.

Abscisic acid (ABA) is implicated in seed dormancy (Bewley and Black, 1982; Tillberg, 1983; Barthe and Buland, 1985; Hole, Smith and Cobb, 1989; Singh and Browning, 1991; Hilhorst and Karssen, 1992; Karssen, 1995). Even in seeds that show little or no dormancy at maturity, e.g. soybean (Eisenberg and Mascarenhas, 1985), rape (Finkelstein *et al.*, 1985), maize (Neill, Horgan and Rees, 1987), wheat (Raikhel and Quatrano, 1986) and rice (Stinissen, Peumans and DeLanghe, 1984), exogenously applied ABA has been shown to suppress the natural germinability of the developing embryo. It should be noted, however, that

sensitivity to ABA, rather than ABA levels *per se*, may be the main determinant in seeds expressing prolonged expression of ABA responsive genes (Morris *et al.*, 1991; Steinbach *et al.*, 1995; review by Kermode, 1995), thereby offering an explanation as to why the ABA levels of some mature dormant and non-dormant seeds are similar (Black, 1983; Walker-Simmons, 1987; Thomas, 1992a).

Abscisic acid can also prevent precocious germination (Ackerson, 1984; Bewley and Black, 1985; Quatrano, 1986; Kermode, 1990; Oishi and Bewley, 1990; Xu, Coulter and Bewley, 1990; Black, 1991; Meurs *et al.*, 1992; Steinbach, Benech-Arnold and Sánchez, 1997). For example, soybean embryos are not capable of germination until the midstage of their development when their ABA content has decreased markedly. However, when the ABA content is reduced experimentally, by washing, the embryos are able to germinate, the percentage germination being correlated with the length of the washing period, which in turn appears to be related to the ABA content (Ackerson, 1984). Similarly, ABA has been shown to inhibit germination of developing caryopses of sorghum, (Steinbach *et al.*, 1995) and when ABA synthesis is inhibited by fluridone, precocious germination occurs (Steinbach *et al.*, 1997).

Evidence exists that ABA is involved in the suppression of α-amylase activity in developing seeds of some species, e.g. triticale (King, *et al.*, 1979) and wheat (King, 1976; 1982; Napier, Chapman and Black, 1989), presumably ultimately by arresting enzyme synthesis. In addition, the enhancement of α-amylase synthesis in barley aleurone layers by GA may be inhibited to varying degrees by the presence of specific concentrations of ABA (Chrispeels and Varner, 1967; Barton

et al., 1973; Jacobsen and Chandler, 1987) or by analogues (Todoroki, Hirai and Koshimizu, 1995) or metabolites of ABA (Walker-Simmons *et al.*, 1997). Further, Hooley, Beale and Smith (1990), using α-amylase cDNA probes, have demonstrated that in protoplasts of wild oat, the stimulatory effect on the level of α-amylase mRNA caused by GA_4 can be overcome, and germination inhibited, by ABA. As suggested in the review by Mayer and Poljakoff-Mayber (1989), it is possible that the ratio or interrelationship between GA and ABA is a key factor which regulates germination.

Abscisic acid appears to be capable not only of preventing protein synthesis, but also of inducing the synthesis of new proteins that may be involved in the regulation of dormancy and germination. Leah and Mundy (1989) and Robertson *et al.* (1989) reported that besides its influence on α-amylase production, ABA may also affect enzyme activity through the induction of amylase inhibitors. In addition, in seeds of chick pea, a synergism between ABA and Ca^{2+} in the synthesis of RNAs related to germination has been demonstrated (Colorado, Nicolas and Rodriguez, 1991).

In addition to antagonising the effects of GAs, ABA is also known as a cytokinin antagonist (Ketring, 1973; reviewed by Thomas, 1992a). Whereas, most studies indicate that GAs and ABA act quite independently, it would appear that cytokinins may interact competitively at the same site as ABA: in this regard the inhibitory effects of ABA can quite often be completely reversed by high concentrations of cytokinins (reviewed by Thomas, 1992a).

The exact relationship between PGRs and the control of metabolic processes leading to germination, can be rather confusing. Complicating the issue is that the PGR may be synthesised in different tissues in the same plant; they elicit different effects in different cells, in some cases the effects of one PGR overlap those of another (Trewavas and Cleland, 1983; Gee, Hagan and Guilfoyle, 1991) and tissue sensitivity to the PGR may change (Pinfield, Stutchbury and Bazid, 1987; Pinfield *et al.*, 1989; Pinfield and Stutchbury, 1990). There is also evidence that, at least for ABA, there are multiple signal transduction pathways resulting in diverse expression of ABA-responsive genes (Gilroy, 1996).

7 THESIS OBJECTIVES

According to De Wet (1995) in the Foreword to a recent text book on sorghum and millets, although there has been substantial research on sorghum in southern Africa, especially related to the industrialisation of the traditional sorghum beer; *"we know very little about what is required in sorghum.... to produce a good malt"*.

The aims of this research work were:

To establish conditions of steeping and germination for sorghum malting with respect to external factors such as time, temperature, moisture and aeration to maximise the quality of the malt for beer brewing.

To investigate the endogenous factors that are involved in sorghum germination with the intention of elucidating the possible control mechanisms and consequently utilising this knowledge to further enhance the quality of malt obtainable from sorghum.

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CHAPTER 2: DETERMINATION OF IMPROVED STEEPING CONDITIONS FOR SORGHUM MALTING

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CHAPTER 2 DETERMINATION OF IMPROVED STEEPING CONDITIONS FOR SORGHUM MALTING

ABSTRACT

The effect of various steeping conditions (time, temperature and aeration) on the quality of sorghum malt for brewing (in terms of diastatic power, free amino nitrogen and hot water extract) was examined. Steeping time and temperature had a highly significant effect on sorghum malt quality. In general, malt quality increased with steeping time (from 16-40 hours). Malt diastatic power increased with steeping temperature (up to 30°C) and free amino nitrogen and extract content peaked at a steeping temperature of 25°C. Aeration during steeping appeared to enhance the extract and free amino nitrogen content of the finished malt. Sorghum malt quality was found to be directly related to the steep-out moisture of the grain.

Abbreviations: AB=air-blast; AR=air-rest; NA=non-aerated; DP=diastatic power; FAN=free amino nitrogen; HWE=hot water extract; SDU=sorghum diastatic units.

INTRODUCTION

In Africa, the cereal, sorghum, is malted widely to provide an important raw material in brewing. In southern Africa, approximately 200,000 tons per annum of malted sorghum are used in the production of traditional (opaque) sorghum beer (Dewar and Taylor, 1993). Since a ban on the importation of barley malt by the Nigerian government in 1988, there has also been growing interest in the use of malted sorghum in the brewing of clear lager-type beers (Ajerio, Booer and Proudlove, 1993).

Malting is the germination of cereal grain in moist air under controlled conditions, the primary objective being to promote the development of hydrolytic enzymes which are not present in the ungerminated grain. The malting process can be divided into three physically distinct operations i.e. steeping, germination and drying.

Steeping (the soaking of grain in water) is widely acknowledged as the most critical stage of the malting process (Briggs *et al.*, 1981; French and McRuer, 1990). This is a consequence of the importance of initiating germination such that modification of the endosperm structure will progress at a rate producing malt of the desired quality. Factors that are important for the successful initiation of germination are adequate moisture, temperature and the presence of oxygen (French and McRuer, 1990). These factors can affect the extract yield, diastatic activity and other important malt quality characteristics (Morrall *et al.*, 1986).

Much emphasis has been placed on the effects of various germination conditions on the quality of sorghum malt (Kneen, 1944; Novellie, 1962a and 1962b; Pathirana, Sivayogasunderam and Jayatissa, 1983; Morrall *et al*, 1986; Ilori *et al.*, 1990; Ratnavathi and Bala Ravi, 1991). However, despite the acknowledgement of the importance of the steeping process in malting, there have, until recently, been only limited studies on the effect of steeping conditions on sorghum malt quality (Ratanavathi and Bala Ravi, 1991; Ezeogu and Okolo, 1994 and 1995; Okolo and Ezeogu, 1995a, 1995b and 1996). Indeed, the work done has been limited in its scope, being concerned virtually only with final warm water steeping (Ezeogu and Okolo, 1994 and 1995; Okolo and Ezeogu, 1995a, 1995b and 1996). This may relate to the assumption made in an early study that regarded steeping time as unimportant in determining sorghum malt quality (Novellie, 1962a). This is reflected in commercial sorghum malting practice in South Africa where steeping times, of a maximum of 24 hours, and sometimes as short as six hours, are employed.

In view of this, a systematic investigation of the effect of steeping conditions on sorghum malt quality was needed. In this study the effect of steeping time, temperature and aeration on sorghum malt quality was investigated.

MATERIALS AND METHODS

Materials

Grain:

Sorghum (*Sorghum bicolor* (L.) Moench) grain of cultivar Barnard Red, of good germinability (germinative energy > 95%), was used.

Steeping equipment:

The steeping apparatus (Fig. 2.1) consisted of three perspex vessels each of 2 kg capacity. Each vessel was fitted with a water-jacket in which attemperated water was circulated to control the temperature of the water within the vessels. A pump connected to an air supply was used to aerate the steeping water (air-blast (AB)). The air-rest (AR) method of aeration was achieved by periodically draining the steeping water, which was not aerated by means of a pumped air-stream, from the grain and allowing the grain to rest (i.e. to be exposed to the air) for a pre-determined period of time before refilling the vessel with fresh attemperated water. Non-aerated (NA) steeping conditions were achieved by steeping the grain for the full steeping period in non-aerated water. The conditions of steep (duration and frequency of air-blast or air-rest periods) within each of the vessels were controlled and monitored by computer.

Fig. 2.1 One of the perspex steeping vessels with air being bubbled through the grain

Methods

Steeping:

Samples of grain (500 g), in nylon mesh bags (400 x 400 mm), were steeped at a pre-determined temperature (20, 25 or 30°C). After the first hour, the steeping vessels were drained and refilled with fresh attemperated tap water. Grain at each of the pre-determined temperatures was steeped for one of three different times (16, 24 or 40 hours), under one of three conditions of aeration (AR, AB or NA conditions). Oxygen levels were not monitored during the different steeping treatments, as, when these experiments were designed, the aeration treatments attempted simply to reproduce the steeping conditions used in South African commercial sorghum maltings. At the end of steeping, the grain was centrifuged in a domestic spin drier (AEG type SD 452BN) for one minute at 300 x g to remove the surface film of moisture.

Germination:

The bag-held grain was germinated for a total of six days (from the initiation of steeping) in a water-jacketed incubator (Forma Scientific, Marrietta, U.S.A.) set at 25°C and 100% relative humidity. Twice daily, the bags were removed from the incubator, the grain turned (to avoid meshing of the roots and shoots) and steeped for 10 minutes in tap water. Following the short steep, the grain was spin-dried (one minute at 300 x g) to remove the excess surface-held water and returned to the incubator.

***Drying*:**

After six days from the beginning of steeping, germination was arrested by drying the malt for 24 hours in a forced-draught oven set at 50°C.

***Analyses*:**

Steep-out moisture: The mass of the spin-dried, steeped grain was determined and the steep-out moisture calculated as a percentage. The results were expressed on a wet weight basis.

Malting loss: The loss of dry grain material as a consequence of the malting processes was calculated as a percentage and expressed on a wet weight basis.

Diastatic Power (DP): DP (joint α- and ß-amylase activity) was determined according to an accepted method for sorghum (Dewar, Taylor and Joustra, 1995a, see Appendix A for a copy of the method), adapted from the South African Bureau of Standards method 235 (1970). This method involves making an extract (in this case using water as the extractant) containing the enzymes and then incubating the extract with soluble starch under standardised conditions of time, temperature and pH. The products of joint α- and ß-amylase activity are then measured in terms of reducing power. The results were expressed as sorghum diastatic units (SDU) g^{-1} dry weight.

Free amino nitrogen (FAN): The FAN content of the malt was determined according to an accepted method for sorghum (Dewar *et al.*, 1995b, see

Appendix B for a copy of the method), adapted from EBC Method for Free Amino Nitrogen (European Brewery Convention, 1987). This method involves making an aqueous extract of milled sorghum malt containing the free amino acids and small peptides. The extract is reacted with ninhydrin reagent to produce a purple colour which is proportional to the quantity of FAN. The intensity of purple colour is determined colorimetrically and the amount of FAN calculated by the use of a standardised solution of an amino acid. The results were expressed as mg FAN 100 g^{-1} dry weight.

Hot water (60 °C) extract (HWE): Samples of malt (7.78 g) were placed in 80 ml plastic tubes containing 62.22 g of distilled water and incubated for 2 h at 60°C. At 15 min intervals the contents of the tubes were mixed by inversion. After incubation, the tubes were cooled in 20-25°C water for 30 min and then centrifuged at 1,500 g for 2 min. Samples of the supernatant (40 ml) were transferred to 50 ml beakers and the specific gravity determined by means of a plummet attached to the beam of an analytical balance. Plato Tables (American Society of Brewing Chemists, 1982) were used to calculated total soluble solids. The results were expressed as percentage dry weight.

Water content: Water content was determined after three hours in an oven at 103°C and expressed on expressed on a fresh weight basis.

Statistical analyses: The effect of the variables of steeping, *viz.* time, temperature and aeration on malt DP, FAN, HWE and malting losses were analysed by means of multivariate analysis of variance. Tukey's method of multiple comparisons

(Graybill, 1976) was used to compare individual levels of these variables and a 95% confidence limit was used. Regression analysis was also conducted on the data.

RESULTS AND DISCUSSION

Effect of steeping conditions on malt quality

Sorghum malt quality for opaque beer brewing is defined primarily in terms of DP and FAN (Novellie, 1962a; Taylor and Boyd, 1986). Diastatic power is a measure of the joint α- and ß-amylase activity (Novellie, 1959), and is especially important in the case of sorghum malt as the level of the ß-amylase enzyme in this cereal is intrinsically low (Novellie, 1960; Palmer, Etokakpan and Igyor, 1989). Free amino nitrogen, the proteolytic breakdown products of endosperm proteins, composed of amino acids and small peptides, is important in brewing since it is the source of nitrogen for yeast during fermentation (Baxter, 1981). In sorghum beer brewing, it is particularly important as the FAN in the wort may be limiting due to the high proportion of unmalted cereal adjunct in the grist (Taylor and Boyd, 1986). In conventional clear lager beer brewing, as opposed to opaque sorghum beer brewing, the most important malt quality criterion is extract (Novellie, 1966). Extract is a measure of how much malt will dissolve during the brewing process. This is a less important measure of malt quality in sorghum beer brewing as the malt makes up only approximately 30% of the cereal grist (Novellie, 1966).

Novellie (1962a) reported that steeping time had little effect on the final DP of sorghum malt. Results of the present study, however, indicated that malt DP

increased significantly with increasing steeping time (Table 2.1; $p<0.001$). In addition to DP, other malt quality parameters important for brewing, FAN and HWE, were improved significantly by increasing the steeping time (Tables 2.2 and 2.3, respectively). Sorghum, unlike barley, must be watered during germination. Novellie (1962a), possibly believing that the grain hydration requirements were met on the floor (i.e. during germination), did not regard steeping time as important in determining malt quality. The present study, however, indicates clearly that the steeping times of 16 hours or less as currently practised in South African maltings (some as low as six hours) are sub-optimal. (Tables 2.1, 2.2 and 2.3). It is important to note, however, that unless the oxygen requirements of the grain can be satisfied, long steeping times may lead to anoxic conditions which may be compounded by microbial proliferation.

Analysis of variance indicated that steeping temperature also had a significant effect on the malt quality (Tables 2.1, 2.2 and 2.3). Malt DP was enhanced not only by an increase in the steeping time but also by an increase in the temperature of the steeping water ($p<0.001$). The maximum DP of 43 SDU g^{-1} was obtained in grains steeped for 40 hours under AR, 30°C conditions (Table 2.1). With respect to malt FAN and HWE, it was apparent that 25°C was optimum (Tables 2.2 and 2.3). The highest values for malt FAN and HWE (120 mg 100 g^{-1} and 62%, respectively), were obtained from grain that had been steeped at 25°C for 40 hours under AR conditions for the former and for 24 hours under aerated conditions for the latter. Steeping temperature has also been shown to be important in that, although cultivar related, steeping sorghum with a final immersion in warm water (40°C for six hours) improves the quality of the malt

Table 2.1 Effect of steeping time, temperature and aeration on the diastatic power of sorghum malt (SDU g^{-1} dry weight)

Steeping time (hours)	Temperature (°C)								
	20			25			30		
	Aeration								
	AR	AB	NA	AR	AB	NA	AR	AB	NA
16	29	24	26	27	33	33	35	31	33
24	32	30	28	31	29	36	38	36	38
40	35	35	31	35	37	37	43	40	39

Analysis of variance table for malt diastatic power

Source of variation	Mean square	DF	F	P
Time	92.06	2	24.17	0.000
Temperature	111.27	2	29.22	0.000
Aeration	2.58	2	0.68	0.534
Time*Temperature	1.72	4	0.45	0.770
Time*Aeration	3.31	4	0.87	0.523
Temperature*Aeration	13.83	4	3.63	0.057
Residual	3.81	8		

DF=degrees of freedom, F=F-test, P=significance level

Table 2.2 Effect of steeping time, temperature and aeration on the free amino nitrogen content of sorghum malt (mg 100 g^{-1} dry weight)

Steeping time (hours)	Temperature (°C)								
	20			25			30		
	Aeration								
	AR	AB	NA	AR	AB	NA	AR	AB	NA
16	68	69	66	99	104	94	92	97	85
24	72	67	69	108	107	103	94	97	94
40	95	95	90	120	117	105	117	111	107

Analysis of variance table for malt free amino nitrogen

Source of variation	Mean square	DF	F	P
Time	1030.57	2	208.82	0.000
Temperature	2146.78	2	435.00	0.000
Aeration	104.67	2	21.21	0.001
Time*Temperature	49.38	4	10.01	0.003
Time*Aeration	18.71	4	3.79	0.051
Temperature*Aeration	10.75	4	2.18	0.162
Residual	4.94	8		

DF=degrees of freedom, F=F-test, P=significance level

Table 2.3 Effect of steeping time, temperature and aeration on the extract content of sorghum malt (% dry weight)

Steeping time (hours)	Temperature (°C)								
	20			25			30		
	Aeration								
	AR	AB	NA	AR	AB	NA	AR	AB	NA
16	48	49	47	58	59	56	57	54	54
24	53	51	48	62	62	58	57	58	55
40	56	56	52	61	61	59	54	61	58

Analysis of variance table for malt extract

Source of variation	Mean square	DF	F	P
Time	33.00	2	12.02	0.004
Temperature	157.46	2	57.34	0.000
Aeration	19.12	2	6.96	0.018
Time*Temperature	5.75	4	2.10	0.173
Time*Aeration	2.27	4	0.83	0.544
Temperature*Aeration	1.79	4	0.65	0.641
Residual	2.75	8		

DF=degrees of freedom, F=F-test, P=significance level

(Ezeogu and Okolo, 1994 and 1995; Okolo and Ezeogu, 1995a, 1995b and 1996). In South Africa, few commercial sorghum malting operations have temperature-controlled steeping vessels. In winter the temperature of the steeping water can be as low as ± 12°C and in summer as high as ± 34°C (personal observation). These results show clearly the need for temperature control during steeping.

It has been stated that adequate oxygen is necessary for the formation of α-amylase and peptidase and that excessive carbon dioxide inhibits the formation of these enzymes even in the presence of sufficient oxygen (Weith and Klaushofer, 1963). In terms of FAN and to a lesser extent HWE, this would appear to hold true in that aeration during steeping improved the quality of the malt ($p<0.001$ and $p<0.05$, respectively) (Tables 2.2 and 2.3). Analysis of variance, however, suggested that aeration during steeping did not significantly affect the DP of the malt ($p>0.05$; Table 2.1). In other studies (Ezeogu and Okolo, 1995; Okolo and Ezeogu, 1995b), however, even the length of the air rests employed during steeping were shown to significantly affect the quality of sorghum malt produced. The apparent discrepancy between these results may be explained by the fact that the FAN and HWE assays measure the products of enzymic hydrolysis, whereas DP measures enzyme activity. During malting, DP has been shown to increase gradually and thereafter reach a plateau (Okun and Uwaifo, 1985; Morrall *et al.*, 1986). Therefore, it is probable that even if the enzyme activity of the grains exposed to different levels of aeration had developed at different rates, this would not be reflected in the DP after six days germination.

Aeration and steeping temperature and the combined effect of time and aeration were found to have a significant effect on malting losses ($p<0.01$, $p<0.001$ and $p<0.01$, respectively; Table 2.4). Malting losses were generally retarded by steeping the grain for increasing periods in non-aerated water and the higher the steeping temperature the higher the losses accrued. These results are not unexpected as malting losses are an outcome of respiratory metabolism and, therefore, any condition that affects respiration, and consequently malt quality, will also affect the losses accrued.

Effect of steeping conditions on steep-out moisture

The steep-out moisture content of the grain was significantly affected by both steeping time and temperature ($p<0.001$). There was a general increase in steep-out moisture with increasing steeping time (16-40 hours) and temperature (20-30°C) (Table 2.5). Analysis of variance indicated that aeration did not significantly affect the steep-out moisture of the grain ($p>0.05$).

It has been reported (Swanston and Taylor, 1990) that the moisture content of barley at the end of steeping (as long as air-rests are employed) is an effective way of selecting the steeping regime giving the highest HWE. In this study, a significant correlation was obtained between the steep-out moisture and malt DP ($R=0.862$; accounting for 74.2% of the variation) (Fig. 2.2). Significant correlations were also found between steep-out moisture and FAN, and HWE ($R=0.736$ and $R=0.578$, accounting for 54.2% and 33.5% of the variation, respectively) (Fig. 2.2). It would appear, therefore, that steep-out moisture may similarly provide the sorghum maltster with a rough tool with which to select the steeping procedure

Table 2.4 Effect of steeping time, temperature and aeration on malting losses (% dry weight)

Steeping time (hours)	Temperature (°C)								
	20			25			30		
	Aeration								
	AR	AB	NA	AR	AB	NA	AR	AB	NA
16	16.2	16.5	16.1	17.7	17.3	17.2	18.0	17.5	17.4
24	16.2	16.1	16.4	17.5	18.2	17.4	17.4	17.5	17.4
40	16.5	16.6	15.8	17.1	17.4	15.5	18.3	17.7	16.3

Analysis of variance table for malting losses

Source of variation	Mean square	DF	F	P
Time	0.29	2	4.14	0.058
Temperature	3.84	2	54.54	0.000
Aeration	1.06	2	15.07	0.002
Time*Temperature	0.30	4	4.26	0.039
Time*Aeration	0.54	4	7.61	0.008
Temperature*Aeration	0.15	4	2.18	0.161
Residual	0.07	8		

DF=degrees of freedom, F=F-test, P=significance level

Table 2.5 Effect of steeping time, temperature and aeration on the steep-out moisture of sorghum grain (% dry weight)

Steeping time (hours)	Temperature (°C)								
	20			25			30		
	Aeration								
	AR	AB	NA	AR	AB	NA	AR	AB	NA
16	30.5	30.9	30.6	31.6	31.9	31.8	33.2	32.8	34.1
24	nd[a]	31.1	30.4	31.8	33.5	32.5	34.6	34.2	34.4
40	33.5	32.9	32.6	34.4	35.0	33.6	35.9	36.5	35.2

[a] = not determined

Analysis of variance table for steep-out moisture

Source of variation	Mean square	DF	F	P
Time	14.54	2	66.60	0.000
Temperature	18.37	2	84.13	0.000
Aeration	0.38	2	1.75	0.242
Time*Temperature	0.16	4	0.74	0.592
Time*Aeration	0.43	4	1.96	0.206
Temperature*Aeration	0.24	4	1.08	0.434
Residual	0.22	7		

DF=degrees of freedom, F=F-test, P=significance level

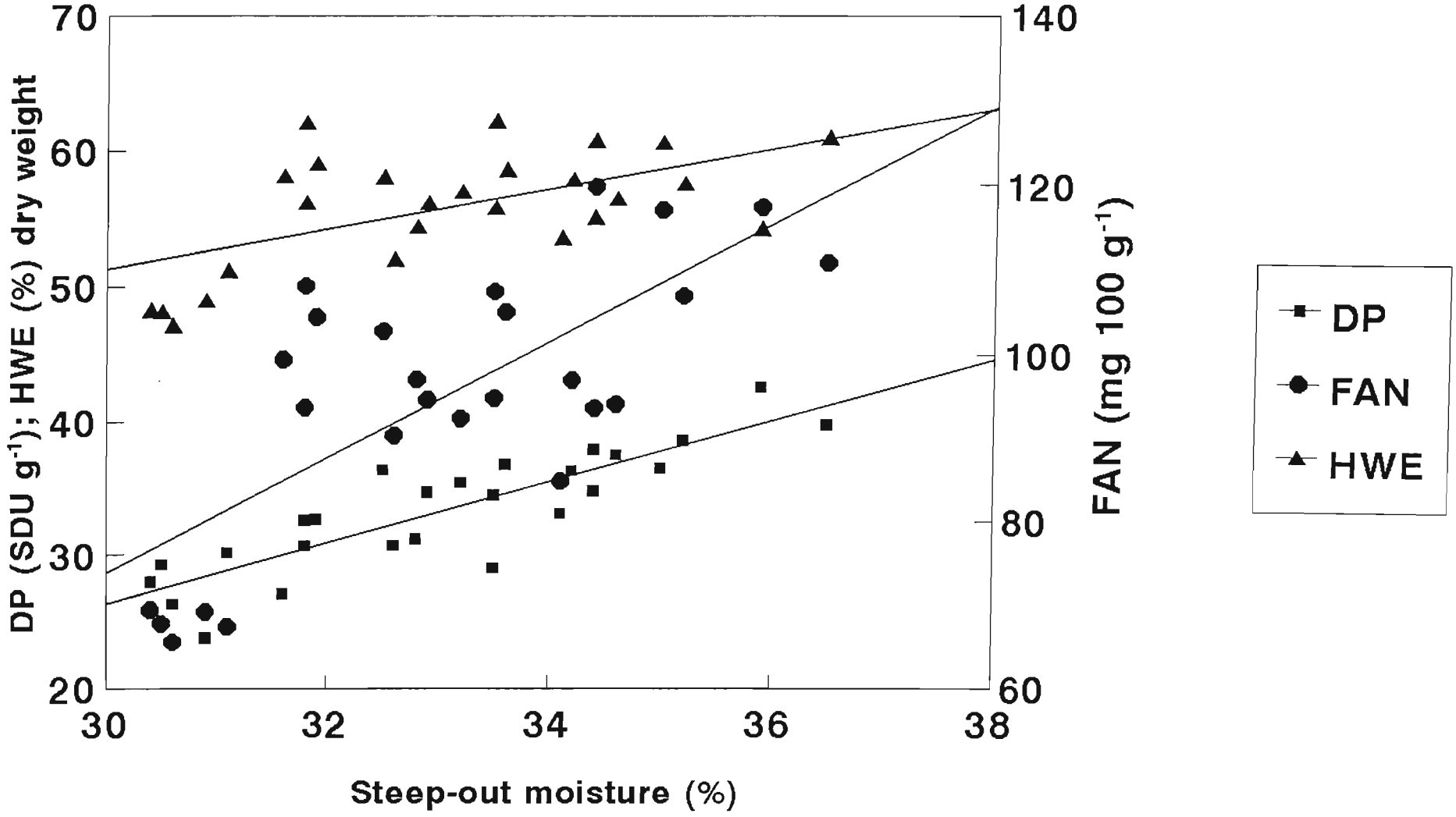

Fig. 2.2 Relationship between steep-out moisture and sorghum malt quality (all data)
DP r=0.862; FAN r=0.736; HWE r=0.578

that will maximise the malting quality of the grain. Although the absolute steep-out moisture may vary depending upon the grain size and cultivar, generally the higher the steep-out moisture the better the quality of the malt produced.

CONCLUSIONS

The findings of this study clearly indicate that in South Africa, the commercial steeping practices are sub-optimal for sorghum. Sorghum malt quality is significantly affected by steeping time and temperature. Malt quality within the range of parameters studied, increases with steeping time (16-40 hours) and the optimum steeping temperature, according to the parameters presently used, is between 25° and 30°C. Aeration during steeping was also shown to be necessary to maximise malt quality. The quality of the sorghum malt was found to be directly related to the steep-out moisture of the grain. Although further work is required, it is suggested that steep-out moisture may provide a means of estimating, at an early stage, the quality of the finished malt. These findings have relevance for sorghum malting for both opaque and clear beer brewing.

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CHAPTER 3: EFFECT OF GERMINATION CONDITIONS, WITH OPTIMISED STEEPING, ON SORGHUM MALT QUALITY - WITH PARTICULAR REFERENCE TO FREE AMINO NITROGEN

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CHAPTER 3 EFFECT OF GERMINATION CONDITIONS, WITH OPTIMISED STEEPING, ON SORGHUM MALT QUALITY - WITH PARTICULAR REFERENCE TO FREE AMINO NITROGEN

ABSTRACT

Using optimised steeping conditions for sorghum, the effect of various germination parameters (time, temperature and moisture) on the quality of sorghum malt for brewing purposes (in terms of diastatic power, free amino nitrogen and hot water extract) and on the associated malting losses, was investigated. Over the range studied (two, four and six days), the quality of the malt and the losses incurred during malting increased with increasing germination time. In general, the optimum germination temperature was between 25 and 30°C, and 18°C was found to be sub-optimal for the development of malt diastatic power. The quality of the finished malt and the associated malting losses were significantly correlated with the moisture content of the green malt. The combined root and shoot portion of the malt was found to be rich in free amino nitrogen (more than four times richer than the berry portion). Although a relatively small proportion of the total weight of the whole malt, the roots and shoots were found to contribute a substantial amount (as much as 61% under certain circumstances) to the whole-malt free amino nitrogen.

Abbreviations: DP=diastatic power; FAN=free amino nitrogen; HWE=hot water extract; TKW=thousand kernel weight.

INTRODUCTION

Malted sorghum is widely used in southern Africa to brew the traditional alcoholic opaque sorghum beer (Novellie and de Schaepdrijver, 1986; Haggblade and Holzapfel, 1989; Daiber and Taylor, 1995). There is also growing interest in the use of malted sorghum in the brewing of clear lager-type beers (Ajerio, Booer and Proudlove, 1993).

The malting process comprises the operations of steeping, germination and drying. A number of factors, such as germination temperature, time and moisture content, are known to have an effect on the appropriate development of the enzymes synthesised during germination and thus to affect the quality of the finished malt (Morrall *et al.*, 1986; Palmer, 1989).

A number of workers have investigated the effects of malting conditions (i.e. germination conditions) on sorghum malt quality (Kneen, 1944; Novellie, 1962; Morrall *et al.*, 1986; Ilori *et al.*, 1990; Muts, 1991). However, until recently (Ratnavathi and Bala Ravi, 1991; Ezeogu and Okolo, 1994 and 1995; Okolo and Ezeogu, 1995a, 1995b and 1996; Dewar, Taylor and Berjak, 1997 (Chapter 2)), there has been little research into the effect of different conditions of steeping on subsequent sorghum malt quality. Dewar *et al.* (1997; Chapter 2) showed that steeping time, temperature and aeration significantly affect the quality of sorghum malt produced in terms of diastatic power (DP), free amino nitrogen (FAN) and hot water extract (HWE). Because the optimum conditions for sorghum germination may be dependent on the prior steeping conditions, the work reported here utilises

the optimised steeping conditions for sorghum (Dewar *et al.*, 1997; Chapter 2) to investigate the effect of various germination conditions (*viz.* germination time, temperature and moisture) on sorghum malt quality.

Morrall *et al.* (1986) found that 24 and 28°C during germination were equally good for the development of sorghum malt DP, FAN and HWE, and that higher temperatures were progressively worse. The effect of malting sorghum at temperatures lower than 24°C, however, has not been addressed, despite the fact that barley is generally malted at much lower germination temperatures (Briggs *et al.*, 1981). In South Africa, during the winter months, sorghum is often malted at temperatures far below the 24 and 28°C reported optimal by Morrall *et al.* (1986). An additional aim of this study was, therefore, to investigate the effect of germinating sorghum at a temperature considered suitable for barley.

A major objective of sorghum malting for opaque-beer brewing is to produce malt with a high FAN content. FAN is produced during malting by the action of the endogenous proteinase and peptidase enzymes on the protein reserves of the grain (Evans and Taylor, 1990a). The breakdown products of the proteolytic action are amino acids and small peptides and are collectively referred to as FAN. During the brewing process, FAN is required by yeast as a source of nutrition in fermentation (Baxter, 1981; Pickerell, 1986). Free amino nitrogen can be particularly limiting in sorghum beer brewing, where there is a low ratio of malt to adjunct (Novellie, 1966 and 1968) and also in lager beer brewing with unmalted grain where only small amounts of sorghum malt are added to supply yeast nutrients (Muts, 1991). In this study, particular emphasis is given to the

development and location of FAN in the malt, which, despite the acknowledgement of its importance in brewing (Baxter, 1981), has received little attention.

MATERIALS AND METHODS

Materials

Grain:

Sorghum (*Sorghum bicolor* (L.) Moench) grain, cultivar Barnard Red, was used. This grain had good germinability (germinative energy > 95%).

Germination vessels:

The germination apparatus comprised six perspex germination vessels (each of 2 kg capacity) and a 600 x 900 x 230 mm stainless steel box (in which the vessels were placed) (Fig. 3.1). Each of the germination vessels had a gridded floor and attemperated, humidified, compressed air was continuously passed through the malt. The temperature of the germinating grain in each of the vessels was continuously controlled and monitored by computer.

Methods

Steeping:

Samples of grain (2 kg) were placed into 600 x 600 mm nylon bags and steeped for 24 hours at 25°C in steeping vessels (of 2 kg capacity). The vessels were

Fig. 3.1 The germination apparatus

drained of water every three hours and refilled with fresh 25°C tap water after one hour of air-rest. At the end of the steeping period, excess surface-held water was removed from the grain by centrifugation in a domestic spin drier (one minute at 200 x g). The conditions of steep were controlled and monitored by computer.

***Germination*:**

Samples of steeped sorghum grain (1.2 kg) were germinated at a pre-determined temperature (18, 25 and 30°C), for one of three different times (i.e. two, four and six days), under either high or low watering conditions. A measured volume of distilled water was sprayed onto the malt by means of an atomiser spray and the grain was turned to ensure similar wetting of all grains. For the low watering treatment, the grain was watered once daily with a set 300 ml volume of water. For the high watering treatment the grain was watered twice daily with an excess of distilled water. Table 3.1 shows the moisture content of the green malt (average of three germination temperatures) for the low and high watering treatments.

Drying:

After the pre-determined malting time, germination was arrested by drying the malt for 24 hours in a forced-draught oven set at 50°C.

***Analyses*:**

Contribution of roots and shoots: A 100 g sample of each of the dried malts was polished as described by Morrall *et al.* (1986) (i.e. the roots and shoots were removed from the berries) and the percent (g 100^{-1}) contribution by weight, of

Table 3.1 Effect of low and high watering levels (high and low) on the green malt moisture content of sorghum malt (% dry mass)

Germination time (days)	Low	High
0	30.4[1]	30.4
2	43.5±1.2[2]	49.2±2.7
4	56.1±1.8	66.2±3.8
6	67.3±2.4	73.8±4.5

[1] = mean of three germination temperatures
[2] = standard deviation

roots and shoots to the whole malt determined.

Malting loss - thousand kernel weight (TKW): Malting loss was estimated by calculating the TKW of the dried, polished malt and comparing this with the TKW of the dry grain.

Diastatic power (DP): DP g^{-1} dry weight was determined - refer to Chapter 2 for details.

Free amino nitrogen (FAN): FAN was determined and expressed as mg 100 g^{-1} dry weight - refer to Chapter 2 for details.

A second set of malt samples (malted previously in our laboratories and stored in airtight containers at 4°C), which had been germinated for various times, at 28°C under the "high"moisture level of Morrall, *et al.* (1986) (where sufficient water was applied every six hours during malting to ensure that none drained off the malt, but at the end of each six hour period, the grain just felt wet) was also analysed to determine the contribution of the various parts of the malt to the FAN content of the whole malt.

Hot water (60 °C) extract (HWE): HWE, percentage dry weight, was determined as detailed in Chapter 2.

Water content: Water content was determined as described in Chapter 2 and expressed on a fresh weight basis.

Statistical analyses: The effect of the variables of germination, *viz*. time, temperature and moisture on malt DP, FAN, HWE and malting losses were analysed by means of multivariate analysis of variance. Tukey's method of multiple comparisons (Graybill, 1976) was used to compare individual levels of these variables and a 95% confidence limit was used. Regression analysis was also conducted on the data.

RESULTS AND DISCUSSION

Diastatic power

A primary objective of malting is to promote the development of hydrolytic enzymes which are not present in the ungerminated grain. The development of the amylase enzymes during malting is of critical importance. These enzymes are required to hydrolyse the malt and adjunct starch to fermentable sugars when the malt is used to brew beer (Briggs *et al.*, 1981). Sorghum malt quality is assessed primarily in terms of DP, which is a measure of the joint activity of α- and ß-amylase. Malt DP was significantly affected by germination time ($p \leq 0.001$), temperature ($p \leq 0.001$), watering level ($p<0.05$), the combined effect of time and temperature ($p<0.05$), and time and watering level ($p<0.05$) (Table 3.2). At 18°C, malt DP increased almost linearly with germination time (Table 3.2). However, at 25°C (no significant difference was found between 25 and 30°C malts) the increase in the DP was more marked over the first four days (Table 3.2). Indeed, it took the 18°C germinated grains at least six days to attain a DP approaching that obtained by a 25 or a 30°C malt in just four days (Table 3.2). Extending the germination time beyond four days, at germination temperatures of 25 and 30°C,

Table 3.2 Effect of germination time, temperature and watering level (low and high) on the diastatic power of sorghum malt (SDU g^{-1} dry weight)

Germination time (days)	Germination conditions					
	18°C		25°C		30°C	
	Low	High	Low	High	Low	High
2	15	12	27	29	31	32
4	30	23	45	46	43	45
6	43	31	45	29	45	39

Analysis of variance table for diastatic power

Source of variation	Mean square	DF	F	P
Time	821.78	2	61.89	0.001
Temperature	624.78	2	47.05	0.001
Watering	80.22	1	12.08	0.025
Time*Temperature	219.22	4	8.26	0.032
Time*Watering	115.11	2	8.67	0.035
Temperature*Watering	30.11	2	2.27	0.219
Residual	26.56	4		

DF=degrees of freedom, F=F-test, P=significance level

did not produce an improvement, and when germination was conducted under the high watering condition, actually occasioned a decline in malt DP (Table 3.2). Of the six-day, 25°C-germinated malts, those that received the high watering treatment had a green malt moisture content of 77% and a DP of 29 SDU g^{-1}, whereas those that received the low watering treatment had a 55% higher DP and a lower green malt moisture content of 69%. The high moisture content late in germination, may have indirectly had a negative effect on the malt DP, as has been suggested by Novellie (1960 and 1962) and Morrall *et al.* (1986). Although not observed in the present investigation, it is worth noting that sorghum maltsters caution that at high germination moistures the production of "slime" on sorghum malt may result in a lowering of malt quality. A more likely explanation for the reduction in malt DP is that even the relatively low drying temperature of 50°C may have been inappropriate for such high moisture content malts, damaging the amylase enzymes in the process. This is supported by the fact that the high moisture continued to have a beneficial effect on FAN, the products of protease activity, late in germination (see below). Irrespective of the reason, it would appear that when sorghum is malted at relatively high germination temperatures it is necessary to control the length of the germination period critically.

Free amino nitrogen

The FAN content of the sorghum grain was significantly enhanced, as much as eight-fold, by malting, i.e. from 28 mg 100 g^{-1} for the ungerminated grain to 230 mg 100 g^{-1} when malted at 25°C under the high watering conditions (Table 3.3). Malt FAN increased significantly with increasing germination time ($p<0.01$). These findings support those of Nout and Davies (1982) and Evans and Taylor (1990b) who reported that the proteolytic activity of sorghum malt increased with germination time. Similarly, Morrall *et al.* (1986) reported an increase in malt

Table 3.3 Effect of germination time, temperature and watering level (low and high) on the free amino nitrogen content of sorghum malt (mg 100 g^{-1} dry weight)

Germination time (days)	Germination conditions					
	18°C		25°C		30°C	
	Low	High	Low	High	Low	High
2	110	96	72	92	102	110
4	176	159	133	165	141	106
6	167	159	177	230	131	213

Analysis of variance table for free amino nitrogen

Source of variation	Mean square	DF	F	P
Time	10372.69	2	22.73	0.006
Temperature	235.44	2	0.52	0.631
Watering	821.88	1	1.80	0.250
Time*Temperature	993.71	4	2.18	0.234
Time*Watering	990.64	2	2.17	0.229
Temperature*Watering	904.98	2	1.98	0.252
Residual	456.30	4		

DF=degrees of freedom, F=F-test, P=significance level

FAN up to six days of germination. In general, malt FAN was not statistically significantly affected by germination temperature, nor the watering treatment administered (Table 3.3). However, when germination was conducted at 25 or 30°C for six days, the high watering treatment did appear to benefit the final FAN content of the malt (Table 3.3). Similarly, Morrall *et al.* (1986) reported that FAN was greatest in malts germinated at high moistures when a high germination temperature (32°C) was employed.

In South Africa, sorghum malt, unlike the normal practice with barley malt, is traded and brewed complete with external roots and shoots. It is known that the roots and shoots are rich in nitrogenous compounds (Taylor, 1983). However, until now the effect of germination conditions on the FAN content of the component parts of the malt has not been studied. Although analysis of variance indicated that neither germination temperature nor the watering regime administered affected the FAN content of the whole malt significantly (Table 3.3), these variables did appear to affect significantly the FAN contributed by the component parts of the malt, particularly the root and shoot portion (Table 3.4). The FAN contributed by the roots and shoots was significantly affected not only by germination time ($p<0.001$), but also by germination temperature ($p<0.01$), especially from 18 to 25°C (25 and 30°C were not significantly different). With respect to the level of watering, it appeared that the high watering level improved the FAN content of the roots and shoots (Table 3.4).

The roots and shoots contained a far higher concentration of FAN than the berries (between four and seven times more) (Table 3.5). Also, the relative contribution of the roots and shoots to the total malt FAN increased with germination time

Table 3.4 Effect of germination time, temperature and watering level (low and high) on the contribution of the root and shoot component to the free amino nitrogen content of whole sorghum malt (mg 100 g^{-1} dry weight)

Germination time (days)	Germination conditions					
	18°C		25°C		30°C	
	Low	High	Low	High	Low	High
2	19 (17)[1]	25 (26)	19 (26)	36 (39)	25 (25)	34 (31)
4	55 (31)	60 (38)	56 (42)	79 (48)	61 (43)	68 (64)
6	81 (49)	74 (47)	79 (45)	95 (41)	87 (66)	90 (42)

[1] = Figures in parentheses represent the percentage contribution of the roots and shoots of the whole malt FAN

Analysis of variance table for free amino nitrogen - roots and shoots

Source of variation	Mean square	DF	F	P
Time	5153.44	2	955.72	0.000
Temperature	140.29	2	26.02	0.005
Watering	339.74	1	63.01	0.001
Time*Temperature	5.27	4	0.98	0.509
Time*Watering	27.56	2	5.11	0.079
Temperature*Watering	119.78	2	22.21	0.007
Residual	5.39	4		

DF=degrees of freedom, F=F-test, P=significance level

Table 3.5 Free amino nitrogen content and relative weight of the component parts of sorghum malt after various germination times (mg 100 g^{-1} dry material)

Germination time (days)	Roots and shoots	Berries
2	585[1] [a 2] (4.6)[3]	79 [x] (95.4)
4	553 [b] (12.0)	106 [y] (8..8)
6	500 [c] (17.1)	127 [z] (82.9)

[1] = Mean of six values
[2] = Dissimilar letters denote a statistically significant difference ($p<0.05$)
[3] = Figures in parentheses represent the relative percentage weight of the total malt mass (g 100^{-1})

(Table 3.4). Thus, although the roots and shoots represented only a relatively small proportion of the total mass of the malt (Table 3.5), after six days of germination at 25°C, under the high watering condition, 95 mg FAN 100 g^{-1} dry weight was contributed by the roots and shoots (41% of the total malt FAN) (Table 3.4), which accounted for only 21% of the total weight of the malt (dry weight basis). The dramatic increase in FAN in the roots and shoots can be explained as a result of translocation of the products of storage protein breakdown from the kernel (Taylor, 1983).

Analysis of the FAN content of a second and independent batch of malt samples, germinated at 28°C under the "high" watering conditions of Morrall *et al.* (1986), support these findings. With respect to the contribution that the roots and shoots made to the FAN content of the whole malt, this initially increased with germination time with a peak of 62% being reached at day four (Fig. 3.2). This is particularly significant when it is realised that at day four the roots and shoots represented only 17.5% of the dry weight of the whole malt.

These findings are of importance to sorghum beer brewing where malt of a high FAN content is required. It is clear that even a relatively small loss of roots and shoots from the malt may represent a relatively large loss of FAN. Therefore, care should be exercised during malt processing to minimise root and shoot losses. In addition, if sorghum malt and large quantities of unmalted cereal adjunct are to be used for conventional clear beer brewing, where the roots and shoots of the malt are routinely removed and discarded, attention should be given to the supply

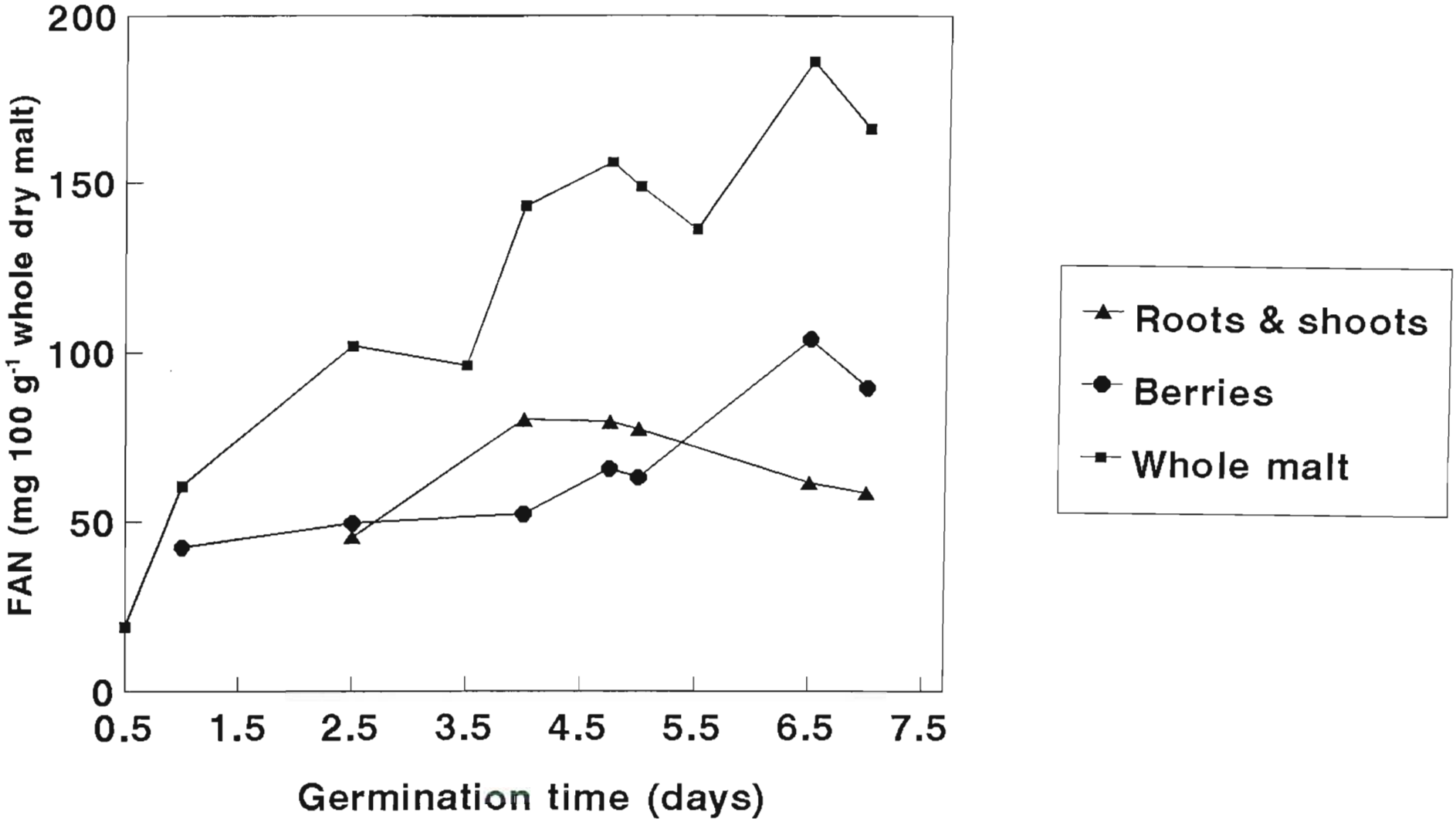

Fig. 3.2 Contribution of the berries and roots and shoots to the FAN content of the whole sorghum malt at 28°C, high moisture (Morrall *et al.*, 1986)

of a source of FAN in the wort. Failure to consider this could result in fermentation problems due to limited yeast growth (Pickerell, 1986).

Extract

Malt HWE, as with DP and FAN, was significantly affected by germination time ($p<0.01$) increasing especially over the first four days of germination (Table 3.6). This result agree with the findings of Jayatissa, Pathirana and Sivayogasunderam (1980) and Morrall *et al.* (1986) who showed that HWE increased with germination time.

Morrall *et al.* (1986) reported that there was no significant difference in sorghum malt HWE when germination was conducted at either 24 or 28°C. However, at temperatures of 32°C and 35-38°C, significantly less was produced. Under the conditions of the present study, the amount of HWE obtainable from the malt was not affected significantly by germination temperature over the range 18, 25 and 30°C. It would appear that unlike the higher germination temperatures (i.e. 32°C and 35-38°C) investigated by Morrall *et al.* (1986), a lower germination temperature of 18°C is not detrimental to the development of malt HWE, possibly because the rate of sugar utilisation could be slower at the lower temperature.

Malting losses

Malting loss, as indicated by the TKW of the malt, was significantly affected by germination time ($p\leq0.001$) and temperature ($p<0.001$) (Table 3.7). As Pathirana, Sivayogasundaram and Jayatissa (1983) have reported, the malting losses (as indicated by a reduction in TKW) were proportional to the number of days allowed for germination. The TKW decreased, and consequently the malting

Table 3.6 Effect of germination time, temperature and watering level (low and high) on the hot water extract content of sorghum malt (% dry weight)

Germination time (days)	Germination conditions					
	18°C		25°C		30°C	
	Low	High	Low	High	Low	High
2	38	36	41	41	54	59
4	68	62	65	61	54	78
6	75	77	70	60	70	77

Analysis of variance table for hot water extract

Source of variation	Mean square	DF	F	P
Time	1163.17	2	33.596	0.003
Temperature	124.78	2	3.604	0.127
Watering	10.89	1	0.315	0.610
Time*Temperature	80.93	4	2.338	0.215
Time*Watering	9.36	2	0.270	0.776
Temperature*Watering	120.67	2	3.485	0.132
Residual	34.62	4		

DF=degrees of freedom, F=F-test, P=significance level

Table 3.7 Effect of germination time, temperature and watering level (low and high) on the thousand kernel weight (g) of sorghum malt (dry weight basis)

Germination time (days)	Germination conditions					
	18°C		25°C		30°C	
	Low	High	Low	High	Low	High
2	29.3	28.0	23.2	22.0	24.0	22.6
4	29.2	30.3	19.6	17.3	20.9	20.5
6	25.3	21.7	16.9	15.1	18.3	17.6

Analysis of variance table for thousand kernel weight

Source of variation	Mean square	DF	F	P
Time	50.25	2	45.27	0.001
Temperature	115.54	2	104.09	0.000
Watering	7.69	1	6.93	0.058
Time*Temperature	4.26	4	3.83	0.110
Time*Watering	0.84	2	0.76	0.525
Temperature*Watering	0.35	2	0.31	0.746
Residual	0.11	4		

DF=degrees of freedom, F=F-test, P=significance level

increased markedly when the temperature was raised from 18 to 25°C, but no significant difference was found when the temperature was raised from 25 to 30°C (Table 3.7).

Malt moisture

It has been shown that the higher the steep-out moisture, the higher the quality of the resulting malt in terms of DP, FAN and HWE (Dewar *et al.*, 1997; Chapter 2). Similarly, in this study highly significant correlations were found between the moisture content of the green malt and DP ($p<0.01$), FAN and HWE ($p<0.001$) (Fig. 3.3). It would appear, therefore, that moisture content, whether it is at the end of steeping (Dewar *et al.*, 1997) (Chapter 2) or at the end of the malting process itself, is an important indicator of sorghum malt quality for brewing purposes. It should be noted that in South Africa, the moisture content of sorghum malted under commercial sorghum malting conditions is often far less than 50% (fresh weight basis, unpublished data). This is considerably lower than that obtained at six days of germination under even the low watering conditions (Table 3.1). It would appear that a useful way of enhancing the quality of sorghum malt produced commercially in South Africa, would be to enhance the moisture content of the germinating grain during malting. However, malting losses, as indicated by TKW, were also significantly correlated with green malt moisture ($p<0.01$) (Fig. 3.3). Therefore, in maximising the quality of the malt, care should be taken not to accrue excessive malting losses.

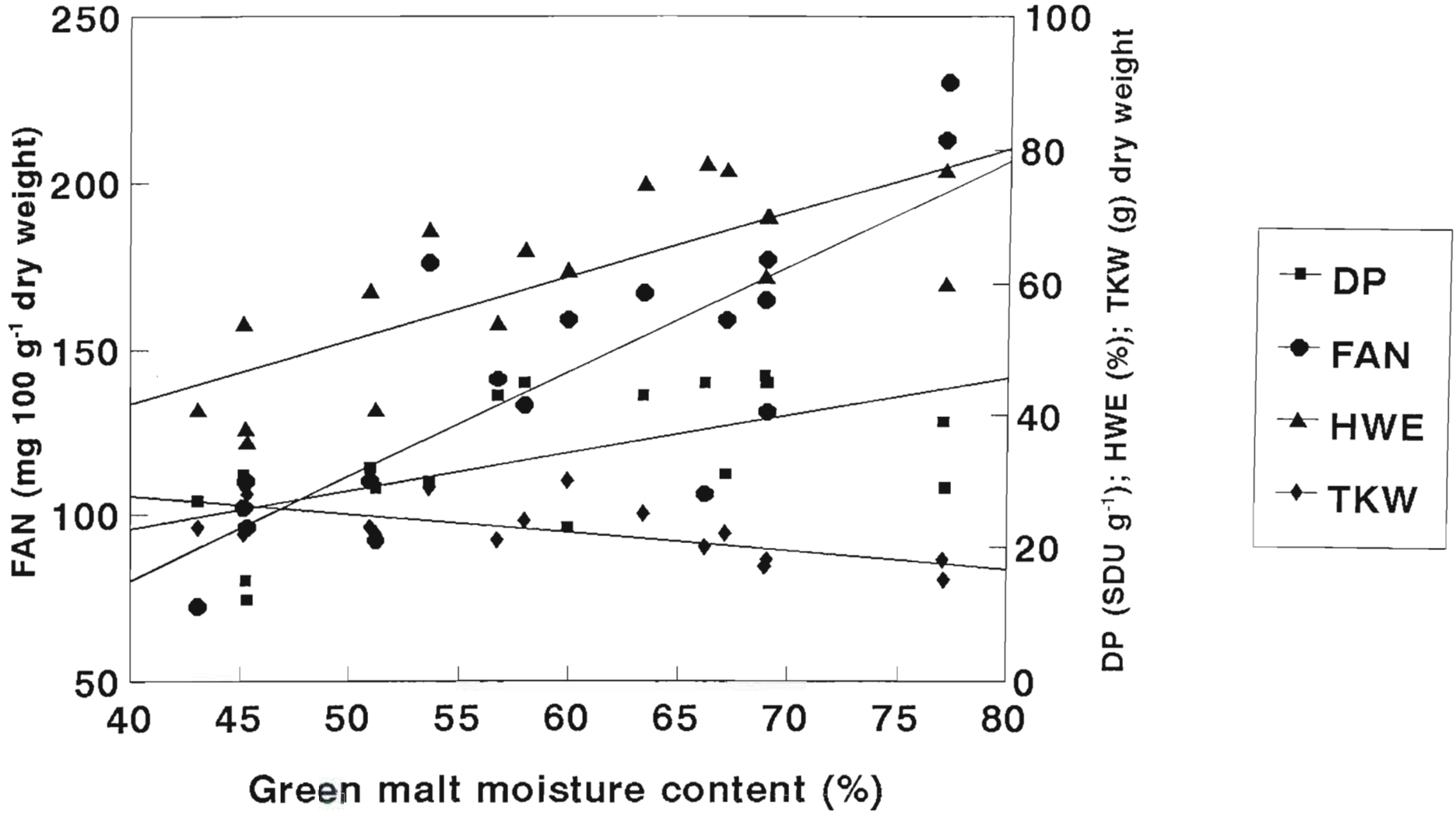

Fig. 3.3 Relationship between green malt moisture content and sorghum malt quality

DP r=0.609; FAN r=0.806; HWE r=0.768; TKW r=0.699

CONCLUSIONS

Under optimum steeping conditions, the optimum germination temperature for sorghum malting is between 25 and 30°C. The findings indicate clearly that where germination is conducted at lower temperatures (18°C), as is sometimes done in South Africa during the winter months, this would be sub-optimal for the development of malt DP. The quality of the malt and the associated malting losses have been found to be directly related to the moisture content of the green malt. The root and shoot portion of the malt was found to be an extremely rich source of FAN, and to contribute a substantial amount to the malt FAN. Thus, where sorghum malt is being used as a critical source of FAN care should be exercised during malt processing to minimise the loss of roots and shoots.

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CHAPTER 4: EFFECT OF ALKALINE STEEPING ON WATER UPTAKE AND MALT QUALITY IN SORGHUM

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CHAPTER 4 EFFECT OF ALKALINE STEEPING ON WATER UPTAKE AND MALT QUALITY IN SORGHUM

ABSTRACT

Condensed-tannin-free and high-tannin sorghum grain was steeped in dilute alkali and the effects on water uptake during steeping and final malt quality were determined. With the condensed-tannin-free sorghum, steeping in dilute alkali led to increased water uptake and an improvement in malt quality in terms of diastatic power and free amino nitrogen. These effects are presumed to be due to the alkali disrupting polysaccharides of the pericarp cell walls, thus allowing more rapid water uptake. Best results were obtained by application of alkali early in steeping, presumably because the grain is less susceptible to alkali toxicity than later in steeping. Steeping high-tannin sorghum in dilute alkali did not improve malt quality. This appeared to be because water uptake was not increased to the same extent as with condensed-tannin-free sorghum, as the alkali reacted with the tannins.

Abbreviations: DP=Diastatic power; FAN=free amino nitrogen.

INTRODUCTION

Recent research has shown that the quality of sorghum malt, with respect to diastatic power (DP), free amino nitrogen (FAN) and hot water extract, is highly significantly correlated with the steep-out moisture content of the grain (Dewar, Taylor and Berjak, 1997; Chapter 2). Other recently published research has indicated that steeping sorghum in dilute alkali can increase the DP (Okolo and Ezeogu, 1996a) and FAN (Okolo and Ezeogu, 1996b) content of the malt, although the effect appeared to be cultivar dependent. These latter publications (Okolo and Ezeogu, 1996a and 1996b) offered no explanation as to the mechanism by which alkali steeping affected sorghum malt quality. In view of the fact that alkali is known to disrupt the molecular structure of the non-starch polysaccharides which constitute sorghum cell walls (Verbruggen, Beldman and Voragen, 1995), the relationship between alkali steeping, water uptake and sorghum malt quality was investigated. An additional aim of this study was to investigate the effect of alkali steeping on two different types of sorghum grain: 1) containing condensed-tannins in the so-called testa (classed as GH sorghum by the South African Sorghum Board) and 2) a condensed-tannin-free grain (classed as GM sorghum by the South African Sorghum Board). Both of these grain types are considered suitable for malting purposes. However, for the GH grain to be used, it is essential the tannins be inactivated during malting, otherwise they bind with the essential malt enzymes during brewing and adversely affect the hydrolysis of starch, proteins and other components of the mash (Daiber, 1975). Treatment with alkali is known to inactivate tannins in sorghum (Price *et al.*, 1979).

MATERIALS AND METHODS

Material

Grain:

Two sorghum cultivars of good germinability (Germinative Energy > 95%) were used: NK 283, a condensed-tannin-free hybrid (GM) and a high-tannin hybrid (1.3% polyphenols; GH) (cultivar unknown).

Methods

Steeping:

Samples of pre-washed, spin-dried (one minute at 300 x g) sorghum grain (100 g), were steeped in a still solution of NaOH [0 (control), 0.1, 0.3 and 0.5% w/v] for a period of eight hours during either the first eight hours (0-8 hours) or during the last eight hours (16-24 hours) of a 24 hour steeping period. After the alkaline steep, the grain was rinsed thoroughly in fresh tap water. During the time the grain did not receive the alkaline treatment, it was steeped in continuously changing tap water (±16°C). At the end of steeping, the grain was spin-dried (one minute at 300 x g) to remove the surface film of moisture.

Germination:

The grain was germinated in a water-jacketed incubator (Forma Scientific, Marrietta, USA) set at 25°C and 100% relative humidity. Twice daily, the bags were removed from the incubator, the grain turned (to avoid meshing of the roots

and shoots) and immersed for 10 minutes in tap water. Following each short immersion, the grain was spin-dried (one minute at 300 x g) to remove the excess surface-held water and returned to the incubator.

Drying:

After six days from the beginning of steeping, germination was arrested by drying the malt for 24 hours in a forced-draught oven set at 50°C.

Analyses:

Diastatic power (DP): Malt DP was determined as detailed in Chapter 2, except that both 2% peptone and distilled water were used as extractants. Extraction in peptone allows the determination of DP, even in high-tannin sorghum malts as the peptone complexes with any condensed-tannins in the sample. In contrast, when extracted in water, the tannins in the malt are not inactivated and consequently an artificially low value for DP is obtained (Taylor, 1989) as the tannins are able to bind with the malt enzymes. Diastatic power was expressed as Sorghum Diastatic Units (SDU) g^{-1} dry weight.

Free amino nitrogen (FAN): The FAN content of the malt was determined and expressed as mg 100 g^{-1} dry weight - refer to Chapter 2.

Water content: Water content was determined as described in Chapter 2 and expressed on a fresh weight basis.

Steep-out moisture: The amount of water taken up during steeping was determined and expressed as a percentage of the pre-washed, non-steeped grain weight.

RESULTS AND DISCUSSION

Figures 4.1 and 4.2 show the effect of steeping the condensed-tannin-free sorghum in different concentrations of NaOH on water uptake during steeping and on DP and FAN in the final malt. It can be seen that water uptake increased almost linearly with increasing NaOH concentration. Whilst not tested, this is possibly as a consequence of disruption of the pericarp non-starch polysaccharide, and other cell wall material by the NaOH. Specifically, alkali is known to saponify acetyl groups and other ester linkages, cause cellulose to swell and disrupt the hydrogen bonds between hemicelluloses, resulting the solubilisation of hemicelluloses (Verbruggen *et al.*, 1995). Both malt DP and FAN were increased substantially by steeping in 0.1% NaOH, by 34% and 33%, respectively when the alkali was administered during the first eight hours of steeping (Fig. 4.1) and by 21% and 22%, respectively when NaOH was administered during the last eight hours of steeping (Fig. 4.2). A concentration of 0.3% NaOH did not increase DP or FAN further. Steeping in 0.5% NaOH resulted in levels of malt DP and FAN which were far lower than when the grain had been steeped in water only, presumably as a result of the phytotoxicity of the high concentration of NaOH.

The fact that DP, a measure of joint α- and ß-amylase enzymic activity (Novellie, 1959), and FAN, a measure of the products of proteolysis (Evans and Taylor,

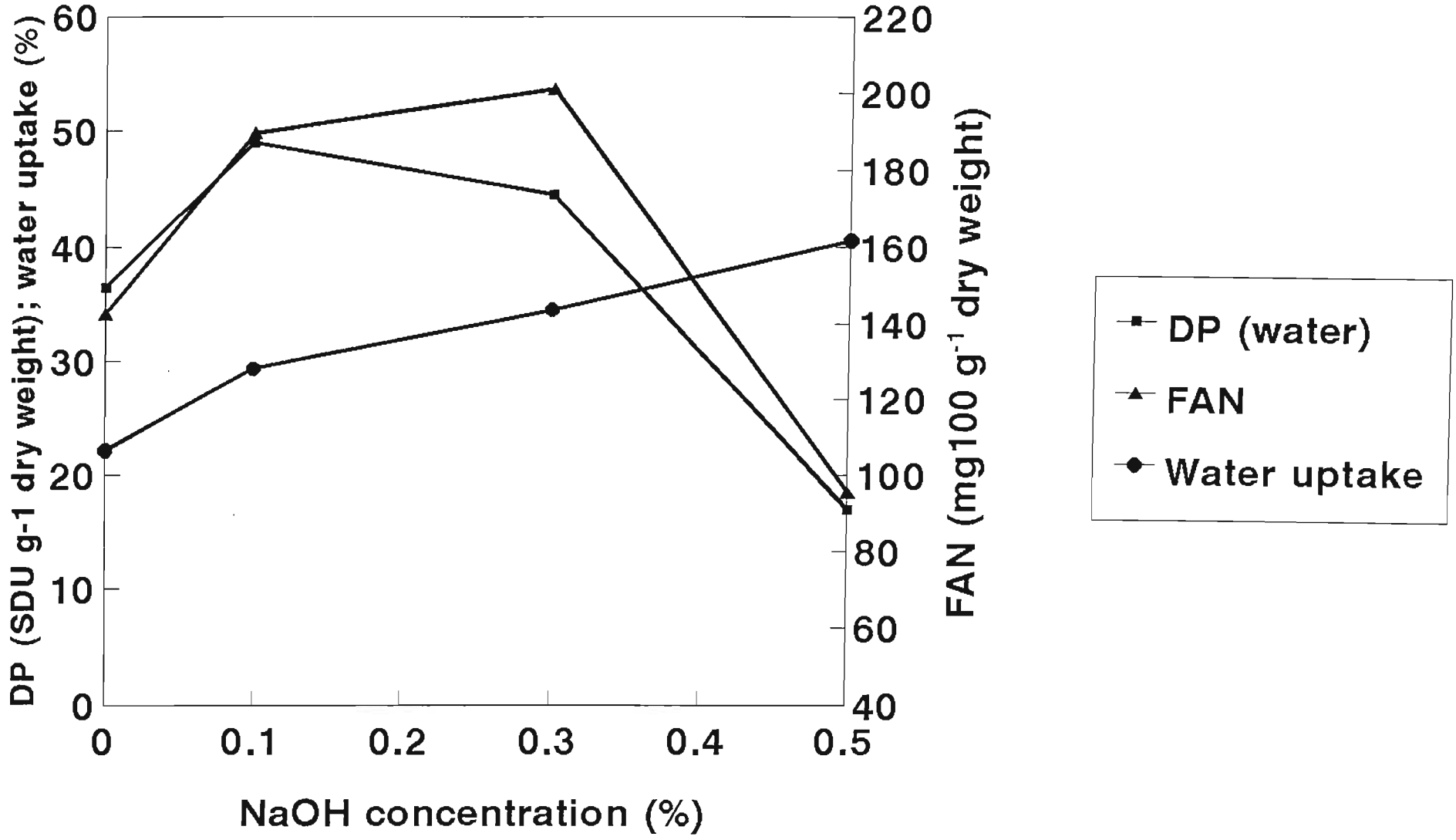

Fig. 4.1 Effect of steeping (0-8 hours) in different concentrations of NaOH on water uptake and sorghum malt quality - condensed-tannin-free (GM) sorghum

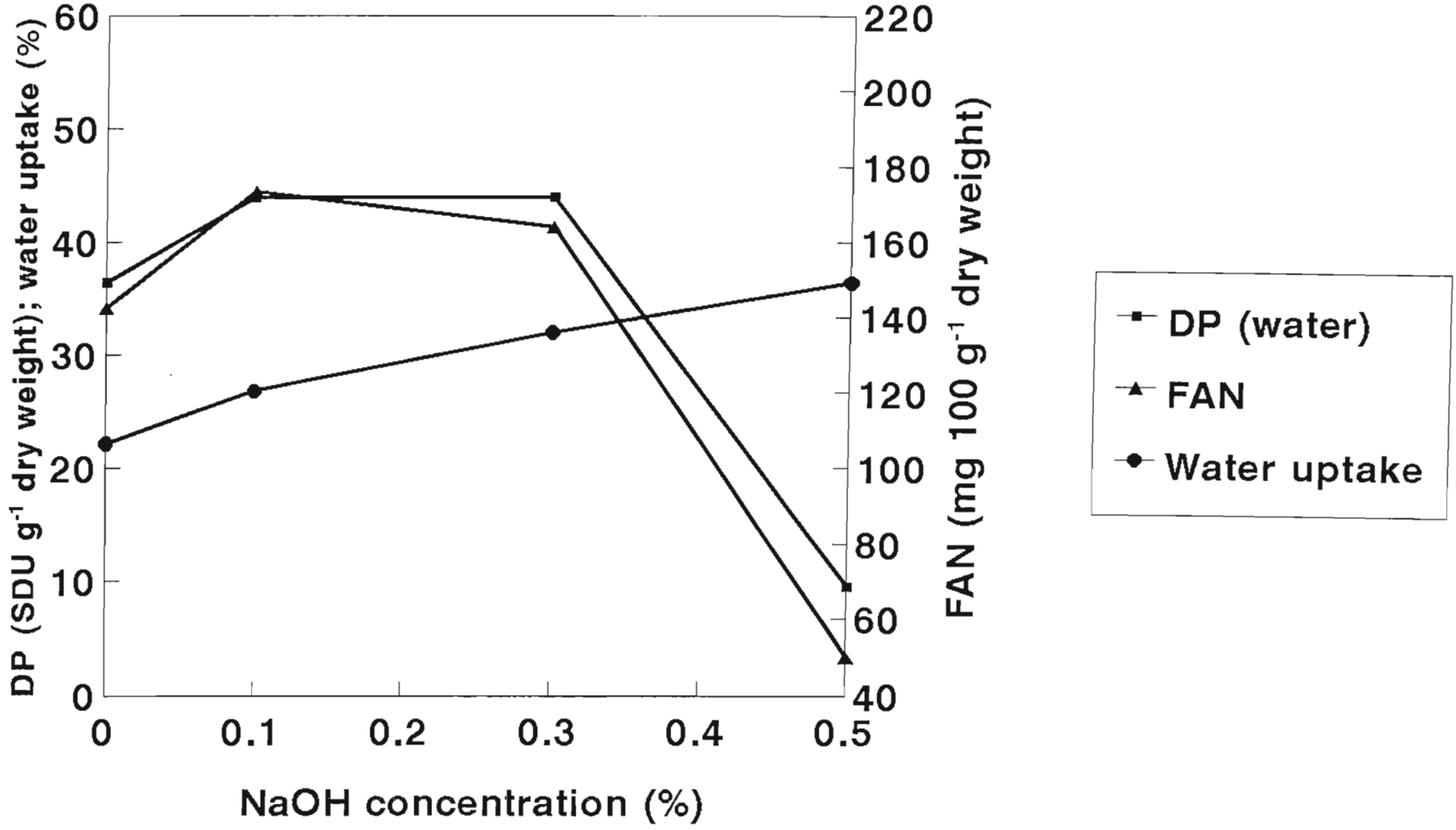

Fig. 4.2 Effect of steeping (16-24 hours) in different concentrations of NaOH on water uptake and sorghum malt quality - condensed-tannin-free (GM) sorghum

1990), were similarly increased by steeping in 0.1% NaOH suggests that the effect was due to increased metabolic activity in the malt. Such an effect can be attributed to the more rapid hydration of the grain brought about by the NaOH. The lower increase in DP and FAN obtained when NaOH was administered during the last eight hours of steeping (Fig. 4.2), as opposed to the first eight hours (Fig. 4.1), is possibly due to the later administration bringing about a smaller increase in hydration. Alternatively or additionally, it could be due to the fact that as the germinating grain became more metabolically active it became more susceptible to the toxic effects of the NaOH. This latter explanation is supported by the fact that the administration of 0.5% NaOH during the first eight hours of steeping gave levels of DP and FAN of 47% and 67%, respectively of the water control, whereas NaOH administration during the last eight hours gave only 26% and 35%, respectively.

With the high-tannin sorghum, the administration of NaOH was examined only over the first eight hours of steeping, in view of the adverse effects that its addition had over the last eight hours with the condensed-tannin-free type. From Fig. 4.3 it can be seen that as with the condensed-tannin-free sorghum, water uptake increased with increasing NaOH concentration, although the increase in water uptake was much less. The water uptake of the high-tannin sorghum control was, however, considerably greater than that of the condensed-tannin-free sorghum control (Fig. 4.1). It has been observed that high-tannin sorghums take up more water than condensed-tannin-free types (Daiber, personal communication[1]). The probable explanation for this is that high-tannin sorghums normally have a softer

[1] K.H. Daiber, Sorghum Beer Unit, CSIR, Pretoria, South Africa (retired)

endosperm texture (Waniska, Poe and Bandyopadhyay, 1989). This is borne out by the fact that this high-tannin sorghum had both a lower thousand kernel weight and hectolitre weight (21.6 g and 70.7 kg versus 24.7 g and 73.4 kg for the condensed-tannin-free sorghum).

Unlike the situation with the condensed-tannin-free sorghum (Fig. 4.1), increasing concentrations of NaOH had little effect on malt FAN and DP (as measured by peptone extraction) (Fig. 4.3). Low concentrations of NaOH did not increase malt DP and FAN, and they were not reduced by high NaOH concentrations. It should be noted that when determining the DP of malts made from high-tannin sorghum it is necessary to extract the amylases in the presence of peptone, otherwise they will be inactivated by the tannins, giving an artificially low value for DP (Taylor, 1989).

The lack of a positive or negative effect of NaOH steeping with the high-tannin sorghum may be attributed to the NaOH reacting preferentially with the tannins. Treatment with alkali is a well-described method of inactivating the tannins in sorghum (Price *et al.*, 1979). This reaction effect is borne-out by the fact that the pH of the high-tannin sorghum steep liquors at the end of steeping from all three alkali steeps (0.1, 0.3 and 0.5% NaOH) were considerably lower than those of the corresponding steep liquors from the condensed-tannin-free sorghum (Table 4.1). In fact, at the end of the eight hours steep, the pH of the high-tannin sorghum 0.5% NaOH steep liquor (pH 11.1) was lower than that of the 0.3% NaOH steep liquor for the condensed-tannin-free sorghum (pH 11.9). The results of the reaction between the NaOH with the condensed-tannins can be seen clearly from

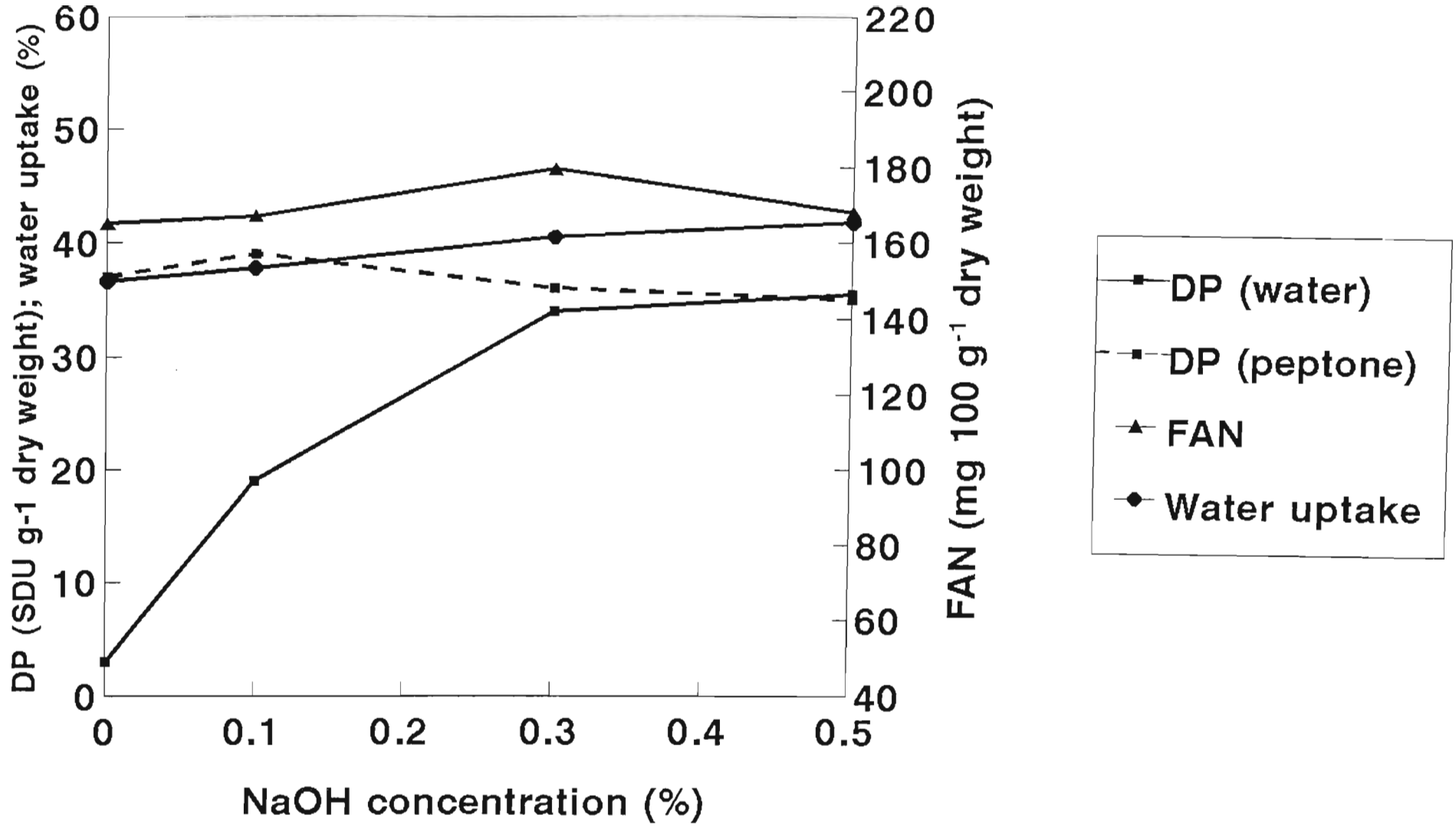

Fig. 4.3 Effect of steeping (0-8 hours) in different concentrations of NaOH on water uptake and sorghum malt quality - high-tannin (GH) sorghum

Table 4.1 pH of the steep liquor of the condensed tannin-free (GM) and the high-tannin (GH) sorghums at the start and the end of eight hours steeping in various concentrations of NaOH

NaOH concentration (%)	pH at start of 8 hours treatment		pH at end of 8 hours treatment	
	GM	GH	GM	GH
0	7.4	7.3	7.3	7.6
0.1	12.0	12.0	10.1	8.9
0.3	12.3	12.3	11.9	10.2
0.5	12.5	12.5	12.2	11.1

the fact that when the grain was steeped in high concentrations of NaOH, the water and peptone extract DPs were virtually the same (34 and 36 SDU g^{-1}, respectively for 0.3% NaOH and 35.5 and 35 SDU g^{-1}, respectively for 0.5% NaOH) (Fig. 4.3). In contrast, when the grain was steeped in water only, the water extract DP was only 3 SDU g^{-1} whereas the peptone extract DP was 37 SDU g^{-1}. It seems therefore that, at the higher NaOH concentrations, the reaction between the NaOH and the tannins prevented reaction between the tannins and the malt amylase enzymes. It is suggested that the preferential reaction between the alkali and the tannins reduced the effect of the alkali on the sorghum cell walls. Hence, the increase in water uptake due to alkali steeping high-tannin sorghum (Fig. 4.3) was substantially lower than for alkali steeping condensed-tannin-free sorghum (Fig. 4.1).

CONCLUSIONS

It appears that the improvement in sorghum malt quality brought about by steeping in dilute alkali is due to increased water uptake during steeping. This is presumed to be as a result of the alkali disrupting the sorghum pericarp cell wall structure. The improvement in malt quality appears only with condensed-tannin-free sorghum, as in high-tannin sorghum the alkali is presumed to react with the tannins. This difference in the effect of alkali between condensed-tannin-free and high-tannin sorghum may account for some of the cultivar effect differences reported in previous work on alkali steeping of sorghum (Okolo and Ezeogu, 1996a and 1996b).

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CHAPTER 5: CHANGES IN SELECTED PLANT GROWTH REGULATORS DURING GERMINATION IN SORGHUM

This Chapter has been published in part in:

Dewar, J., Taylor, J.R.N and Berjak, P. (1998). Changes in selected plant growth regulators during germination in sorghum. *Seed Science Research*, **8**, 1-8. (See Appendix F for copy of the paper)

CHAPTER 5 CHANGES IN SELECTED PLANT GROWTH REGULATORS DURING GERMINATION IN SORGHUM

ABSTRACT

The technique of radioimmunoassay following sample resolution by HPLC was used to determine the amounts of cytokinins, zeatin (Z), zeatin riboside (ZR) and isopentyladenine (IPA), the combined amounts of gibberellins$_{1+3}$ (GA_{1+3}), and the levels of indole acetic acid (IAA) and abscisic acid (ABA) during germination in grains of sorghum. Although the GA_{1+3} concentrations were very low there was some indication that peaks were related to the time of germination. In the mature, non-germinated grain, the concentration of each of the other plant growth regulators was much higher in the smaller component comprised of the embryonic axis and scutellum than in the much larger endosperm (plus aleurone) tissue. During the germination period studied (64 hours), these concentrations declined, with a peak in the amount of the cytokinin IPA and a small peak in Z+ZR (24 hours) in the embryo following the first visible signs of root protrusion and coincident with a large enhancement in amylase activity. The high concentration of the germination inhibitor, ABA, in the embryo tissue prior to germination was noteworthy. It is suggested that the interaction of ABA and the cytokinins, IPA+Z+ZR, and possibly gibberellins, GA_{1+3}, may play a significant rôle in controlling sorghum germination.

Abbreviations: PGR=plant growth regulator; RIA=radioimmunoassay; Z=zeatin; ZR-zeatin riboside; IPA=isopentyladenine; GA_{1+3}=gibberellin$_{1+3}$; IAA=indole acetic acid; ABA=abscisic

INTRODUCTION

A variety of internal and external factors is known to be important in controlling germination in seeds. Already in the 1960s, Paleg (1960), MacLeod, Duffus and Johnston (1964) and Chrispeels and Varner (1967) demonstrated that gibberellic acid (GA_3) induces barley aleurone cells to produce several endosperm-degrading enzymes.

During the process of malting (i.e. the germination of cereal grain in moist air under controlled conditions) a number of hydrolytic enzymes develop and degrade the reserve food materials of the endosperm (Bewley and Black, 1978). Barley malting practices have taken advantage of the knowledge that the application of the plant growth regulator (PGR) GA_3 can dramatically enhance the synthesis of the hydrolytic enzyme, α-amylase. The action of the enzyme α-amylase is of critical importance in malting as it initiates the breakdown of starch granules. This modification is essential to hydrolyse the starch into fermentable sugars when malt is used by the brewing industry for beer making.

In Africa, sorghum malt is used for the brewing of the traditional opaque-type beers (Novellie and de Schaepdrijver, 1986; Haggblade and Holzapfel, 1989; Daiber and Taylor, 1995). Recently, in tropical countries which are not suitable for the cultivation of barley, there has been interest in substituting sorghum malt for barley malt for the brewing of "clear" beers (Ajerio, Booer and Proudlove, 1993). Compared with barley malt, sorghum malt has low ß-amylase activity, the α-amylase activity being similar (Aniche and Palmer, 1990) and, therefore, worts

(unfermented malt infusions) produced using sorghum malt generally have lower fermentability compared with barley malt. There have been reports that the application of GA_3 can promote shoot growth in sorghum (Morgan, Miller and Quinby, 1977; Wright *et al.*, 1983; Rood, 1995). However, unlike the situation for barley, there is contradictory evidence as to whether the application of GA_3 to germinating sorghum grain increases amylase activity. Daiber and Novellie (1968) and Aisien, Palmer and Stark (1983) found little stimulation of amylase activity. However, other studies have indicated that application of GA_3 can be used to improve the amylase activity of sorghum malt (Agu *et al.*, 1993; Nzelibe and Nwashike, 1995). The stimulatory effect, however, appears to be somewhat variety-dependent and in some cases, the application of GA_3 actually reduced amylase activity (Nzelibe and Nwashike, 1995). Hence, current sorghum malting practices tend to rely almost entirely on the provision of suitable environmental conditions to initiate germination and promote the development of the essential malt hydrolytic enzymes [Morrall *et al.*, 1986; Dewar *et al.*, 1997a (Chapter 2) and 1997b (Chapter 3)].

This study was initiated in an attempt to elucidate the possible control mechanisms involved in sorghum germination. A combined HPLC-radioimmunoassay (RIA) technique was used to determine the levels of selected endogenous PGRs from the groups auxin, cytokinins, gibberellins and abscisic acid in sorghum at various different stages of germination.

MATERIALS AND METHODS

Material

Grain:

Sorghum grain (cv. NK 283), a cultivar widely grown in South Africa and recommended for malting purposes, was used.

To minimise fungal contamination during germination, the grain was subjected to a hot water treatment (i.e. immersed in 55°C water for a 10 minute period) (Berjak, Whittaker, Mycock, 1992; Erdey, Mycock and Berjak, In Press) prior to being used.

Methods

Germination Conditions:

Samples of hot-water-treated grains were set out immediately and germinated (in triplicate) for different lengths of time (0, 8, 16, 24, 32, 40, 48, 56 and 64 hours) under controlled conditions. The grains were germinated in Petri dishes (130 per 90 mm diameter dish) on two black filter paper discs (Whatman No. 29; 90 mm diameter) which had been evenly wetted with 4 ml distilled water, and maintained in an incubator set at 25°C and 100% relative humidity.

Analyses:

Germination: Upon sampling, the percentage of germinated grains (i.e. those grains that showed the protrusion of the root and/or shoot) was determined.

Amylase activity: The joint α- and ß-amylase activity g^{-1} (dry weight) of the samples was determined according to the method for diastatic power (DP) - refer to Chapter 2 for details.

Extraction, purification and HPLC separation of PGRs: Each grain (130 grains per sample) was separated into three parts [i.e. the embryo (including the scutellum and the roots and shoots), the proximal, and the distal endosperm (including the pericarp and the aleurone layer)]. (Financial constraints made it impossible to assay more than one pooled sample per PGR). The samples were freeze-dried and maintained at -70°C until required. The freeze-dried sorghum samples were finely ground with a mortar and pestle and extracted in 10 ml 90% methanol in water (containing 50 mg butylhydroxytoluene and 100 mg sodium ascorbate $litre^{-1}$). Extraction was done in the dark at 4°C for 16 hours. The extracted samples were centrifuged at 20,000 x g for 10 minutes. The supernatant was collected and reduced to dryness in a Savant vacuum concentrator. The dried extracted samples were stored at -4°C until required.

Each of the dried extracts was dissolved in 1 ml 90% HPLC-grade methanol. An internal standard (i.e. 100 µl ^{14}C-ABA and [^{3}H](diH)Z; 10,000 dpm each) was added to each of the samples (so as to determine recoveries), the samples were

passed through a 0.45 µm polytetrafluoroethylene disposable filter and the filtrate was injected onto the HPLC column. The PGRs were separated on a Waters gradient HPLC instrument fitted with a 10 x 250 mm Zorbax 5 µm semiprep. ODS column (Dupont, Wilmington, USA.) and a U6K variable volume injector. The column was eluted with a gradient of methanol in 0.1 M acetic acid (buffered to pH 3.5 with triethylamine) starting at 10% and changing to 50% methanol over 120 minutes at a flow rate of 1 ml min^{-1}. Immunohistograms were obtained from the series of two minute fractions collected from 20-80 minutes. Retention times of zeatin (Z), zeatin riboside (ZR), isopentyladenine (IPA), indole acetic acid (IAA), abscisic acid (ABA) and gibberellic acid (GA_1 + GA_3) were determined using authentic standards as described by Farrant *et al.* (1993). Fractions corresponding to the elution times of each of the PGRs were collected and dried in a Savant concentrator and used for the quantification of the PGR by radioimmunoassay (RIA).

Quantification of PGRs by RIA: The HPLC-separated, dried, samples were reconstituted in 2 ml 100% methanol, 100 µl aliquots pipetted into Greiner tubes, dried in a Savant concentrator and subjected to RIA according to verified protocols for each of the PGRs tested (Cutting *et al.*, 1983; Hofman, Featonby-Smith and van Staden, 1985; Cutting *et al.*, 1986; Cutting and Bower, 1989). Antibodies specific for each of the PGRs were raised, according to the verified protocols, in the laboratories of Dr. J.G.M. Cutting[1].

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[1] J.G.M. Cutting, Department of Horticulture, University of Natal, Pietermaritzburg, South Africa

Although the antibodies, antigen tracers and standard curves were specific for each type of the PGR, the RIA assay procedure was similar for each of the PGRs. The general protocol (done in triplicate) is detailed below.

A standard curve (ranging from 0.01 to 100 ng per 100 µl) was generated for each PGR. 100 µl aliquots of standard were pipetted into Greiner tubes and dried in a Savant concentrator.

The Greiner-tube-contained samples and standards were dissolved in 100 µl of methanol and treated as follows:

1 100 µl of dilute [^{3}H]-PGR (antigen-tracer) of known concentration and specific activity, and 100 µl of diluted antibody [in 0.1% bovine serum albumin (BSA) in 0.01 M phosphate buffered saline pH 7.2 (PBS)] were added.

2 500 µl BSA in PBS were added, mixed by vortexing and then incubated at 37°C for 30 minutes.

3 850 µl 90% ammonium sulphate was added, vortexed and protein precipitation allowed to occur for 30 minutes at room temperature.

4 The tubes were centrifuged at 4 000 g for 10 minutes and the supernatant discarded.

5 The precipitated protein pellet was washed with 1.5 ml 50% ammonium sulphate, vortexed and recentrifuged at 4 000 g for 10 minutes. The supernatant was discarded.

6 The washed pellet was dissolved in 250 µl distilled water.

7 2 ml of Picoflor 40 scintillant was added, the tubes capped, shaken, inserted into scintillation vials and counted in a Packard scintillation counter.

The amount of non-specific binding (NSB) was determined by replacing 100 µl of distilled water instead of antibody in step 1 above. The remainder of the protocol (steps 2-7) was the same.

The total tracer activity (Ta) was determined by adding 250 µl distilled water and 2 ml scintillant immediately into Greiner tubes in step 1 above. The tubes were capped, mixed and counted in the scintillation counter. A Ta of 10 000 cpm was used.

The PGRs were quantified using the standard curves generated. The Securia data reduction radioimmunoassay computer package (Packard Instrument Company, 1986 publication no. 169-3016) was used to analyse the raw data and correct for cross-reactivity and recoveries

RESULTS AND DISCUSSION

Germination and Amylase Activity

The first sign of germination occurred after 16 hours, when approximately 89% of the grains had germinated (Fig. 5.1). Germination increased to 96% over the remaining germination period. Virtually no amylase activity was detected in mature, non-germinated grains (Fig. 5.1). This is in agreement with the findings of other workers (Daiber and Novellie, 1968; review by Palmer, 1989). Indeed, little amylase activity was detectable in the grain up to 24 hours germination. Thereafter, the amylase activity of the grain increased more or less linearly up to 64 hours germination, supporting the observation that in sorghum, α- and ß-amylases develop during germination (Dufour, Mélotte and Srebrnik, 1992).

Cytokinins

Note that for the sake of clarity the quantities of Z and ZR present in the samples have been combined and will be referred to as a combined total. The concentration of IPA (Fig. 5.2) and Z+ZR (Fig. 5.3) was highest in the embryo and very much lower in both the proximal and distal endosperm tissues. In the mature sorghum grain the combined amount of the cytokinins IPA and Z+ZR (836 ng g^{-1} dry mass) was high compared with the 57 ng g^{-1} dry mass reported by Hocart *et al.* (1988) for maize. Both IPA and Z+ZR declined with germination time. There was a small peak in the concentration of Z+ZR and a relatively larger peak in IPA concentration at 24 hours, following the onset of the first visible sign of root protrusion and coinciding with the initiation of the more or less linear increase in amylase activity (Fig. 5.1). Upon further germination, the amounts of these PGRs

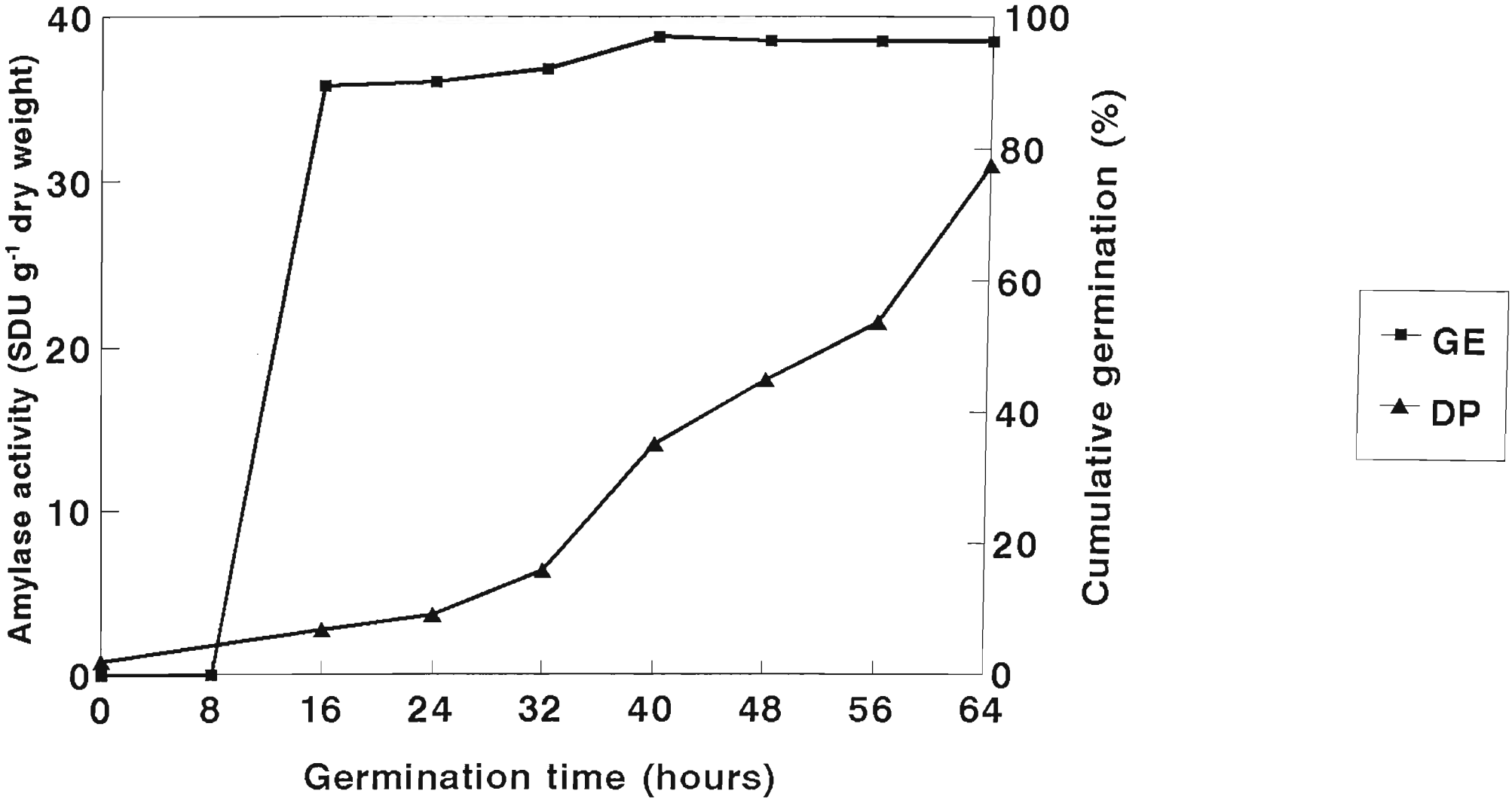

Fig. 5.1 Effect of germination time on the cumulative germination (GE) and the amylase activity (DP) of sorghum

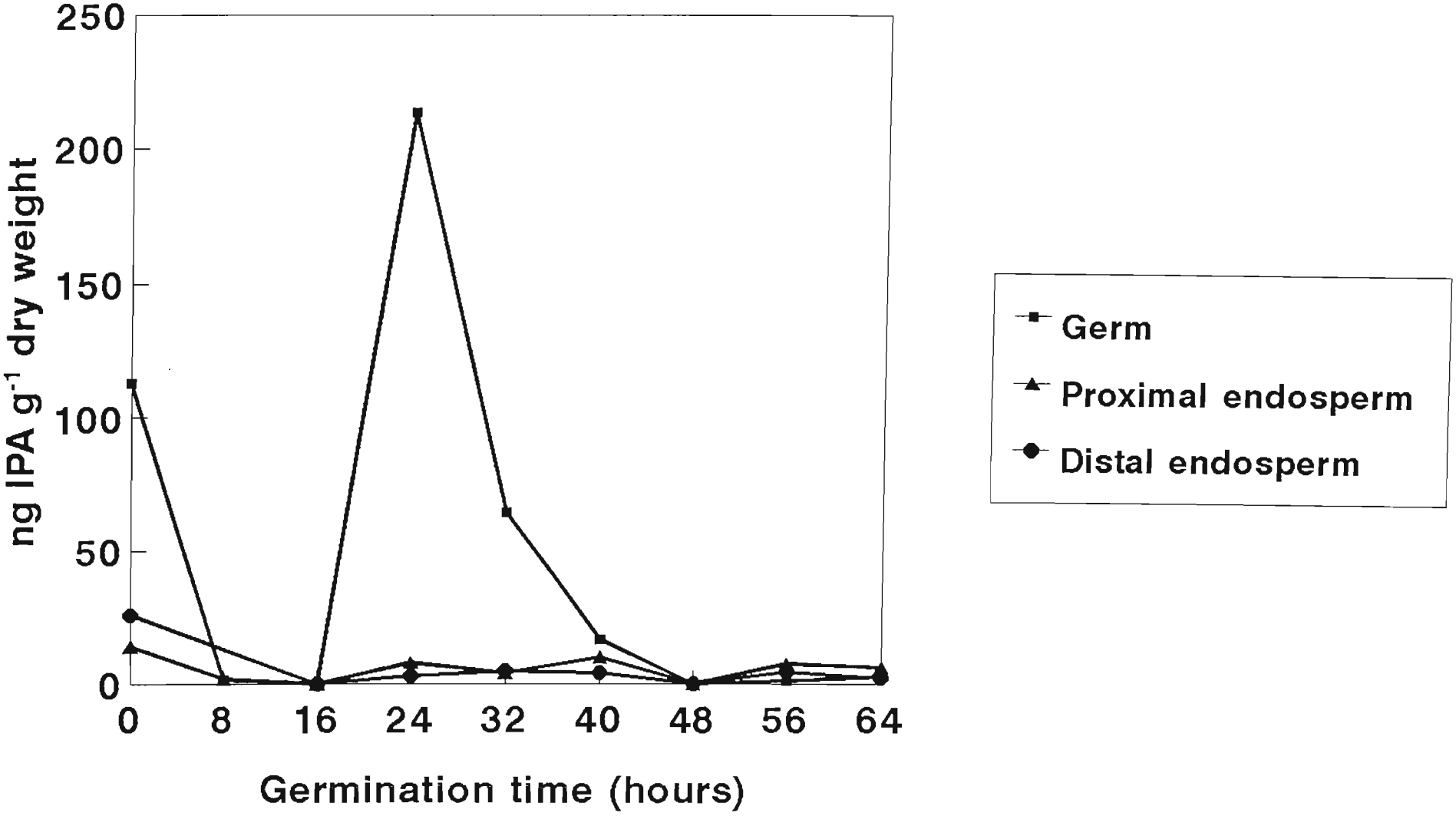

Fig. 5.2 Changes in the concentration of isopentyladenine (IPA) during germination in the grain parts of sorghum

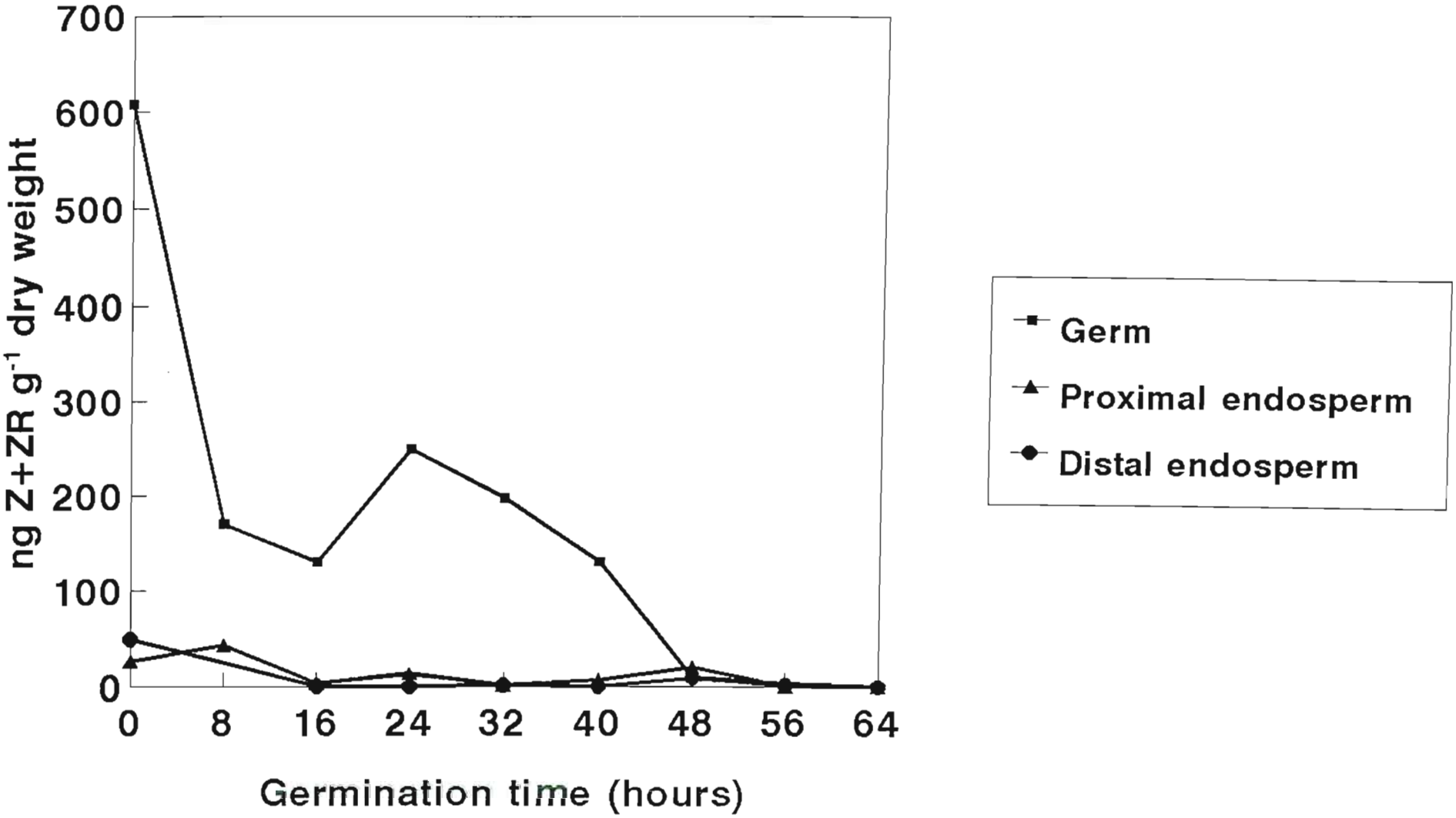

Fig. 5.3 Changes in the concentration of zeatin and zeatin riboside (Z+ZR) during germination in the grain parts of sorghum

declined again. At 24 hours germination, the embryo represented only 6.8% of the grain mass yet it contributed 76% and 77% of the IPA and Z+ZR amounts of the grain, respectively. However, it is probable that most of the cytokinins (and indeed, the other PGRs investigated in this study) in the combined endosperm portions might actually have been concentrated in the aleurone layer, and that the content in the endosperm proper, was negligible.

Cytokinins have been implicated in radicle growth and seedling establishment during germination (Hocart and Letham, 1990). The peaks in the amounts of IPA and Z+ZR following the onset of root protrusion suggests that these cytokinins may play a rôle in the cell division and elongation processes effecting root growth in sorghum. There have been reports that suggest that cytokinins are implicated in mobilisation of storage reserves for utilisation during germination (Fincher, 1989; Hocart, Letham and Parker, 1990). When expressed on a per-grain-component basis, there was a decline in cytokinin concentration in the endosperm and a subsequent increase in the embryo tissue at around the time of root protrusion in sorghum. This might aid in the formation of an embryo sink for nutrients and facilitate the polarised movement of reserve breakdown products from the endosperm to the embryo. It is possible that the cytokinins are re-distributed within the embryo to regions (e.g. the root meristematic region) where they effectively concentrate and direct root growth (see review by Letham, 1978). This would obviate the necessity for much overall increase in the levels of the cytokinins, IPA and Z+ZR. Future studies should determine whether the cytokinin content of the root tips differs markedly from the elongated parts of the roots.

Auxin

As was the case for the cytokinins, the concentration of IAA in the mature, non-germinated, sorghum grain was much higher in the embryo (343 ng g^{-1} dry mass) than in the proximal or distal endosperm (69 and 6 ng g^{-1} dry mass, respectively) (Fig. 5.4). The concentration of IAA in both the embryo and the endosperm declined dramatically upon the grain being set out to germinate. When sampled after eight hours (i.e. the first sampling point), before the first visible sign of root protrusion (Fig. 5.1), virtually no IAA was detected in the component parts of the sorghum grain (Fig. 5.4). Concentrations of IAA in mature seeds appear to vary among species from approximately 7 and 1,572 ng g^{-1} dry mass in the French bean and maize, respectively (Tillberg, 1977) to approximately 1,700 ng g^{-1} dry mass in rice (Bandurski and Schilze, 1977). The IAA concentration in sorghum appears somewhat low when compared with rice, which is similar to sorghum in that they are both tropical cereals. Most reports of IAA concentrations in mature orthodox seeds indicate that the amounts are low relative to earlier developmental stages (Cohen and Bandurski, 1982; Bialek and Cohen, 1989). Likewise, it is possible that the level of IAA in sorghum could have been higher during the developmental phase.

Gibberellic acid

Note that the quantities of GA_1 + GA_3 have been combined and will be discussed as one (i.e. GA_{1+3}). Gibberellic acid was detected in the embryo and the endosperm components of sorghum grain after eight hours germination and at several stages during germination (Table 5.1). However, compared with reports on other seed types, where the levels of endogenous GAs have been represented

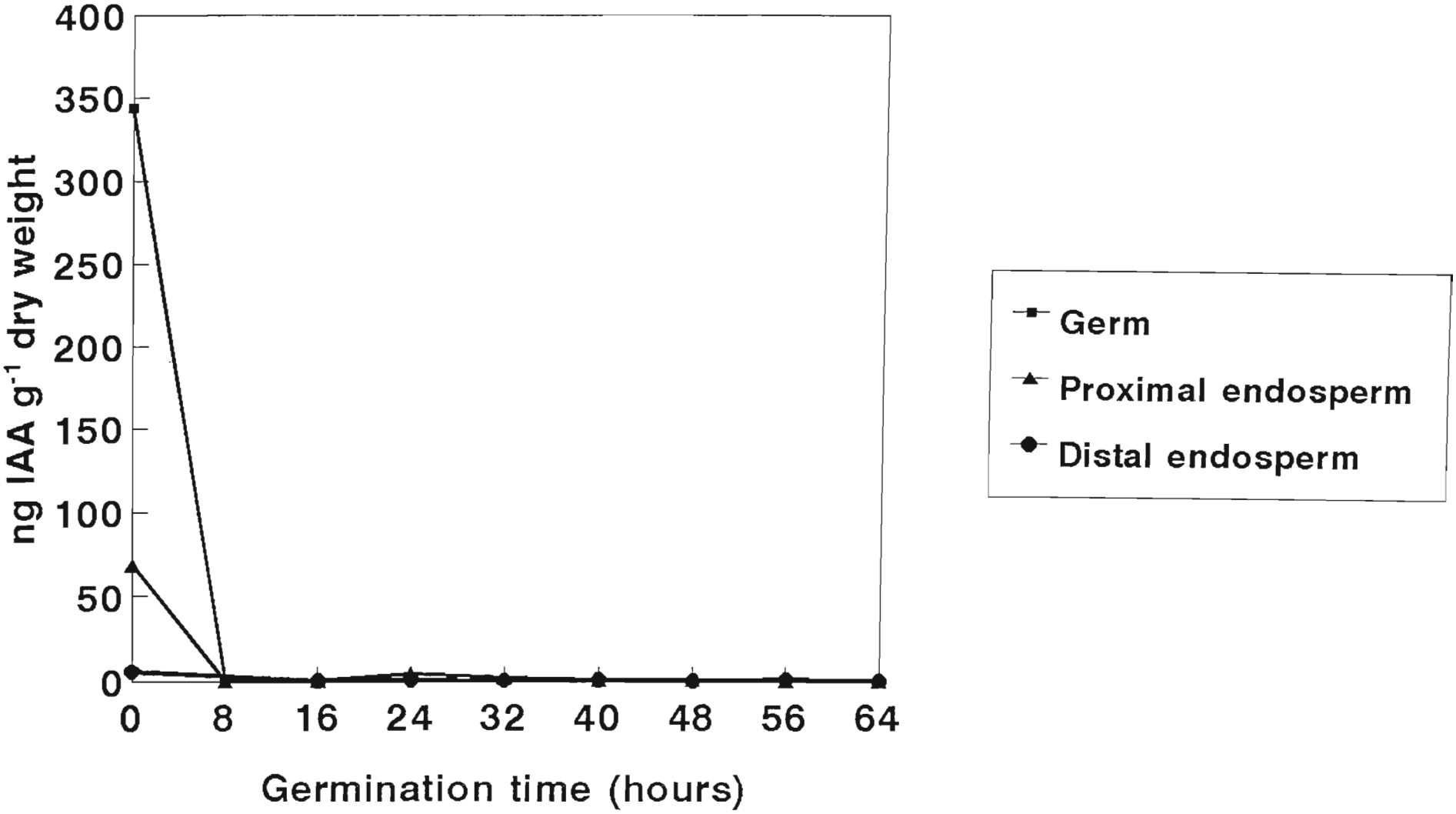

Fig. 5.4 Changes in the concentration of indole acetic acid (IAA) during germination in the grain parts of sorghum

Table 5.1 **Changes in the concentration of gibberellins$_{1+3}$ during germination in the grain parts of sorghum (cv. NK 283)[1]**

Germination time (hours)	Embryo	Endosperm	
		Proximal	Distal
0	-	-	-
8	-	4.2	N/A
16	18.3	2.8	-
24	-	22.2	1.2
32	-	3.4	1.5
40	4.4	-	-
48	7.8	N/A	3.4
56	1.0	-	-
64	-	-	-

[1] Results are for a single analysis per sample and are expressed as ng GA_{1+3} g^{-1} dry weight

\- Indicates below detection level

N/A No data available

on a mass basis, the GA_{1+3} concentrations detected in germinating sorghum (Table 5.1) appear to be relatively low. For example, a peak in GA-like activity of 500 $\mu g.g^{-1}$ fresh mass has been reported for pea seeds (Eeuwens and Schwabe, 1975) and Piagessi *et al.* (1989) have reported 100 $\mu g.g^{-1}$ fresh mass of bioactive $GA_{1+4+5+44}$ in suspensor cells of *Phaseolus coccineus*. Other forms of gibberellic acid, not presently assayed, may also occur in sorghum grains. However, the forms GA_1 and GA_3 are considered to be highly active in seeds (Pharis and King, 1985; Sponsel, 1987). It is possible that in germinating sorghum the gibberellins, GA_1 and GA_3, occur in predominantly conjugated forms as has been proposed by Rood (1995) for GA_8 in sorghum shoots. This may offer an explanation as to why free GA_{1+3} was detected only sporadically during sorghum germination.

In germinating barley, gibberellins are thought to be transported from the scutellum or embryonic axis to the aleurone where they induce synthesis of specific enzyme proteins (e.g. α-amylase) which are active during germination (MacLeod *et al.*, 1964; Palmer, 1982). Good evidence exists that the enzyme distribution in sorghum is not compatible with the concept of *de novo* α-amylase formation in the aleurone layer (Daiber and Novellie, 1968; see review by Palmer, 1989). Those authors postulated that in sorghum, amylases are synthesised in the embryo and then diffuse to the endosperm, unlike barley where the aleurone is predominantly responsible for α-amylase production. Although further work is clearly required, it is possible that the endogenous GA_{1+3} peaks detected in germinating sorghum, first in the embryo at 16 hours and later in the proximal endosperm at 24 hours (Table 5.1) may be related to the marked increase in amylase activity detected from 24 hours onwards (Fig. 5.1); thereby indicating that gibberellins may play a

rôle in the production of amylase enzymes during sorghum germination.

Abscisic acid

The amount of ABA in the embryo tissue of the mature, non-germinated, sorghum grain was relatively high (1,399 ng g^{-1} dry mass) compared with the proximal and distal endosperm tissues (219 and 79 ng g^{-1} dry mass, respectively) (Fig. 5.5). Indeed, even although relatively small in proportion to the whole grain (8.1%), the embryo contributed a high proportion of the ABA content of the whole sorghum grain at maturity (38%). Although the values for ABA content on 8 and 16 hours germination were not available, during the first 32 hours of germination the concentration of ABA in the embryo declined markedly, approaching levels comparable to those of the endosperm parts. This decline in the embryo ABA to apparently negligible amounts, coincided with a marked increased in amylase activity following the onset of germination (Fig. 5.1).

The ABA content of seeds is usually highest during development and low or even absent at maturity (Black, 1983). Although the embryo ABA concentration of mature sorghum grain reported in this study appears relatively high, it is similar to embryo ABA levels reported by Steinbach *et al.* (1995) for developing caryopses of sorghum. Physiologically mature grain from one season was reported to have an embryonic ABA content of more than 600 ng g^{-1} dry mass and more than 1,200 ng g^{-1} dry mass in a second season. ABA prevents precocious germination (Bewley and Black, 1985; Quatrano, 1986; Kermode, 1990). Studies of developing rape seeds have shown that *in planta* the embryos are held in embryogenic growth

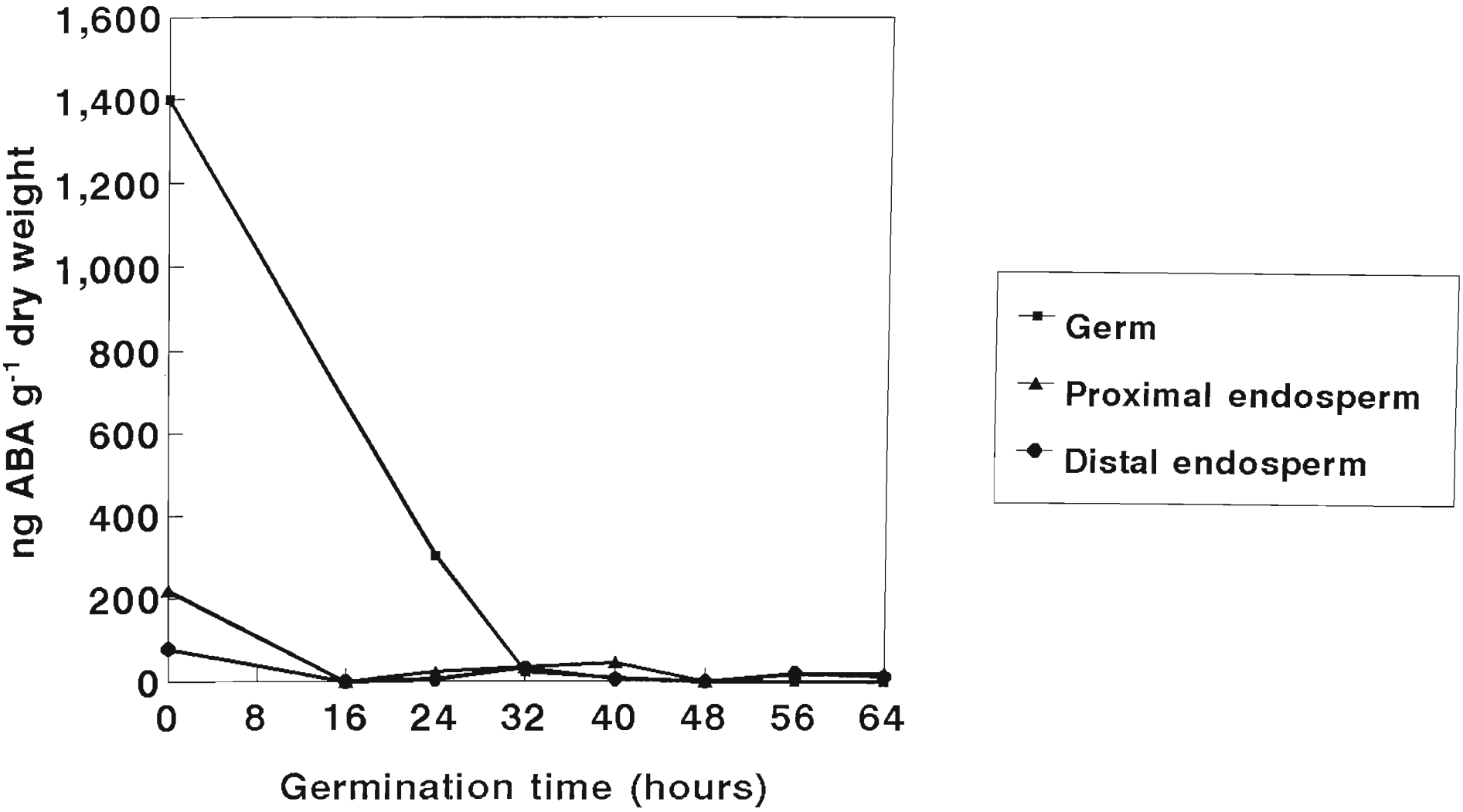

Fig. 5.5 Changes in the concentration of abscisic acid (ABA) during germination in the grain parts of sorghum

by the native ABA. However, when isolated they lose the ABA and germinative growth occurs (see review by Black, 1991). Similarly, for developing caryopses of sorghum, ABA has been shown to inhibit embryo germination (Steinbach *et al.*, 1995) and when ABA synthesis is inhibited by fluridone, precocious germination occurs (Steinbach *et al.*, 1997). The extent of inhibition, however, appears to be related to the embryo sensitivity to ABA (Steinbach *et al.*, 1995).

Although a high concentration of ABA in the embryo tissue of mature sorghum grains does not completely inhibit germination (i.e. impose dormancy) it may constitute an endogenous mechanism that will delay germination until the ABA level has declined significantly. Similarly, studies of several seed species (rape, soybean and dwarf French bean), have shown that the lag phase prior to axial extension is related to the native concentration of ABA at the time the seed is removed from the parent plant; the higher the initial ABA concentration the longer it takes in culture before germination is initiated (see review by Black, 1991). If the lag period prior to germination in sorghum is likewise related to the ABA content, malting quality could perhaps be enhanced by reducing the concentration of endogenous ABA at maturity. Steinbach *et al.* (1995) have suggested, from their study on immature caryopses of sorghum, that differences in pre-harvest germinative behaviour may not be related simply to the endogenous ABA concentration but rather to the rate at which the concentration of ABA is decreased. For example, embryo ABA decreased faster and germination was initiated sooner in a pre-harvest sprouting-susceptible sorghum variety than in more resistant varieties.

The production of hydrolytic enzymes, such as the amylases, is one of the main objectives of malting. Evidence exists that ABA is involved in the suppression of α-amylase activity in developing seeds of some species, e.g. triticale (King, *et al.*, 1979) and wheat (King, 1976; 1982; Napier, Chapman and Black, 1989), presumably by arresting enzyme synthesis. Oxygenated metabolites of ABA, 7'-hydroxy-ABA and phaseic acid, have also been shown to be effective in suppressing α-amylase activity (Nolan and Ho, 1988; Hill *et al.*, 1992; Hill *et al.*, 1995; Todoroki, Hirai and Koshimizu, 1995; Walker-Simmons *et al.*, 1997). It would appear that constraints, one of which is thought to be ABA (Garcia-Maya, Chapman and Black, 1990), operate on the embryo *in planta* to suppress amylase production. The present evidence suggests that ABA plays a similar rôle in sorghum, taking into account the high concentration of ABA in the embryo tissue (Fig. 5.5) and the absence of amylase activity in the mature, non-germinated sorghum grain (Fig. 5.1). Thus the sooner the endogenous ABA content is reduced, the faster germination would occur (Steinbach *et al.*, 1995) and the greater the potential would be to produce the required malt hydrolytic enzymes.

It has been proposed that during germination, cytokinins and gibberellins may play a rôle in countering the effect of germination inhibitors (Khan, 1975). It is thus possibly of significance that the increase in the amounts of the cytokinins IPA and Z+ZR (Figs 5.2 and 5.3) and GA_{1+3} (Table 5.1) coincided with the decrease in the concentration of ABA (Fig. 5.5) and the concomitant increase in amylase activity (Fig. 5.1).

CONCLUSIONS

Although in this study a direct causal relationship between germination, amylase activity and PGRs was not investigated, from the data it is suggested that germination in sorghum is under embryo control, as the concentration of the PGRs in the mature grain (with the exception of GA_{1+3}) is much higher in the relatively small embryo tissue compared with the larger mass of endosperm tissue, including the aleurone. Further, it appears that the high concentration of ABA at maturity, and the interaction of ABA and the cytokinins, IPA and Z+ZR, and possibly GA_{1+3}, may play a significant rôle in amylase production during sorghum germination. This possible causal relationship requires further investigation. In view of the financial constraints at the time, that curtailed the RIA assays to one pooled sample per PGR, and the unfortunate loss of ABA data for 8 and 16 hours germination, these assays ideally, need to be repeated. However, the trends presently reported, are borne out by the work described in Chapter 6.

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CHAPTER 6 EFFECT OF THE APPLICATION TIME OF GIBBERELLIC ACID ON THE DIASTATIC POWER AND FREE AMINO NITROGEN CONTENT OF SORGHUM MALT

The material embodied in this Chapter is to remain confidential until January 2000.

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ABSTRACT

Sorghum malt is widely used in southern Africa to brew traditional sorghum (opaque) beer. There is also interest in utilising sorghum malt in the brewing of clear lager-type beers. A problem with malt is that it is low in diastatic power (amylase activity), but unlike the situation with barley, application of exogenous gibberellic acid to improve malting quality has produced anomalous results. The effect on malt quality [*viz.* diastatic power (amylase activity) and free amino nitrogen] of administering gibberellic acid to sorghum grain of two different cultivars at various stages throughout malting, was investigated. The diastatic power and the free amino nitrogen content of the resulting malts was enhanced by the gibberellic acid treatment. The timing of the treatment, however, was found to be critical. Although there was some differences between the two cultivars, generally, no significant improvement was observed unless the treatment was administered towards the end of a 24 hour steep or on the subsequent day of malting, suggesting that an endogenous inhibitor may first have to decline before sorghum can respond to the stimulatory effect of gibberellic acid. The present finding is of importance to the sorghum malting industry, as it may be possible to

enhance the quality of malt obtained from a particular sorghum or reduce the time require to obtain a specific quality.

Abbreviations: ABA=abscisic acid; DP=diastatic power; FAN=free amino nitrogen; GA_3=gibberellic acid; PGR=plant growth regulator; SDU=sorghum diastatic units.

INTRODUCTION

During malting (i.e. the germination of cereal grain in moist air under controlled conditions) a number of hydrolytic enzymes develop and degrade the reserve nutrients of the endosperm (Bewley and Black, 1978). The development of the enzymes α- and ß-amylase during malting are of critical importance as they initiate the breakdown of starch granules during germination and further, are required to hydrolyse starch to fermentable sugars during the beer brewing process.

Gibberellins (GAs), a group of plant hormones involved in plant growth and development, are defined by their chemical structure rather than biological activity (Sponsel, 1987). They are diterpenoids, composed of four isoprene units, usually arranged to form three rings, with an additional lactone bridge (Khan, 1982). The functions of many of the different GAs, at least 72 having been chemically characterised, appear to be largely unknown with many having little to no biological activity (Khan, 1982). A large proportion of the characterised GAs have been found to occur in seeds (Khan, 1982; Sponsel, 1987). One of these, namely gibberellic acid (GA_3), has been shown to induce barley aleurone cells to produce several endosperm-degrading enzymes (Paleg, 1960; MacLeod, Duffus and Johnston, 1964; Chrispeels and Varner, 1997). Gibberellic acid-treated barely aleurone cells have been found to contain elevated levels of α-amylase mRNA, apparently largely through a stimulation of α-amylase gene transcription (Jacobsen and Beach, 1985; Zwar and Hooley, 1986). The barley malting industry (economically the most important malting industry) has taken advantage

of the knowledge that application of the plant growth regulator (PGR) GA_3 during germination, can dramatically enhance the synthesis of the critically important hydrolytic enzyme, α-amylase (Palmer, 1974).

In Africa, malt made from sorghum (*Sorghum bicolor* (L.) Moench) is used for the brewing of the traditional opaque-type beers (Schwartz, 1956; Novellie, 1968; 1977; Novellie and de Schaepdrijver, 1986; Haggblade and Holzapfel, 1989; Taylor, 1989; Daiber and Taylor, 1995). Barley is the traditional material of choice for producing clear lager-type beers (Narziss, 1976). Barley, however, is suited to temperate conditions and does not grow well under tropical and subtropical climatic conditions. In Africa, the possibility of substituting sorghum malt for barley malt for the brewing of "clear" lager-type beers, therefore, has received some attention (Skinner, 1976; Okafor and Aniche, 1980; Ajerio, Booer and Proudlove, 1993; Dufour, Mélotte and Srebrnik, 1992).

Compared with barley, sorghum is indigenous to, and grows well in, the semi-arid tropics. Indeed, it produces a crop under conditions where other crops fail. Sorghum malt, however, has low diastatic power (amylase activity) (Novellie, 1960: Jayatissa, Pathirana and Sivayogasunderam, 1980; Aniche and Palmer, 1990) and worts produced using sorghum malt generally have lower fermentability (and extract) compared with barley malt. Unlike the situation with barley, the evidence is inconsistent as to whether the application of GA_3 to germinating sorghum grain increases amylase activity. There have been several reports that GA_3 does not stimulate amylase activity in sorghum (Daiber and Novellie, 1968; Palmer and Bathgate, 1976; Aisien and Palmer, 1983; Aisien, Palmer and Stark,

1983). However, there have been some recent reports that application of GA_3 can be used to improve the amylase activity of sorghum malt (Agu *et al.*, 1993; Nzelibe and Nwashike, 1995). The stimulatory effect, however, appears to be variety-dependent. As an example of an extreme case, Nzelibe and Nwashike (1995) reported that in some cases, application of GA_3 actually reduced amylase activity. Current sorghum malting practices, therefore, have had to rely almost entirely on supplying the germinating sorghum grain with the most suitable environmental conditions (i.e. time, temperature, moisture and aeration) to initiate germination and promote the development of the essential malt hydrolytic enzymes [Morrall *et al.*, 1986; Dewar, Taylor and Berjak, 1997a (Chapter 2); 1997b (Chapter 3)].

To elucidate the possible control mechanisms involved in sorghum germination, a combined HPLC-radioimmunoassay (RIA) technique was used to determine the levels of selected endogenous plant growth regulators in sorghum at various different stages of germination (Dewar, Taylor and Berjak, 1998; Chapter 5). Levels of the gibberellins$_{1+3}$ were low throughout germination and did not appear to be related to the germination time. Interestingly, a relatively high level of abscisic acid (ABA) was detected in the embryo tissue prior to germination. As ABA is thought to be an inhibitor of precocious germination (Bewley and Black, 1985; Quatrano, 1986; Kermode, 1990), it was suggested that the high ABA level may have to be overcome before the application of an exogenous PGR would have a stimulatory effect on germination and sorghum malt quality (Dewar *et al.*, 1998; Chapter 5).

The present study was undertaken to determine whether the effect of applying GA_3 to germinating sorghum, in terms of diastatic power (amylase activity) and free amino nitrogen content and therefore malt quality, could be dependent upon the time of application.

MATERIALS AND METHODS

Phase ONE

Materials

Grain:

Sorghum grain of cultivar NK 283, which is widely grown in South Africa and recommended for malting purposes, was used. This grain germinated at better than 95%.

Gibberellic acid:

Gibberellic acid at a 900 U mg^{-1} (min) was supplied by Enzymes SA (Pty) Limited, Marlboro, South Africa and used in both Phases ONE and TWO.

Methods

Prewash:

The grain was washed by agitating it for five minutes in running tap water to remove the dust, loose glumes and light grains. Excess water was removed by spinning the grain in a domestic spin drier (AEG type SD 452BN) for one minute at 300 x g.

Treatment:

One hundred grams of pre-washed grain (see above) per sample was placed into nylon mesh bags (180 x 250 mm) and steeped in a still solution of GA_3 (20 mg l^{-1}; prepared immediately before use) for the final two hours of a 24-hour steep (the first 22 hours being in continuously changing 25°C tap water). Alternatively, GA_3 was applied on one of the subsequent days during the germination period (i.e. on day 2, 3, 4 or 5, counted from the beginning of steeping). After steeping, the grain was spin-dried (one minute at 300 g) and placed in a cabinet set at 25°C and 99% relative humidity. The grain was wetted twice daily by steeping it for 15 minutes in either tap water or GA_3 (20 mg l^{-1}). After the wetting period, the grain was spin-dried (one minute at 300 x g) and returned to the cabinet. Germination was stopped after a total of six days from the beginning of steep by drying the malt in a force-draught oven set at 50°C.

Grain that had been steeped and wetted in water only throughout the steeping and germination periods (i.e. not treated with GA_3) served as a control.

Phase TWO

Materials

***Grain*:**

Sorghum grain of cultivars Barnard Red and NK 283, which are widely grown in South Africa and recommended for malting purposes, were used. Note that cultivar NK 283 was obtained from an industrial maltster and was not from the same batch as used in Phase ONE above.

Methods

Prewash:

As for Phase ONE above.

Treatment:

Gibberellic acid (20 ppm; prepared immediately before use) was applied to sorghum grain at different times during malting. That is, at one of four times during a 24 hour steeping period (*viz.* 0-2, 4-6, 18-20, and 22-24 h) or on one of the subsequent days during the germination period (*viz.* day 2, 3, 4 and 5, counted from the beginning of steeping). Fifty grams of pre-washed grain per sample was placed into nylon mesh bags (180 x 250 mm) and steeped and germinated as described in Phase ONE, above. After the treatment (when administered during

steeping), the grain was removed from the GA_3 solution, rinsed thoroughly in tap water and returned to the tank for the remainder of the steeping period.

In view of the suggestion that sorghum grain/malt moisture content during malting may affect the quality of the resulting malt (Dewar *et al.*, 1997a, b; Chapters 2 and 3, respectively), the grain was not spin-dried (as was done in Phase ONE and in the work described in Chapters 2, 3 and 4) at the end of the 24 hour steeping period or after each of the wettings. Instead, the grain was placed on absorbent paper towelling for 10 minutes and allowed to drain before being placed in the cabinet. To determine the effect of not spin-drying the grain to remove the surface-held water, and to ensure that any beneficial effect of the GA_3 treatment was not simply a result of enhancing the moisture content of the grain, a second set of control samples (i.e. those that did not receive the GA_3 treatment and was steeped and wetted with water only) were malted. At the end of the steep and after each of the wettings during the germination period, the surface-held water, from this set of controls, was removed by spin-drying the grain (as was done in Phase ONE above). The malting trial was done in duplicate.

Note: When applied during the steeping period (for both Phases ONE and TWO), the GA_3 treatment time was 120 minutes and when applied during the germination period the treatment time was two 15 minute (i.e. a total of only 30 minutes) application time

Analyses:

Diastatic Power (DP): Malt DP g^{-1} dry weight was determined - refer to Chapter 2 for details.

Free amino nitrogen (FAN): The FAN content of the malt was determined and expressed as mg 100 g^{-1} dry weight - refer to Chapter 2.

Water content: Water content was determined as described in Chapter 2 and expressed on a fresh weight basis.

Water uptake: The water uptake of the grain during steeping and malting was monitored and expressed as a percentage of the pre-steep weight.

Statistical analysis: The effect of GA_3 application time on the diastatic power and free amino nitrogen content of the malt was analysed by one-way analysis of variance. Least square difference at a 95% confidence level was used to compare individual levels.

RESULTS AND DISCUSSION

Application of GA_3 to germinating sorghum enhanced the DP of the malt only when it was administered either late during steeping (cv. NK 283; Figs 6.1 and 6.2) or on or about the second day of malting (cv. NK 283; Figs 6.1 and 6.2, and

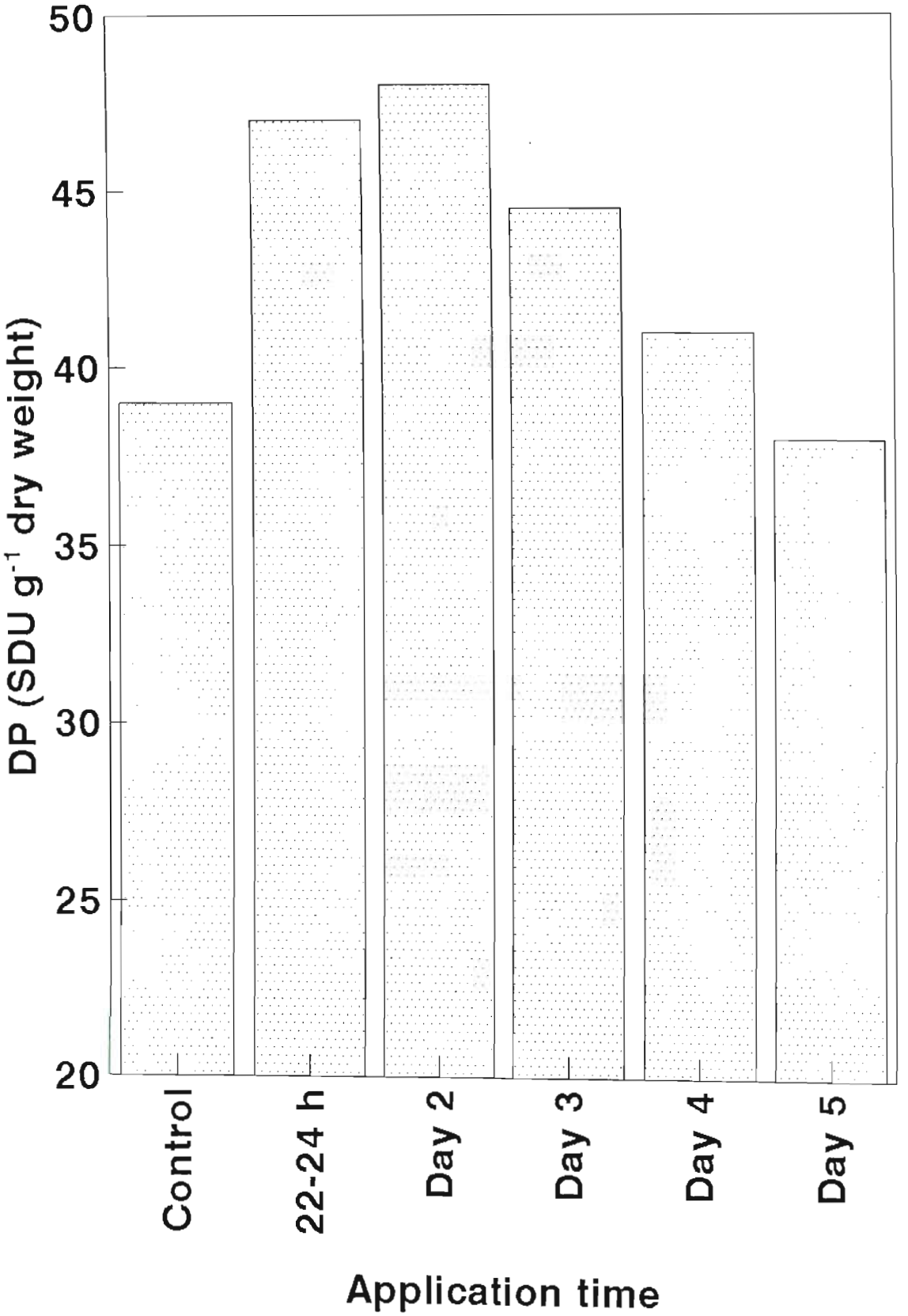

Fig. 6.1 Effect of GA_3 application time on the diastatic power of sorghum malt (cv. NK 283) (Phase ONE)

Error bars: 1 standard deviation from the mean

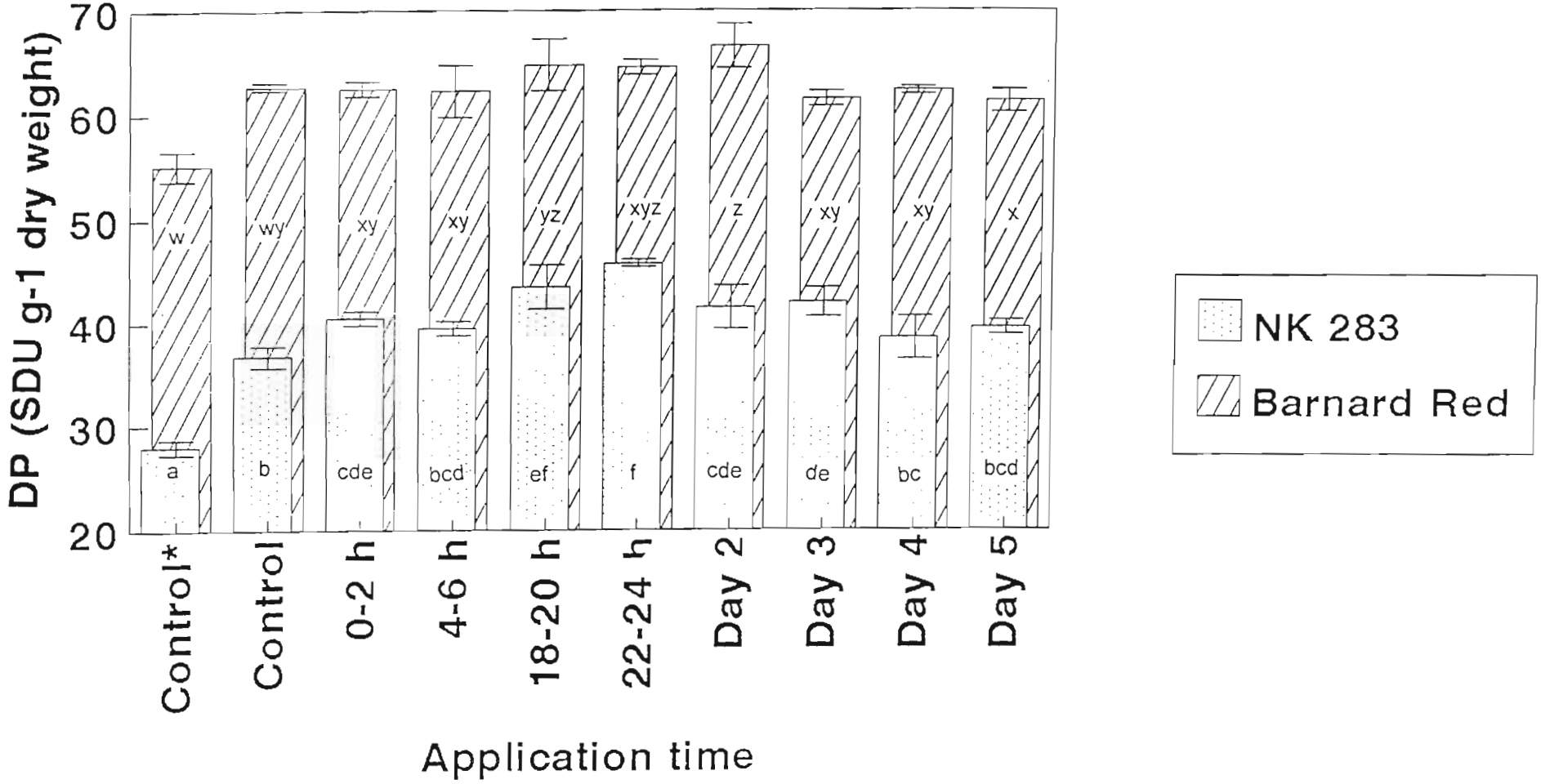

Fig. 6.2 Effect of GA_3 application time on the diastatic power of sorghum malt (Phase TWO)

* spin-dried

dissimilar letters denote a significant difference

cv. Barnard Red; Fig. 6.2). For cultivar NK 283 (Phase ONE), the greatest enhancement in malt DP occurred when GA_3 was administered on the second day where a DP of 48 SDU g^{-1} was obtained compared with 39 SDU g^{-1} for the control (i.e. a 23% enhancement) (Fig. 6.1). In Phase TWO, where the effect of administering GA_3 at one of four different times during a 24 hour steeping period was investigated, it was found that for cultivar NK 283, the greatest increase in malt DP occurred when the two hour treatment was administered during the last six hours (i.e. 18-20 or 22-24 hours) (Fig. 6.2). For cultivar Barnard Red (Phase TWO), GA_3 treatment during steeping did not have a statistically significant effect (Fig. 6.2). It did, however, have a significant effect when applied during the second day of malting as was the case for cultivar NK 283 (Phases ONE and TWO; Figs 6.1 and 6.2, respectively). In all cases, the enhancement in malt DP occasioned when the treatment was administered on the second day of malting is rather surprising when it is realised that the total application time of the treatment was only 30 minutes (two fifteen minute periods) as opposed to the 120 minutes which was the application time when the treatment was administered during the steeping period.

Irrespective of whether or not the surface-held moisture was removed from the germinating grain by spin-drying (Phase ONE; Fig. 6.1) or not (Phase TWO; Fig. 6.2), the grains showed a similar trend in that they responded to GA_3 only when it was administered during a certain critical time. As mentioned in the Materials and Methods section, the NK 283 grain used in Phases ONE and TWO were not from the same batch. Indeed, a comparison of the DP of the spin-dried

control grains shows that the malting quality of the NK 283 grain used in Phase TWO (Fig. 6.2) was not nearly as good as the one used in Phase ONE (Fig. 6.1). The data, therefore, cannot be compared directly. Some indication of the effect of allowing the grain to take up additional water during malting, however, can be obtained by expressing the DP results from Phase TWO as a percentage increase over that of the control sample that was spin-dried. By not spin-drying the grain, the DP of the malt was increased by 14% for Barnard Red and 31% for NK 283. It is unlikely that the reduced malting quality of the spin-dried grain is related to hydrostatic pressure as it has been shown that exposing sorghum grain to as much as 500 kPa of hydrostatic pressure for a period of five minutes at the end of steep, has no adverse affect on the malting quality of the grain (Dewar, Joustra, and Taylor, 1995). Rather, it is suggested that the increase in malt quality of the control samples that were not spin-dried during malting, is related to the increased moisture content of these grains during malting (Fig. 6.3a and 6.3b). In previous studies conducted in our laboratories, it was shown that in general the higher the moisture content of sorghum grain at the end of steeping (Dewar, *et al.*, 1997a; Chapter 2) and at the end of malting itself (Dewar *et al.*, 1997b; Chapter 3), the higher the quality of the resulting malt.

It would appear, however, that the enhancement in malt DP by the GA_3-treated grain is not simply related to an increase in grain moisture content. The moisture uptake of the GA_3-treated grains that were not exposed to spin-drying did not differ significantly from their non-GA_3-treated controls (Fig. 6.3a and 6.3b). In Phase TWO, for cultivar NK 283, the DP of the malt was increased by as much as 63%

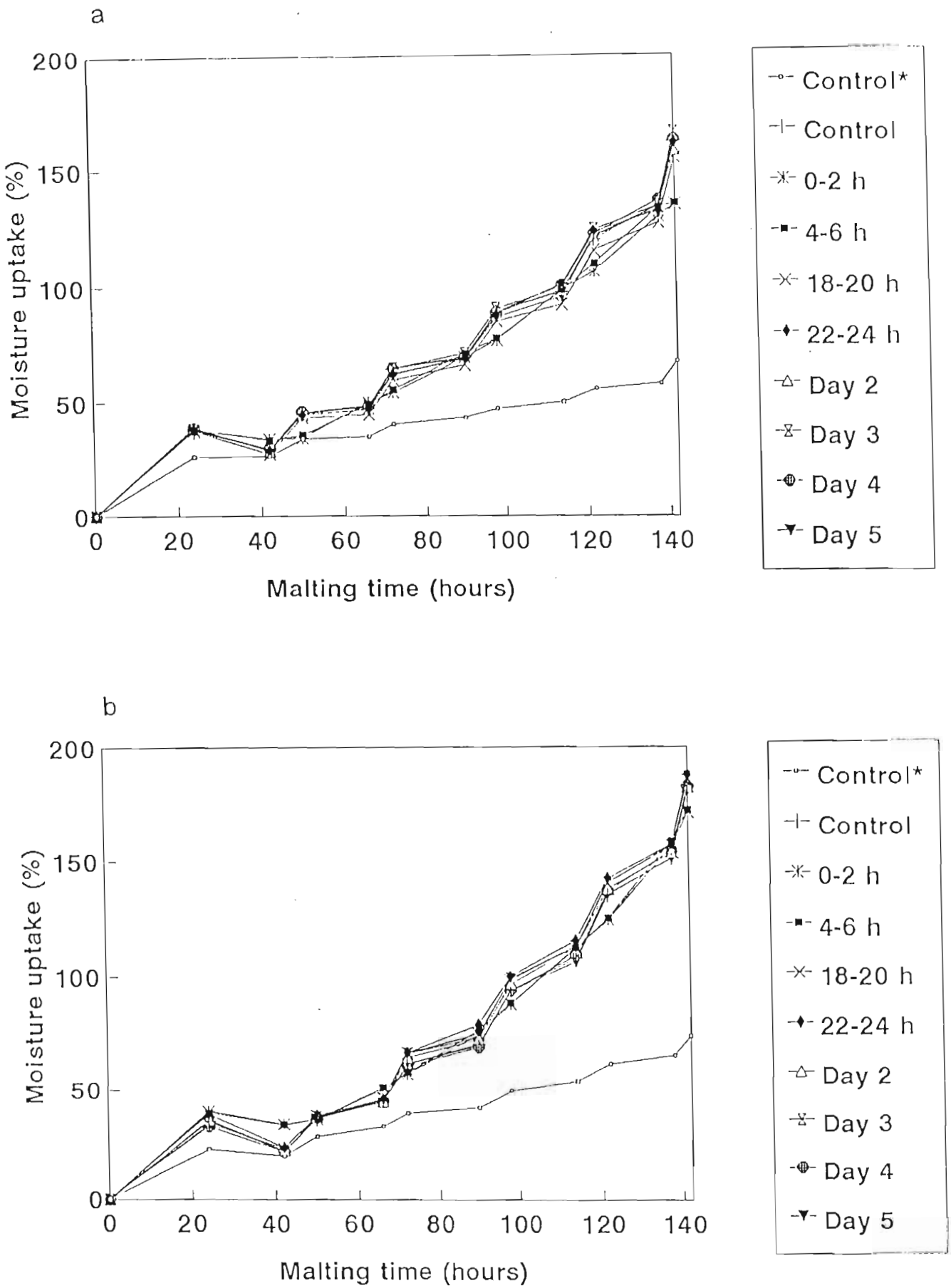

Fig. 6.3 Effect of GA_3 application time and spin-drying on moisture uptake during sorghum malting a) cv. NK 283 and b) cv. Barnard Red

* spin-dried

the GA_3 treatment had a significant effect on malt FAN. For NK 283 the increase in FAN was particularly evident when GA_3 was administered during the last two hours of the steep or on the second or third day of malting (Fig. 6.4). For cultivar Barnard Red, analysis of variance indicated that the FAN content was significantly enhanced only when GA_3 was administered on the second day of malting (Phase TWO) (Fig. 6.4).

Evans and Taylor (1990) showed that proteinase activity is present in ungerminated sorghum grain and that the level of activity increases moderately during germination, whereas carboxypeptidase activity increases greatly. In barley, synthesis of the proteinase enzymes is thought to be controlled by GA_3 (Jacobsen and Varner, 1967) and to be increased substantially when GA_3 is administered to the germinating grain (Baxter, Booer and Wainwright, 1978; Burger and Schroeder, 1976). For sorghum, it has been reported that GA_3 does not appear to be involved in the synthesis of protease enzymes during germination (Koehler, 1981) and Nzelibe and Nwashike (1995) found GA_3 to actually inhibit the proteolytic activities of sorghum. However, from the present assessments of the FAN status, it would appear that GA_3 application, at the appropriate time, in addition to simulating the synthesis of amylase enzymes, can stimulate the synthesis of protease enzymes during sorghum germination, as occurs in barley (Jacobsen and Varner, 1967).

Allowing the grain to take up additional moisture during malting (i.e. not spin-drying the grain to remove excess surface-held water), as well as enhancing the

finished malt DP, also enhanced the malt FAN. Grain that was not spin-dried and did not receive treatment with GA_3, produced malt that was approximately 37% for cultivar NK 283 and 24% for cultivar Barnard Red richer in FAN (Fig. 6.4). When GA_3 was administered on the second day of malting for a total application time of 30 minutes, to grains that were not spin-dried during malting, the final malt FAN was enhanced by as much as 64% for grains of cultivar NK 283 and 41% for Barnard Red. At least for these two cultivars it would appear that the application of GA_3 to germinating sorghum preferentially enhanced the malting quality (in terms of both DP and FAN) of the poorer malting quality cultivar (*viz.* NK 283).

On further testing, if the application of GA_3 during malting is found not only to enhance the malting quality of sorghum, but also to enhance the quality of inherently poor grain preferentially, this would be of great importance to the sorghum malting industry. Not only would it be possible to raise the quality of sorghum malt produced and reduce the time required to produce malt of a specified quality, it may be possible to use sorghum grain that has previously been considered unsuitable for malting purposes and produce malt of an acceptable quality.

Further work needs to be done to optimise the process for industrial application. The 20 mg l^{-1} GA_3 used in this investigation was considerably higher than the dosage used by other workers [i.e. 2 ppm (2 mg l^{-1}) GA_3; Daiber and Novellie, 1968 ; 0.20 mg l^{-1} GA_3; Agu *et al.*, 1993; 1 ppm (1 mg l^{-1}) GA_3; Nzelibe and Nwasike, 1995]. It is possible that the 20 mg l^{-1} GA_3 dosage used in the present

study (i.e. a 100 fold higher concentration than found optimal by Agu *et al.*, 1993) may be too high to observe the most significant and cost-effective outcome on sorghum malt quality. In addition to optimising the GA_3 dosage, future research could investigate the effect of administering a cytokinin-based PGR to germinating sorghum grain. Dewar *et al.* (1998; Chapter 5) indicated that cytokinins may be involved in sorghum germination. The decline in embryo ABA to its lowest level (prior to a linear increase in amylase activity) was accompanied by a peak in the concentration of measured cytokinins (*viz.* isopentenyladenine and the combined concentration of zeatin and zeatin riboside) in the embryo. Preliminary studies conducted in our laboratories (unpublished data) have indicated that application of the cytokinin, 6-benzylaminopurine, together with GA_3, can improve sorghum malt quality, in terms of DP and FAN, over and above the enhancement occasioned when GA_3 is administered alone. As with the findings of the present study, the timing of the treatment was found to be important.

CONCLUSIONS

Application of GA_3 to malting sorghum enhances the quality of the malt produced in terms of DP and FAN. The timing of the GA_3 treatment is, however, important. No significant improvement in malt quality occurs until the treatment is administered towards the end of steep or on the second or third day of malting, indicating that in sorghum, the amylase and the protease enzymes are not immediately inducible by GA_3 and may require the removal of an inhibitor. No

increase occurs when GA_3 is administered after approximately two or three days of malting. There is some evidence that GA_3 treatment may preferentially enhance the malting quality of relatively poor malting sorghum.

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CHAPTER 7 DISCUSSION

CHAPTER 7 DISCUSSION

The present study examines the parameters affecting the production of sorghum malt, in order to optimise its quality for commercial brewing. These parameters include a variety of exogenous and endogenous factors operative in the grain during both the steeping (imbibition) and germination periods of malting. Coincidentally, the aims of the work involve gaining a better understanding of the germination biology of this important African cereal, particularly i.e. in relation to what is known about the temperate grain, barley, which is crucial to the clear, lager-type brewing industry.

With regard to the effect of steeping time on malt quality (Chapter 2), the present findings contradict an early publication (Novellie, 1962) which reported that steeping time has little influence on the final amylase activity, i.e. diastatic power (DP), of sorghum malt. Indeed, not only did steeping time have a significant effect on malt DP, but the quality of the malt in terms of free amino nitrogen (FAN) and hot water extract (HWE), were significantly affected. In all cases the quality of the malt increased with increasing steeping time over the range studied (16, 24 and 40 hours). The present findings agree with those of Pathirana, Shivayogasundaram and Jayatissa (1983) who found, for a sorghum variety established previously to be suitable for malting (Jayatissa, Pathirina and Sivayogasundaram, 1980), that when using a steeping temperature of 30°C, the quality of the resulting malt was significantly affected by the steeping time employed. Improved cold and hot water extracts and DP values, both indications of malt quality, were obtained in those samples steeped for longer periods (i.e. 18

or 32 hours as opposed to 8 hours). Indeed, Pathirana *et al.* (1983) suggested that by the correct use of steeping period, it may actually be possible to reduce the germination time required to produce malt of a stipulated quality. Although under the conditions of the present study (Chapter 2), malt quality was assessed only at the end of a total of six days of malting (inclusive of steeping time), the effect of steeping time was found to be highly significant. In the investigation of Pathirana *et al.* (1983), however, in which the effect of steeping times were assessed as germination progressed (i.e. after 2, 3, 4, 5 and 6 days), it was observed that with increasing germination time, the early differences in DP occasioned by the different steeping times became reduced. It is possible that because Novellie (1962), assessed the effect of steeping time generally after seven days of germination (after a steep of 8-18 hours), the early effect of steeping time on sorghum malt quality had become insignificant. This, therefore, may offer an explanation as to why Novellie (1962) suggested steeping time to be unimportant with respect to sorghum malt DP. If the present study were to be repeated, with periodic assessment of malt quality made during the six day period, it is likely that even more significant values pertaining to DP, might be obtained (see below).

In addition to steeping time, the temperature of the steeping water was found to affect the quality of the resulting malt. To optimise sorghum malt DP, HWE and FAN, whilst minimising malting losses, it would appear that the temperature of the steeping water should be at approximately 25°C (Chapter 2). This is similar to optimum germination temperatures reported previously for sorghum malting (Novellie, 1962; Morrall *et al.*, 1986).

Aeration during steeping was found to improve the FAN and HWE quality of the resulting sorghum malts (Chapter 2). Although analysis of variance indicated that aeration during steeping did not significantly affect the DP of the malt it is suggested that this could be misleading. The FAN and HWE assays measure the products of enzymic hydrolysis, whereas DP measures enzyme activity. During sorghum malting, DP has been shown to increase gradually and thereafter reach a plateau (Pathirana *et al.*, 1983; Okun and Uwaifo, 1985; Morrall *et al.*, 1986; Chapter 3, for grain germinated at 25 or 30°C). It is thus probable that enzyme activity of the grains exposed to different levels of aeration might well have developed at different rates, but that this would no longer be reflected in the DP after six days of germination. The present observations that aeration generally improved the malt quality, are supported by the findings of Ezeogu and Okolo (1995) and Okolo and Ezeogu (1995), who showed that even the length of the air rest periods employed during steeping affect the quality of sorghum malt produced significantly.

As has been reported (reviewed by Bewley and Black, 1978; 1994; Mayer and Poljakoff-Mayber, 1982; 1989; Bradford, 1995), the imbibition of an adequate amount of water into a seed is extremely important in initiating the metabolic events which facilitate the growth processes of germination. Seeds generally exhibit a three-phase process of water uptake (reviewed by Bewley and Black, 1994; Bradford, 1995) or possibly four phases (Yeoung, Wilson and Murray, 1995). Initially water enters the seed rapidly (Phase I); thereafter, the seed enters Phase II, a plateau phase with respect to water uptake. While some metabolism can take place during Phase I, it is during Phase II that major metabolic events

occur in preparation for radicle protrusion. It is only once radicle emergence has occurred in Phase III, that the seed takes up water rapidly again. It is during Phase II that the seed is sensitive to factors that affect germination: however, just prior to the end of Phase I, the water uptake rate is affected by, *i.a.* enzyme activity and respiration rate (Hadas, 1982 cited in Yeoung *et al.*, 1995). Similarly, Yeoung *et al.* (1995) have shown for muskmelon seeds, that water uptake is increased by oxygen during 2-15 hours (prior to the plateau) and inhibited by cyanide, indicating that even prior to Phase II, water uptake is driven by respiration. From the present studies, it is suggested that in combination, steeping time, temperature and the use of aeration had a significant effect on sorghum malt quality because these factors affected the rate of water uptake of the grain, its entry into a state of active metabolism and probably minimised the length of the lag phase (Phase II) prior to radicle protrusion. The exact nature of the water uptake characteristics, however, remains to be established.

As discussed in Chapter 1, for a grain to germinate, an expenditure of energy is required. This usually occurs via the utilisation of adenosine triphosphate (ATP) which is generally formed as a consequence of aerobic oxidation processes (reviewed by Bewley and Black, 1978; 1994; Mayer and Poljakoff-Mayber, 1982; 1989). In the absence of sufficient oxygen, or in the presence of carbon dioxide, normal aerobic respiration is replaced to varying degrees by anaerobic respiration (Chapon, 1963 cited in Hofmeyer, 1970; Hourmant and Pradet; 1981; Pradet and Raymond, 1983; Kennedy, Rumpho and Fox , 1992), the energy released from which, is only about one-third of that released by aerobic respiration. It is therefore reasonable to suppose that realisation of the energy required for

metabolic processes to occur would be dependent upon aeration during the steep. Indeed, according to Corbineau and Côme (1995), the seeds of only six species of plants are known to germinate under anoxic conditions and most seeds do not germinate when denied oxygen (Al-Ani *et al.*, 1985). It has been shown that for barley, aeration during steeping is necessary for the growth processes of germination (Enari, Linnahalme and Linko, 1961; Abdul-Baki, 1969). Reynolds and MacWilliam (1966), who examined the development of amylase and other enzymes systems in barley during steeping, reported that enzymic activity increases with moisture content during early physical water uptake and is independent of the metabolic activity of the grain, but thereafter is dependent primarily on the availability of oxygen. The actual oxygen requirement of the seed, however, appears to be affected by environmental factors such as temperature, osmotic pressure and light. For example, when placed on a medium of low water potential, sunflower and muskmelon seeds require more oxygen for germination (Smok *et al.*, 1993; Yeoung *et al.*, 1995, respectively).

In the present study (Chapter 2), the fact that DP, a measure of joint α- and ß-amylase enzymic activity (Novellie, 1959), and FAN, a measure of the products of proteolysis (Evans and Taylor, 1990), were generally affected similarly by the conditions of steeping suggests that the effect was due to increased metabolic activity in the grain. Further, the fact that aeration during steeping appeared to enhance the HWE and the FAN content of the malt would seem to suggest that, (especially for the high temperature, long steeps), enough water had been imbibed during steeping to initiate metabolic events such that the demands for oxygen were increased. In addition, malting losses, which are basically accrued

as an outcome of respiratory metabolism, were found to be generally retarded by steeping sorghum grain for increasing periods in non-aerated water and to be enhanced by high steeping temperatures, as long as aeration was provided. In view of the fact that the solubility of oxygen in the aqueous phase decreases with increasing temperature (reviewed by Corbineau and Côme, 1995) and the respiration of microorganisms growing on the surface of the grain increases at elevated temperatures (Heydecker and Chetram, 1971), it is probable that the availability of oxygen to the respiring embryo may become limiting, especially during the long, high temperature steeps. These findings tend to support the suggestion that aeration is required to optimise the quality of sorghum malt.

In those steeps that were considered sub-optimal to maximise malt quality (i.e. low temperature, short periods), it is likely that insufficient water had entered the grain and active metabolism, although initiated [integrated metabolism (respiration) can occur at water potentials as low as -15 MPa, far lower than would be required for radicle emergence (Vertucci and Roos, 1990; Vertucci and Farrant, 1995], was retarded. This suggestion that adequate water uptake is important for a germination rate that maximises malt quality, is further supported by the finding that the quality was directly related to the moisture content of the grain at the end of steeping; the higher the steep-out moisture, the higher the malt quality.

It would appear, therefore, that as for barley (Briggs *et al.*, 1981; French and McRuer, 1990), steeping is a critical stage of the malting process for sorghum and the conditions of temperature, time and aeration should be controlled in order to optimise the quality of the resulting malt.

There have been several reports on the effects of germination conditions on sorghum malt quality (Kneen, 1944; Novellie, 1962; Morrall *et al.*, 1986; Ilori *et al.*, 1990; Muts, 1991). However, there has been no investigation of germination conditions that has specifically utilised optimised steeping conditions.

When use was made of optimised steeping conditions (Chapter 3), it was found that malt quality was affected in a manner similar to that reported for sorghum by Morrall *et al.* (1986) in their comprehensive investigation of the effects of germination (but not steeping) conditions. In the present study, and that of Morrall *et al.* (1986), malt quality and the associated malting losses tended to increase with increasing germination time. With respect to germination temperature, Morrall *et al.* (1986) found 24-28°C to be optimum for sorghum malt quality, with temperatures above this range (32°C and 35-38°C) giving progressively lower quality malt. In the present study, where germination temperatures of 18, 25 and 30°C were investigated, it was determined that both 25 and 30°C gave similar optimal results and that 18°C retarded malt quality. Considering that the 18°C germinated grains took at least six days to attain a DP approaching that attained by the grains germinated at 25 or 30°C in just four days, it is apparent that by the appropriate manipulation of germination temperature it may actually be possible to reduce the time required to produce malt of a required quality.

As reviewed by Mayer and Poljakoff-Mayber (1982;1989) and Bewley and Black (1994), there is generally a range of temperature within which a particular seed will germinate and within this range, there will usually be an optimal temperature for germination. As far as sorghum is concerned, in terms of both steeping

(Chapter 2) and germination (Morrall *et al.*, 1986; Chapter 3), it would appear that the optimum temperature, at least for subsequent malt quality, is in the range 24 to 30°C. A temperature of 18°C (and possibly lower), reported as optimal for barley malting (Briggs *et al.*, 1981), is sub-optimal for sorghum, as are temperatures of 32°C and higher (Morrall *et al.*, 1986). The relatively high temperature optimum of sorghum [in general agreement with temperature optima reported for this grain by other investigators (Kasalu, Mason and Ejeta, 1993; Anda and Pinter, 1994; Brar and Stewart, 1994; Nassar and El-Far, 1995)] over that of barley, is not surprising. According to Kigel (1995), most summer germinators in xeric sub-tropical regions are C_4 or CAM species. It is possible that the high temperature requirement of sorghum, a C_4 plant (Doggett, 1988), relative to the temperate species, barley, may be an adaptation preventing germination under conditions of temperature sub-optimal for photosynthesis and seedling growth.

Another noteworthy finding of this study was that highly significant correlations exist between the moisture content of the green malt (i.e. prior to the drying process) and malt DP, FAN and HWE. It would appear, therefore, as was found for steeping (Chapter 2), that the moisture content of sorghum during the germination period is an important indicator of malt quality for brewing purposes. In South Africa, the moisture content of sorghum malted under commercial conditions is often far less than 50% (fresh weight basis, personal observation). This is considerably less than that obtained after six days of germination under even the lower of the two different watering regimes investigated (Chapter 3).

As mentioned earlier, in producing the malt quality required, the grain expends energy, generally by means of aerobic respiration. In doing so, dry matter is lost as carbon dioxide and water. In this study a highly significant correlation was found, not only between green malt moisture and malt quality, but also between green malt moisture and malting losses.

The hypothesis that an enhancement in sorghum moisture content during malting can improve the quality of the resulting malt (Chapters 2 and 3) was investigated further by alkaline steeping conditions, as described in Chapter 4. Recent publications have indicated that steeping sorghum in a dilute solution of NaOH improves the DP (Okolo and Ezeogu, 1996a) and FAN (Okolo and Ezeogu, 1996b) content of the malt. However, no explanation as to the mechanism by which alkali steeping affects sorghum malt quality was offered. It was presently shown (Chapter 4) that for condensed-tannin-free sorghum (classified as GM by the South Africa Sorghum Board), the improvement in malt DP and FAN occasioned by steeping the grain in dilute NaOH was accompanied by an increased water uptake during steeping. Alkali is known to disrupt the molecular structure of the non-starch polysaccharides which make up the cell walls (Verbruggen, Beldman and Voragen, 1995). As discussed (Bewley and Black, 1978; 1994; Mayer and Poljakoff-Mayber, 1982; 1989; Bradford, 1995), the amount of imbibition that will occur in a seed is related to, *i.a.* the permeability of the seed coat. It is suggested, therefore, that the NaOH disrupted the sorghum pericarp cell wall structure and, consequently, allowed water to enter the seed more rapidly during steeping, but not at a rate causing any significant imbibitional damage. The moisture content of a grain is known to affect the rate of respiration

(Reynolds and MacWilliam, 1966; Abdul-Baki, 1969; Bewley and Black, 1978; Mayer and Poljakoff-Mayber, 1982; 1989; Bradford, 1995; Yeoung *et al.*, 1995). Enhanced imbibitional hydration of the grain (as was suggested previously; Chapter 2), brought about in this case by steeping in dilute NaOH, is suggested to facilitate the onset of the stage of active metabolic activity more rapidly, thereby producing the malt quality required more quickly.

Sorghums classified by the South African Sorghum Board as GH are considered suitable for malting purposes. However, they contain condensed tannins in the layer immediately below the outer pericarp layer, the testa. If these grains are to be malted successfully it is essential that the tannins be inactivated (Daiber, 1975a), otherwise when the malt is milled and mixed with water during brewing, the tannins will bind with the malt enzymes, adversely affecting hydrolysis of starch, proteins and other components of the mash. Daiber (1975b) patented a process of inactivating the tannins by soaking sorghum grain for a four to six hour period at the end of steeping in a very dilute solution of formaldehyde (0.03-0.08%, depending upon the percentage polyphenolics in the grain). In recent years, however, the use of formaldehyde has not been viewed favourably. Indeed, in some countries, its use has been banned (van de Venter, personal communication[1]). Therefore, alternative methods of inactivating tannins have, and still are, being sought, treatment with alkali being one approach (Price *et al.*, 1979). The findings of the present study on the effect of alkaline steeping on sorghum malt quality (Chapter 4), provided further evidence that NaOH may be

[1]T. van de Venter, Director, Department of National Health and Population Development, Pretoria, South Africa

a potential replacement for formaldehyde. Although treatment of the GH sorghum grain with NaOH did not enhance the quality of the resulting malt (possibly because it did not enhance the moisture content of the grain significantly), it did appear to inactivate the tannins in the grain, as indicated by the DPs of the water and peptone extracted malts being similar. That is, the tannins in the malt seem not to have complexed with the malt enzymes.

It is suggested that the inconsistent results reported by other workers concerning the ability of NaOH to improve sorghum malt quality (DP; Okolo and Ezeogu, 1996a and FAN; Okolo and Ezeogu, 1996b) may indeed, be related not merely to cultivar differences in general (as suggested by those authors) but rather, in some of the cases, more specifically, to the grain being of the high-tannin (GH) type.

The FAN content is an important component of malt quality, as it is required during the fermentation stage of the brewing process as a source of yeast nutrition (Daiber and Novellie, 1968; Baxter, 1981; Pickerell, 1986). The level of sorghum malt FAN is particularly important in opaque-beer brewing, where it can be limiting, because only a small amount of the grist is malt (Novellie, 1966 and 1968; Taylor, 1989; Daiber and Taylor, 1995). Adequate FAN levels are especially necessary for lager beer brewing processes, which use unmalted grain (sorghum or maize), with only a small amount of sorghum malt (Muts, 1991). The roots and shoots of sorghum malt are known to be rich in nitrogenous compounds (Taylor, 1983). Although the roots and shoots of sorghum together represented only a small proportion of the total mass of the malt (generally less than 20%) they were found

to contribute as much as 61% to the FAN content of the whole malt (Chapter 3). The dramatic increase in FAN in the roots and shoots during germination can be explained as a result of translocation of the products of storage protein breakdown from the kernel during germination (reviewed by Mayer and Poljakoff-Mayber, 1982; 1989; Taylor, 1983).

In addition to time and exogenous factors such as temperature, aeration and moisture affecting the germination and malting quality of grain, there is a great deal of evidence supporting the rôles of the plant hormones or plant growth regulators (PGRs), in controlling germination (reviewed by Bewley and Black, 1978; 1994; Mayer and Poljakoff-Mayber, 1989; Fincher and Stone, 1993; Kermode, 1995). In practice, the barley malting industry has taken advantage of the knowledge that the PGR, gibberellic acid (GA_3), can improve malting quality significantly, particularly the production of the critically important hydrolytic enzyme, α-amylase (Paleg, 1960; MacLeod, Duffus and Johnston; 1964; Briggs *et al.*, 1981; Palmer, 1990). The sorghum malting industry, however, does not make use of exogenous GA_3 during malting. This is largely because of research that indicated that provision of GA_3 did not significantly improve the amylase activity of sorghum (Daiber and Novellie, 1968; Aisien and Palmer, 1983; Aisien, Palmer and Stark, 1983).

To elucidate possible endogenous control mechanisms involved in sorghum germination, the technique of radioimmunoassay following sample resolution by HPLC was used to assay the levels of selected PGRs during germination (Chapter 5). It was suggested that in sorghum, germination is under embryo

(inclusive of scutellum) control as the concentration of the PGRs in the mature grain (with the exception of GA_{1+3}) was much higher in the relatively small embryo in relation to the total mass of the grain. It is probable, however, that most of the PGRs in the combined distal and proximal endosperm portions might actually have concentrated in the aleurone layer, and that the content in the endosperm proper, was negligible.

Throughout germination, the levels of the combined amounts of the gibberellins$_{1+3}$ (GA_{1+3}) were relatively low and somewhat erratic. Although it requires verification, it is possible that peaks in the levels of GA_{1+3} in the embryo and proximal endosperm were related to the time of germination and activity of amylase enzymes. It would appear that the levels of the PGRs, abscisic acid (ABA) and the cytokinins exemplified by zeatin (Z), zeatin riboside (ZR) and isopentenyladenine (IPA), may be important in controlling sorghum germination. A small peak in the combined concentrations of Z+ZR and a relatively larger peak in IPA concentration, following the onset of the first visible sign of root protrusion, coincided with the initiation of a more or less linear increase in amylase activity. Abscisic acid levels are usually highest during development (Black, 1983) and extremely low at maturity (Zeevaart and Creelman, 1988; reviewed by Black, 1991; Kermode, 1995). The finding that the level of ABA in the embryo tissue prior to germination was particularly high was especially noteworthy and supports the data of Steinbach *et al.* (1995) for sorghum. A significant feature was the decline in the level of embryo ABA to almost negligible amounts over the first 32 hours, which coincided with a marked increased in amylase activity, following the onset of germination. It is possible that the changing balance of ABA and Z+ZR and possibly GA_{1+3}, during the onset of germination could enable the modulation between repression and expression of amylase activity. The

from these data (Chapter 5) are strongly borne out by the work reported on the importance of timing in the application of exogenous gibberellic acid (Chapter 6).

As reviewed in Chapter 1, ABA, is implicated in the inhibition of germination. Not only is it involved in seed dormancy (Bewley and Black, 1982; 1994; Tillberg, 1983; Barthe and Buland, 1985; Hole, Smith and Cobb, 1989; Singh and Browning, 1991; Hilhorst, 1995; Wang, Heimovaara-Dijkstra and Duijn, 1995), it is also accepted as an inhibitor of precocious germination in both dormant and non-dormant seeds during development (Ackerson, 1984; Bewley and Black, 1985; 1994; Quatrano, 1986; Kermode, 1990; 1995; Black, 1991; Oishi and Bewley, 1990; Xu, Coulter and Bewley, 1990; Meurs *et al.*, 1992; Karssen, 1995; Steinbach *et al.*, 1997). When exogenously applied, ABA suppresses the germinability of the developing embryo (Stinissen, Peumans and DeLanghe, 1984; Eisenberg and Mascarenhas, 1985; Finkelstein *et al.*, 1985; Raikhel and Quatrano, 1986; Neill, Horgan and Rees, 1987). The lag phase prior to axial extension has, in several seeds (rape, soybean and dwarf French bean), also been related to the native concentration of ABA at the time of abscision of the seed from the parent plant (reviewed by Black, 1991). The higher the ABA content, the longer it took before germination was initiated. In terms of α-amylase activity, there is evidence that ABA can suppress the activity of the enzyme in developing seeds (King, *et al.*, 1979; King, 1976; 1982; Napier, Chapman and Black, 1989). Additionally, in barley aleurone cells, synthesis of α-amylase, stimulated by GA_3 can be inhibited, at least to some extent, by ABA (Chrispeels and Varner, 1967; Barton *et al.*, 1973; Jacobsen, 1983; Jacobsen and Beach, 1985; Jacobsen and Chandler, 1987; Jacobsen and Close, 1991).

In view of the high level of embryo ABA in the mature sorghum grain (Chapter 5), possibly as a consequence of water stress during development (King, 1982; Robertson *et al.*, 1989), it was suggested that application of a germination stimulator would probably have an effect only once the endogenous level of ABA had declined significantly. This suggestion is supported by the work described in Chapter 6, where application of GA_3 was found to enhance both the DP and FAN contents of sorghum malt. As had been suggested from the results reported in Chapter 5, the timing of the treatment was found to be important. Generally, no significant improvement in malt quality was observed unless the treatment was administered towards the end of a 24 hour steep or subsequently, during early germination, indicating that in sorghum, the amylase and the protease enzymes are not immediately inducible by GA_3 and may require the removal of an inhibitor. In sorghum, neither α- nor ß-amylase enzymes are thought to be present in the ungerminated mature sorghum grain (Novellie, 1960). There is good evidence to suggest that these enzymes are synthesised *de novo* during germination (Daiber and Novellie, 1968; see review by Palmer, 1989; Dufour, Mélotte and Srebrnik, 1992). Application of GA_3 to germinating sorghum, may, therefore, be enhancing the actual synthesis of the amylase enzymes.

It should be noted that the moisture content of the GA_3-treated and the non-GA_3-treated controls did not differ significantly from each other. The enhancement in malt DP and FAN occasioned by the GA_3 treatment cannot, therefore, be related to an increased grain moisture content (refer to Chapters 2, 3 and 4). It would appear, therefore, that the increase in malt quality brought about by GA_3 treatment is related to a real increase in amylase and protease activity and not to it causing the grain to take up additional moisture.

However, when the GA_3 treatment was combined with a malting procedure that allowed the grain to take up additional water during both the steeping and germination periods, it was possible to enhance the improvement in sorghum malt quality over and above that which was obtained by administering GA_3 alone. Indeed, for one of the cultivars of sorghum investigated (NK 283), the DP of the malt was increased by as much as 63% when GA_3 was administered during the last two hours of the steep and when the grain was allowed to take up additional water during malting.

It is possible that the GA_3-stimulated enhancement in amylase activity in sorghum was mediated through a different route to that of barley. There are two main groups of α-amylase isoenzymes in cereal grains, the high pI and the low pI group (Hill and MacGregor, 1988; Fincher, 1989). Both types have been found in temperate cereals such as barley, wheat, rye and oats: however, only the low pI isozyme is produced during germination in more tropical cereals such as maize, rice, sorghum and millet (reviewed by Hill *et al.*, 1993). The high pI type, concentrated predominantly in the endosperm, [approximately 85-90% of α-amylase in barley endosperm is the high pI group (MacGregor and Ballance, 1980)], is highly responsive to GA and ABA and appears to be responsible for most of the starch degradation occurring during germination in temperate cereals (review by Ziegler, 1995). The low pI isozyme is produced early in germination, does not show much response to GA stimulation (Jacobsen and Higgins, 1982) and is more representative of scutellar production (Hill and MacGregor, 1988). Although GA_3 has been found to enhance the amylase activity of sorghum malt [Chapter 6 and in some other instances (Agu *et al.*, 1993; Nzelibe and Nwashike,

1995)], and other tropical cereals [rice (Tanka, Ito and Akazawa, 1970); pearl millet (Thulaseedharan and Mehta, 1992)], the degrees of improvement are low in comparison to barley. Together with the evidence that early synthesis of α-amylase occurs primarily in the embryo and is possibly primarily localised to the scutellum in sorghum (Daiber and Novellie, 1968; Daiber, Malherbe and Novellie, 1973, Aisien and Palmer, 1983; Aisien *et al.*, 1983) and rice (Akazawa and Hara-Nishimura, 1985), it is suggested that, at least in terms of α-amylase activity, the GA_3-stimulated enhancement reported presently, may have occurred via stimulation of the low pI isozyme.

The finding that endogenous cytokinin levels peaked prior to an almost linear increase in amylase activity (Chapter 5) may also be significant. There have been suggestions that cytokinins may interact competitively at the same site as ABA, the inhibitory effects of ABA being relatively frequently reported as becoming completely reversed by high concentrations of cytokinins (reviewed by Thomas, 1992). Additionally, it is reported that the level of sensitivity of a grain to ABA, rather than ABA levels *per se*, may be the main determinant in seeds exhibiting prolonged expression of ABA responsive genes (Walker-Simmons, 1987; Morris *et al.*, 1991; Benech-Arnold, 1993; Benech-Arnold *et al.*, 1995; Steinbach *et al.*, 1995), perhaps as a consequence of changes in phosphorylation of ABA receptors or other upstream regulatory proteins (Verhey and Walker-Simmons, 1997). In the present study, the stimulatory response of sorghum elicited by exogenously applied GA_3, may be related not only to the decline in the endogenous embryo ABA levels, but also to the sensitivity of the tissues to GA_3

perhaps changing as a consequence of the peak in the levels of the endogenous cytokinins.

It was suggested (Chapter 5) that the high concentration of ABA in the embryo tissue of the mature sorghum grain may constitute an endogenous mechanism that will delay germination until the level of this inhibitor has declined sufficiently. Indeed, axis protrusion was not observed until the embryo ABA concentration had declined appreciably. There have been reports that water uptake may be hormonally regulated and there is some indication that ABA may lower the ability of the embryo to take up water under osmotic stress (Schopfer and Plachy, 1984; 1995; McIntyre and Hsiao, 1985). It is possible that the high ABA level in the embryo tissue of the mature, ungerminated, sorghum grain may be a factor which accounts for the slow rate of water uptake reported (Hofmeyer, 1970). In view of the importance of the seed imbibing an adequate amount of water to initiate the metabolic processes of germination (reviewed by Bewley and Black, 1978; Mayer and Poljakoff-Mayber, 1982; 1989; Bradford, 1995), it is possible that one mechanism by which the high ABA concentration in the mature, ungerminated sorghum embryo delays germination, is by limiting the rate of water uptake. As discussed by Kigel (1995), once radicle protrusion occurs, the seed becomes highly vulnerable to drying. Under arid conditions, it is possible that the prolonged imbibition required by sorghum may be a mechanism that will ensure that germination will take place only after an adequate rainfall.

Abscisic acid has also been shown to raise the water potential level required for root protrusion (Ni and Bradford, 1992; 1993). The high ABA level in the embryo

of the mature sorghum grain may affect the threshold water potential thus retarding radicle emergence. Seeds can progress metabolically toward germination even at water potentials too low for radicle protrusion (Heydecker, 1973; Hegarty, 1978; Bradford, 1986; Khan, 1992; Bradford and Haigh, 1994). There is a great deal of evidence to suggest that during hydration at sub-optimal water potentials [and sub-optimal temperatures (Dahal, Bradford and Haigh, 1993; Dahal and Bradford, 1994)], "hydrothermal time", is retained during subsequent drying and when the seed is rehydrated, the time for radicle protrusion is reduced (Heydecker, 1974; Wilson, 1973; Bradford, 1986; Dahal *et al.*, 1993; Dahal and Bradford, 1994; Mauromicale and Cavallaro, 1995; XiangRu *et al.*, 1995; Lanteri *et al.*, 1996; Battaglia, 1997). It is suggested that in sorghum, as is the case for other seeds adapted to intermittent rainfall (Wilson, 1973; McKeon, 1985; Allen, Debaene and Meyer, 1993; Dubrovsky, 1996; Veenendaal, Ernst and Modise, 1996), during precipitation inadequate for germination, hydrothermal time may be accumulated which will result in faster and more uniform germination when there is sufficient rainfall. It is suggested that the accumulation of hydrothermal time, and the gradual leaching of the water-soluble inhibitor ABA from the mature sorghum grain, may constitute a germination control mechanism that accounts for the suitability of sorghum to be cultivated in areas where rainfall is too low for other staples (House, 1985; Dendy, 1995).

Steinbach *et al.* (1997) have reported that not only is the concentration of endogenous ABA important in determining the pre-harvest germinative behaviour of sorghum, but also the rate at which it declines. In immature caryopses of sorghum, endogenous ABA decreased faster in pre-harvest sprouting-susceptible

caryopses than it did in less sprouting-susceptible varieties (Steinbach *et al.*, 1997). In addition, in an earlier publication (Ackerson, 1984) it was reported that immature soybean embryos can be made capable of premature germination if their endogenous ABA content is reduced artificially by washing, the percentage germination being found to be correlated with the length of the washing period. Further Visser *et al.* (1996), using an ABA-insensitive mutant and a wide-type barley showed that when excised embryos were imbibed in water, although the rate at which ABA was leached out was similar, the rate at which the ABA level in the incubation medium was reduced was faster for the mutant, indicating that the rate at which the ABA is turned over outside of the embryo is a determining factor in the germination of barley seeds. In view of these findings, it is suggested that if the rate at which the endogenous ABA content of the mature sorghum grain declines and is removed from the surrounding medium could be increased (perhaps by leaching it out of the grain using frequent rinsing during steeping), it is possible that sorghum may be able to respond to GA_3 treatment at an earlier stage. Rapid removal of ABA could perhaps also, increase the rate at which water is imbibed and active metabolism is initiated.

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CHAPTER 8: CONCLUSIONS AND RECOMMENDATIONS

CHAPTER 8: CONCLUSIONS AND RECOMMENDATIONS

The commercial steeping conditions used for sorghum in South Africa, where grain is imbibed for relatively short periods (as little as six hours), without temperature control and without means of aeration, are sub-optimal to maximise sorghum malt quality. Malt quality, in terms of DP, FAN and HWE, increases with steeping time (16-40 hours) and the optimum steeping temperature is between 25 and 30°C. Aeration during steeping is also necessary to maximise malt quality.

The finding that under the best steeping conditions, the optimum germination temperature for sorghum malting is between 24 and 30°C (the same as for steeping) and that a temperature of 18°C is sub-optimal, is also of significance for the sorghum maltster, and highlights the need for temperature control during germination to optimise the malting potential of the grain. In South Africa during the winter months, germination is sometimes conducted at low temperatures (even lower than 18°C); this is clearly sub-optimal in terms of producing the best sorghum malt quality.

The finding that the quality of the sorghum malt is directly related to both the steep-out moisture of the grain and the green-malt moisture of the malt is particularly significant. It would appear, therefore, that the imbibition of an adequate amount of moisture during steeping and, the continued watering of the grain during germination, is essential for the optimal development of the critical malt enzymes. It is suggested that a useful way of enhancing the quality of

sorghum malt produced commercially in South Africa, would be to enhance the moisture content of the germinating grain during malting.

A way of enhancing the water uptake of condensed tannin-free (GM) sorghum grain during steeping and enhancing the subsequent malt DP and FAN is to steep the grain in a dilute solution of NaOH. This treatment also appears to offer the sorghum maltster a way (other than using formaldehyde) of inactivating the tannins in high-tannin (GH) grain and thus facilitating the use of these grains for malting purposes.

The root and shoot portion of sorghum malt was found to be an extremely rich source of FAN, and to contribute substantially to the total malt FAN. Thus, where sorghum malt is being used as a critical source of FAN, it is clear that care should be exercised during malt processing to minimise the loss of roots.

Contrary to some previous research, it has been clearly shown that sorghum malt quality in terms of DP and FAN can also be improved by the use of exogenous GA_3. The timing of the treatment, however, is critical. No significant improvement in malt quality occurs until the treatment is administered towards the end of the steep or on the second or third day of malting (i.e. measured from the beginning of steeping), indicating that in sorghum, the amylase and the protease enzymes are not immediately inducible by GA_3 and may require the removal of an inhibitor. This may explain why other workers have reported that GA_3 has little or no effect on sorghum malt quality. It appears that application of GA_3 to malting sorghum may have a significant effect on sorghum malt quality (at least in terms of amylase

activity) only after the endogenous embryo ABA level has declined significantly, and perhaps as a consequence of an interaction between the endogenous ABA and cytokinins IPA and Z+ZR and possibly gibberellins GA_{1+3}. Irrespective of the exact mode of action, the finding that application of GA_3 alone can improve the malting quality of sorghum by approximately 20-30% is of importance to the sorghum malting industry.

By optimising steeping and germination conditions and through steeping in NaOH or adding GA_3, not only should it be possible to enhance the quality of malt obtained from a particular sorghum, it should be possible to reduce the time required to obtain the specific quality. This offers a saving to the maltster in terms of operational costs and enhances the total throughput possible from the malting plant.

APPENDIX A

Dewar, J. Taylor, J.R.N. and Joustra, S.M. (1995). Determination of diastatic power of sorghum malt. Method No. 4. *In Accepted Methods of Sorghum Malting and Brewing Analysis.* Pretoria, CSIR Food Science and Technology.

METHOD NO. 4

DETERMINATION OF DIASTATIC POWER OF SORGHUM MALT

Method accepted September 1994

1. INTRODUCTION

Diastatic Power is a measure of the joint amylase enzymatic activity (mainly α- and ß-amylase) in the malt.

The term **Sorghum Diastatic Power** is to be used to distinguish this method from others which determine Diastatic Power.

2. REAGENTS

SABS method 235 (Appendix 1) is used with the following amendments:

A standard starch should be used *viz.* Merck 1252.

A standard peptone extractant should be used *viz.* Difco Bacto-peptone.

It is recommended that "Titrisol" type sodium thiosulphate solution be used.

3. METHOD

SABS method 235 is used with the following amendments:

Peptone Extraction
The amylase enzymes from malt made from high tannin (bird-proof) sorghum varieties are partially or wholly inactivated by the tannins. To estimate the Diastatic Power of such malts it is necessary to prevent inactivation of the amylase enzymes. The amylases of such malts can be measured by extracting in a 2% solution of peptone. Peptone has no effect on the determination of the amylases in tannin-free (non bird-proof) sorghum malts. Hence, Diastatic Power determination by peptone extraction is suitable for the analysis of all types of sorghum malts.

Water Extraction
Water extraction estimates only those amylases which have not been inactivated by tannins.

Note: It should be stated whether peptone or water has been used as the extractant.

A 5 g malt sample size should be used and a 100 ml extraction volume, instead of 25 g and 500 ml as described in SABS method 235. The apparatus is to be reduced in size accordingly.

Diastasis
Both the diastasis and the blanks are to be performed in duplicate.

4. REPEATABILITY AND REPRODUCIBILITY

Repeatability: ± 1 SDU/g

Reproducibility: ± 2 SDU/g

5. BIBLIOGRAPHY

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SOUTH AFRICAN BUREAU OF STANDARDS

STANDARD TEST METHOD FOR THE DETERMINATION OF THE DIASTATIC POWER OF MALTS PREPARED FROM KAFFIRCORN (SORGHUM) INCLUDING BIRD-PROOF VARIETIES, AND FROM MILLET

SECTION 1. OUTLINE OF THE METHOD

1.1 The malt is ground to a standard fineness and an aqueous peptone extract of it made and filtered. This extract is allowed to act on a standard buffered starch solution for 30 minutes at 30°C. At the end of this period the action is stopped by the addition of sodium hydroxide solution. The sugars that have been produced by the malt extract are estimated by boiling an aliquot of the solution with ferricyanide and determining the unchanged ferricyanide by titration. A blank is carried out in order to make allowance for preformed sugar, i.e. that already present in the malt extract The diastatic power is then calculated according to the formula given.

SECTION 2. REAGENTS

2.1 GENERAL. Unless otherwise stated, reagents used shall be of recognized analytical reagent quality.

2.2 PEPTONE. Dissolve 10 g. peptone [1] in 500 ml water.

2.3 STARCH. The starch used shall be a reference starch [2] or any starch which, when tested in parallel with the reference starch, gives results differing by not more that 5 per cent from those obtained with the reference starch. Starches giving results differing by more than 5 per cent from those obtained with the reference starch, shall not be used.

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1) The names of manufactures of suitable grades of peptone may be obtained from the *South African Bureau of Standards.*
2) Samples of reference starch may be obtained from the *South African Bureau of Standards*

2.3.1 **Reference Starch**. The reference starch shall comply with the following requirements:

(a)	Solubility, minimum	: 1 part in 50 parts of water.
(b)	Dextrins	: shall be absent.
(c)	Reducing substances, maximum	: 0.75 per cent (calculated as maltose).
(d)	Moisture content (determined in same manner as described in Section 5)	: 10 to 12 per cent.

2.4 BUFFERED STARCH SOLUTION. Prepare the solution as follows:

Weigh out a quantity of starch representing 2 g. of dry starch per 100 ml. of final solution. Macerate the starch with a little water to form a smooth, thin paste. Pour this with constant stirring into boiling freshly-distilled water representing approximately 75 per cent of the final volume of the starch solution, at such a rate that boiling does not cease. Continue boiling for 2 minutes after the final paste has been introduced, then add an additional 10 per cent of the final volume of cold, freshly-distilled water and transfer to a glass-stoppered volumetric flask. Mix by inverting the flask several times, wash down the neck, cool to 30°C, add 2 ml. of buffer solution (2.5) for each 100 ml. of the final solution and make up to volume. Mix again and store at 30°C.

2.5 BUFFER SOLUTION FOR STARCH. Dissolve 68 g. of chemically pure sodium acetate trihydrate ($CH_3\ COON_a.3H_20$) in 500 ml. of normal acetic acid and dilute to 1 litre with distilled water. (The pH value of this solution is approximately 4.7.)

2.6 SODIUM HYDROXIDE SOLUTION (0.5N) FOR STOPPING DIASTASIS. Dissolve 20 g. sodium hydroxide in distilled water and make up to 1 litre.

2.7 REAGENTS FOR SUGAR DETERMINATION

2.7.1 Alkaline Ferricyanide Solution, 0.05N. Dissolve 16.5 g. potassium ferricyanide and 22 g. anhydrous sodium carbonate in distilled water and dilute to 1 litre. Store in an amber bottle away from light.

Standardization. To 10 ml. of the potassium ferricyanide solution add 25 ml. of acetic acid-salt solution (2.7.4), 1 ml. potassium iodide solution (2.7.6) and 2 ml. of starch indicator (2.7.3). Titrate with sodium thiosulphate solution (2.7.2) until the blue starch-iodine colour is discharged.

2.7.2 Sodium thiosulphate Solution, 0.05N. Dissolve 12.41 g. pure pea crystals of sodium thiosulphate ($Na_2S_2O_3.5H_2O$) and 3.8 g. of borax (as preservative) in water and dilute to 1 litre.

Standardization. Dissolve 0.1000 g. pure dry reagent-grade potassium dichromate in water and make up to 50 ml. Add 2 g. potassium iodide and 8 ml. concentrated hydrochloric acid, mix thoroughly and titrate with the thiosulphate until the colour changes to yellow green. Add a few ml. of starch indicator (2.7.3) and titrate to a light green shade when the colour of the starch-iodine is discharged. Use the relationship, 1 ml. 0.05N sodium thiosulphate = 0.00245 g. potassium dichromate, to calculate the normality of the sodium thiosulphate.

2.7.3 Starch Indicator. Prepare a paste of 1 g. of soluble starch with a little water. Pour the paste with constant stirring into boiling water. Cool and make up to 100 ml. Prepare a fresh solution for each series of standardizations.

2.7.4 Acetic Acid Salt Solution. Dissolve 200 ml. of glacial acetic acid, 70 g. potassium chloride and 20 g. zinc sulphate ($ZnSO_4.7H_20$) in distilled water and dilute to 1 litre.

2.7.5 Concentrated Sodium Hydroxide Solution. Dissolve 50 g. sodium hydroxide in 50 ml. of water. Cool before use.

2.7.6 Potassium Iodide Solution. Dissolve 50 g. potassium iodide in water, add 1 or 2 drops of concentrated sodium hydroxide solution (2.7.5) and dilute to 100 ml. The solution should be colourless.

SECTION 3. APPARATUS

3.1 MILL: STANDARDIZATION OF SETTING[3] Pass about 70 g. of malt through the mill. Mix well and then sieve 50 g. of the ground sample through a No.30 B.S. sieve. Shake the sieve, lid and pan by hand in a horizontal plane for 5 minutes, tapping it on a table top every 15 seconds. Remove the pan and cover and shake the sieve for a short while over a sheet of paper until no further malt passes through the sieve. The mill shall be considered as having a standardized setting when the portion of ground malt remaining on the sieve is between 4.5 and 5.5 g. (i.e. 9 to 11 per cent). The mill shall be standardized at 6-monthly intervals.

3) The names of manufactures of suitable mills bay be obtained form the South Africa Bureau of Standards

3.2 GLASSWARE. All glassware shall be cleaned in chromic acid cleaning solution, rinsed with tap water not less that four times and finally rinsed with distilled water. Dry the glassware in a hot-air oven.

SECTION 4. METHOD

4.1 PREPARATION OF THE MALT EXTRACT. Take about 35 g. of the sample after grinding in a standardized mill (3.1). Weigh accurately an amount of 25 ± 0.05 g of the ground malt and transfer quantitatively to an extraction flask. (Use the remaining malt for the determination of moisture in accordance with Section 5.) Avoid delays which may alter the moisture content of the malt. Add 500 ml. of freshly prepared peptone solution (2.2) to the extraction flask and let the infusion stand for 2 and a half hours at 30 ± 0.2°C. Agitate by gentle rotation at intervals of 20 minutes. *Do not mix by inverting the flask.* Gentle swirling without splashing will give sufficient mixing. At the end of 2 and a half hours filter the infusion by transferring the entire charge on to a 32 cm. fluted filter paper in a funnel with a diameter of 175 mm. Return the first 50 ml. of the filtrate to the filter. Collect the filtrate until 3 hours have elapsed from the time that the peptone solution and ground malt were first mixed. Prevent evaporation during the filtration period as far as possible by placing a watch glass over the funnel and a suitable cover resting on the neck of the receiver around the stem of the funnel.

4.2 DIASTASIS. Using a accurately graduated measuring cylinder, transfer 200 ml. of buffered starch solution to a 250 ml. volumetric flask. Now add distilled water in such a quantity that the volume of this water plus that of the malt extract to be added, is equal to 10 ml. (see Table 1). Place the flask in a waterbath of which the temperature is maintained at 30 ± 0.2°C. When the temperature of the contents of the flask has reached the temperature of the bath, add the malt extract (also at 30°C) mixing the solutions by rotating the flask during the addition and at the same time start a stop watch. Stop the rotation after exactly 30 minutes by adding 20 ml. of 0.5N sodium hydroxide and mixing thoroughly. Make up to volume and shake.

TABLE 1

Diastatic power of malt KDU per gram	Volume of malt extract to be used, ml.	Volume of water to be added, ml.
30 or above	2	8
20 to 29	5	5
0 to 19	10	0

4.3 DETERMINATION OF SUGARS. Pipette 5 ml. of the digested starch solution into a 125 ml. Erlenmeyer flask. Add 10 ml. alkaline ferricyanide (2.7.1) reagent mix well and immerse the flask in a vigorously boiling waterbath for exactly 20 minutes (timed with a stop watch) so that the level of the boiling water is slightly above the level of the mixture in the flask. Cool under running water and add 25 ml. of the acetic acid salt solutions (2.7.4) and 1 ml. of potassium iodide solutions (2.7.6). Mix and titrate with 0.05N sodium thiosulphate (2.7.2) until the blue colour of the starch iodine complex is discharged. Use a 10 ml. semi micro burette for the titration. Note the number of millilitres of 0.05N sodium thiosulphate used. Designate this volume as A.

4.4 DETERMINATION OF BLANK. Prepare a blank by proceeding exactly as describe in 4.2 but add the sodium hydroxide before adding the malt extract. Pipette 5 ml. of the blank into a 125 ml. Erlenmeyer flask. Then add 10 ml. of ferricyanide solution (2.7.1). Boil, cool and titrate as described in 4.3. Designate the number of the millimetres of 0.05N sodium thiosulphate used as B.

SECTION 5. DETERMINATION OF MOISTURE IN MALT

5.1 APPARATUS

(a) *Weighing Bottle.* The weighing bottle may be of glass or aluminium and must be provided with a tightly fitting cover. It should have a diameter of approximately 40 mm. for a 5 g. sample or 50 mm. for a 10 g. sample.

(b) *Oven.* The oven shall be provided with a thermo-regulator capable of keeping the temperature to within 0.5°C of the temperature required. The oven shall be of such a size that all samples can be accommodated on one shelf in order to give comparable results on duplicate samples. The thermometer bulb shall be at the level of the shelf.

(c) *Standardization of Oven.* To standardize the oven, place weighed duplicate samples in the oven at 103 to 104°C and dry for 3 hours. Weigh and dry again for 1 hour. If the moisture content has increased by more than 0.1 per cent, raise the temperature of the oven 1°C and again test with new duplicate samples. The lowest temperature below 106°C giving a moisture content which after 3 hours, drying is within 0.1 per cent of the value obtainable at the same temperature within 4 hours shall be taken as standard for the oven concerned. The ventilators on the oven shall be left open during the entire experiment. The door shall be kept closed during all drying periods.

5.2 PROCEDURE. Determine the moisture content of the malt on duplicate 5- or 10 g. samples, in accordance with the procedure followed in the standardization of the oven but omit the reheating of the samples.

SECTION 6. CALCULATION OF RESULTS

6.1 Express the diastatic power of the malt in kaffircorn diastatic units (KDU) per gram, one kaffircorn diastatic until (KDU) being taken as the amount of enzymatic activity which under the conditions of test described above, produces a quantity of sugar equivalent to 0.5 ml. of 0.05N sodium thiosulphate. Calculate the results as follows:

$$\textit{Diastatic power} = 40\ (B-A)\ \frac{F}{V}\left(\frac{100}{100-M}\right)\ \textit{KDU per gram}$$

where

A = millilitres of sodium thiosulphate used for the determination (4.3),
B = millilitres of sodium thiosulphate used for the blank (4.4),
F = the factor of the 0.05N sodium thiosulphate used,
M = per cent moisture in malt sample used and
V = millilitres of malt extract used for diastasis

This standard test method was approved on 2nd February 1959 at its 133rd meeting by the *Standards Council*, a committee which was established in terms of Section *eleven* of the Standards Act No 24 of 1945 as amended, and which acts in pursuance of the power assigned to it by the *Council for Scientific and Industrial Research* under Sub-section (2) of that section.

It has been published in loose-leaf form by the *Standards Council* and is obtainable from the *South African of Standards*, Private Bag 191 Pretoria South Africa at a price of three cents per leaf, post free.

APPENDIX B

Dewar, J. Taylor, J.R.N. and Joustra, S.M. (1995). Determination of free amino nitrogen in sorghum malt. Method No. 5. *In Accepted Methods of Sorghum Malting and Brewing Analysis*. Pretoria, CSIR Food Science and Technology.

METHOD NO. 5

DETERMINATION OF FREE AMINO NITROGEN IN SORGHUM MALT

Method accepted September 1994

1. INTRODUCTION

Free amino acids and small peptides (free α-amino nitrogen or FAN) are the source of nitrogen for yeast in brewing. Pre-formed FAN in the sorghum malt is a major source of FAN in the wort, the presence of which is necessary for normal yeast growth and fermentation.

2. APPARATUS

Analytical balance
Water bath at 30°C
Bench-type centrifuge
Spectrophotometer covering the visible light range
10 mm cuvettes

3. REAGENTS

Extractant
Prepare 1 litre of a 5% (m/v) solution of trichloroacetic acid in distilled water.

Caution: Care must be taken when handling this acid in its solid and diluted form as it can cause severe burns.

Ninhydrin colour reagent
Dissolve in distilled water: 100 g disodium hydrogen phosphate ($Na_2HPO_4.12H_2O$); 60 g potassium dihydrogen phosphate (KH_2PO_4); 5 g ninhydrin (Merck cat. 6762); 3 g fructose (Merck cat. 5323) and make up to 1 litre. This colour reagent will keep for 2 weeks if stored at 0-4°C in an amber or foil-covered bottle.

Diluent
Dissolve 2 g potassium iodate (KIO_3, Merck cat. 5051) in 600 ml distilled water and add 400 ml 96% ethanol.

Standard for ninhydrin assay
Dissolve 107.2 mg glycine (Merck cat. 4201) in 100 ml distilled water in a volumetric flask. This is a stock solution and will keep for 1 week if stored at 0-4°C. For each set of analyses carried out, 2 ml of this stock solution is diluted to 100 ml with distilled water in a volumetric flask.

4. METHOD

Extraction of FAN from malt
The 5% trichloroacetic acid solution is warmed to temperature in the 30°C water bath. One gram of milled malt (milled according to the specification in SABS Method 235) is weighed into a 100 ml glass Erlenmeyer flask. Exactly 40 ml of the warmed 5% trichloroacetic acid is added to the malt. The flask is then tightly stoppered.

Extraction is carried out for 1 hour with the flasks immersed in the 30°C water bath. At 20 minute intervals the flasks are swirled so as to suspend the contents.

After extraction, a portion of the supernatant (10 ml) is centrifuged in the bench centrifuge for 10 minutes.

After centrifugation, 1 ml of clear supernatant is diluted to 25 ml in a volumetric flask with distilled water.

Ninhydrin assay
Pipette 2 ml of the **diluted** standard into a test tube (do in triplicate). Pipette 2 ml of the distilled water (the Blank) into a test tube (do in triplicate). Pipette 2 ml of the diluted sample into a test tube (do in triplicate). Add 1 ml ninhydrin colour reagent to all the test tubes and mix well. Cover the test tubes with glass marbles to prevent evaporation and heat for exactly 25 minutes in a constantly boiling water bath.

Cool the test tubes for 20 minutes in a water bath at room temperature, then add 5 ml of the diluent to each tube and mix well.

Within 1 hour read the samples and standards against a blank (which should be virtually colourless) at 570 nm. The contents of each test tube should be mixed thoroughly by inversion (wear disposable gloves), prior to placing the contents in a cuvette. The standard should give an absorbance between 0.9 and 1.1.

5. CALCULATION

The results are calculated as mg FAN/100 g dry mass of malt, which can be obtained from the following expression:

$$\frac{\text{Absorbance of sample} \times 400}{\text{Mean of absorbance of standard}} \times \frac{100}{(100 - \text{moisture})}$$

The results are reported as:
Malt FAN = x mg/100 g (dry basis)

6. REPEATABILITY AND REPRODUCIBILITY

Repeatability: ± 5 mg FAN/100 g malt

Reproducibility: ± 10 mg FAN/100 g malt

7. BIBLIOGRAPHY

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APPENDIX C Dewar, J., Taylor, J.R.N. and Berjak, P. (1997). Determination of improved steeping conditions for sorghum malting. *Journal of Cereal Science*, **26**, 129-136.

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Determination of Improved Steeping Conditions for Sorghum Malting

J. Dewar*†, J. R. N. Taylor‡ and P. Berjak§

*CSIR Food Science and Technology, P.O. Box 395, Pretoria, 0001, South Africa, ‡University of Pretoria, Department of Food Science, Cereal Foods Research Unit, Pretoria, 0002, South Africa, and §University of Natal, Department of Biology, Plant Cell Biology Research Unit, Durban, 4000, South Africa

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ABSTRACT

The effect of various steeping conditions (time, temperature and aeration) on the quality of sorghum malt for brewing (in terms of diastatic power, free amino nitrogen and hot water extract) was examined. Steeping time and temperature had a highly significant effect on sorghum malt quality. In general, malt quality increased with steeping time (from 16–40 h). Malt diastatic power increased with steeping temperature (up to 30°C) and free amino nitrogen and extract content peaked at a steeping temperature of 25 °C. Aeration during steeping appeared to enhance the extract and free amino nitrogen content of the finished malt. Sorghum malt quality was found to be directly related to the steep-out moisture of the grain.

Keywords: sorghum, steeping, malting.

INTRODUCTION

In Africa, the cereal sorghum is malted widely to provide an important raw material in brewing. In southern Africa, approximately 200 000 tonnes per annum of malted sorghum are used in the production of traditional (opaque) sorghum beer[1]. Since a ban on the importation of barley malt by the Nigerian government in 1988, there has also been growing interest in the use of malted sorghum in the brewing of clear lager type beers[2].

Malting is the germination of cereal grain in moist air under controlled conditions, the primary objective being to promote the development of hydrolytic enzymes which are not present in the ungerminated grain. The malting process can be divided into three physically distinct operations, i.e. steeping, germination and drying.

Steeping (the soaking of grain in water) is widely acknowledged as the most critical stage of the malting process[3,4]. This is a consequence of the importance of initiating germination such that modification of the endosperm structure will progress at a rate producing malt of the desired quality. Factors that are important for the successful initiation of germination are adequate moisture, temperature and the presence of oxygen[3]. These factors can affect the extract yield, diastatic activity and other important malt quality characteristics[3].

Much emphasis has been placed on the effects of various germination conditions on the quality of sorghum malt[5–11]. However, despite the acknowledgement of the importance of the steeping process in malting, there have, until recently, been only limited studies on the effect of steeping conditions on sorghum malt quality[11–16]. Indeed, the work done has been limited in its scope, virtually

ABBREVIATIONS USED: AB = air-blast; AR = air-rest; NA = non-aerated; DP = diastatic power; FAN = free amino nitrogen; SDU = sorghum diastatic units.

† *Current mailing address*

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Table I Effect of steeping time, temperature and aeration on the diastatic power of sorghum malt (SDU/g)

Steeping time (h)	Temperature (°C)								
	20			25			30		
	Aeration								
	AR	AB	NA	AR	AB	NA	AR	AB	NA
16	29	24	26	27	33	33	35	31	33
24	32	30	28	31	29	36	38	36	38
40	35	35	31	35	37	37	43	40	39

AR = air-rest, AB = air-blast, NA = non-aerated.

Analysis of variance for malt diastatic power

Source of variation	Mean square	DF	F	P
Time	92·06	2	24·17	0·000
Temperature	111·27	2	29·22	0·000
Aeration	2·58	2	0·68	0·534
Time*Temperature	1·72	4	0·45	0·770
Time*Aeration	3·31	4	0·87	0·523
Temperature*Aeration	13·83	4	3·63	0·057
Residual	3·81	8		

DF = degree of freedom, F = F-test, P = significance level.

only being concerned with final warm water steeping[12,16]. This may relate to the assumption made in an earlier study that regarded steeping time as unimportant in determining sorghum malt quality[7]. This is reflected in commercial sorghum malting practice in South Africa where steeping times, of a maximum of 24 h, and sometimes as short as 6 h, are employed.

In view of this, a systematic investigation of the effect of steeping conditions on sorghum malt quality is needed. In this study the effect of steeping time, temperature and aeration on sorghum malt quality was investigated.

EXPERIMENTAL

Materials

Grain

Sorghum (*Sorghum bicolor* (L.) Moench) grain of cultivar Barnard Red was used.

Steeping equipment

The steeping apparatus consisted of three perspex vessels each of 2 kg capacity. Each vessel was fitted with a water-jacket in which attempered water was circulated to control the temperature of the water within the vessels. A pump connected to an air supply was used to aerate the steeping water (air-blast (AB)). The air-rest (AR) method of aeration was achieved by periodically draining the steeping water from the grain and allowing the grain to rest for a predetermined period of time in the air before refilling the vessel with fresh attempered water. Non-aerated (NA) steeping conditions were achieved by steeping the grain for the full steeping period in non-aerated water. The conditions of steep (duration and frequency of air-blast and air-rest periods) within each of the vessels were controlled and monitored by computer.

Methods

Steeping

Samples of grain (500 g), in nylon mesh bags (400 × 400 mm), were steeped at a pre-determined temperature (20, 25 or 30 °C). After the first hour, the steeping vessels were drained and refilled with fresh attempered tap water. Grain at each of the pre-determined temperatures was steeped for one

of three different times (16, 24 or 40 h), under one of three conditions of aeration (AR, AB or NA conditions). Oxygen levels were not monitored during the different steeping treatments, as the aeration treatments attempted to reproduce the steeping conditions used in South African commercial sorghum maltings. At the end of steeping, the grain was centrifuged in a domestic spin drier (AEG type SD 452BN) for one min at 300 × *g* to remove the surface film of moisture.

Germination

The bag-held grain was germinated in a water-jacketed incubator (Forma Scientific, Marrietta, U.S.A.) set at 25 °C and 100% relative humidity. Twice daily, the bags were removed from the incubator, the grain turned (to avoid meshing of the roots and shoots) and steeped for 10 min in tap water. Following the short steep, the grain was spin-dried (1 min at 300 × *g*) to remove the excess surface-held water and returned to the incubator.

Drying

After 6 d from the beginning of steeping, germination was arrested by drying the malt for 24 h in a forced draft oven set at 50 °C.

Analyses

Steep-out moisture. The mass of the spin-dried, steeped grain was determined and the steep-out moisture calculated as a percentage. The results were expressed on a wet weight basis.

Malting loss. The loss of dry grain material as a consequence of the malting processes was calculated as a percentage and expressed on a wet weight basis.

Diastatic Power (DP). Diastatic power (DP) was determined according to the South African Bureau of Standards method 235[17], except that water was used as the extractant, and 5 g of malt used and the extraction volume reduced accordingly. The results were expressed as sorghum diastatic units (SDU)/g dry weight.

Free amino nitrogen (FAN). The FAN content of the malt was determined according to the ninhydrin method described by Morrall *et al.*[5], except that 1 g of malt was used. The results were expressed as mg FAN/100 g dry weight.

Hot water (60 °C) extract. Samples of malt (7·78 g) were placed in 80 mL plastic tubes containing 62·22 g of distilled water. Extraction was conducted at 60 °C and the specific gravity of the extract determined as described by Morrall *et al.*[5]. The results were expressed as percentage dry weight.

RESULTS AND DISCUSSION

Effect of steeping conditions on malt quality

Sorghum malt quality for sorghum beer brewing is defined primarily in terms of DP and FAN[7,18]. DP is a measure of the joint *alpha*- and *beta*-amylase activity[19]. DP is especially important in the case of sorghum malt as the level of the *beta*-amylase enzyme in sorghum is intrinsically low[20,21]. FAN, the proteolytic breakdown products of endosperm proteins, composed of amino acids and small peptides, is important in brewing since it is the source of nitrogen for yeast during fermentation[22]. In sorghum beer brewing, it is particularly important as the FAN in the wort may be limiting due to the high proportion of unmalted cereal adjunct in the grist[18]. In conventional clear lager beer brewing, as opposed to opaque sorghum beer brewing, the most important malt quality criterion is extract[24]. Extract is a measure of how much malt will dissolve during the brewing process. This is a less important measure of malt quality in sorghum beer brewing as malt makes up only approximately 30% of the cereal grist[23].

In 1962 Novellie[7] reported that steeping time had little effect on the final DP of sorghum malt. Results of the present study, however, indicate that malt DP increased significantly with increasing steeping time (Table I; $P<0{\cdot}001$). In addition to DP, other malt quality parameters important for brewing, FAN and extract content, were improved significantly by increasing the steeping time (Tables II and III, respectively). Sorghum, unlike barley, must be watered during germination. Novellie[7], possibly believing that the grain hydration requirements were met on the floor (i.e. during germination), did not regard steeping time as important in determining malt quality. The present study, however, indicates clearly that the steeping times of 16 h or less as currently practised in South African maltings (some as low as 6 h) are sub-optimal. It is important to note, however, that unless the oxygen requirements of the grain can be satisfied, long steeping times may lead to anoxic

Table II Effect of steeping time, temperature and aeration on the free amino nitrogen content of sorghum malt (mg/100 g)

Steeping time (h)	Temperature (°C)								
	20			25			30		
	Aeration								
	AR	AB	NA	AR	AB	NA	AR	AB	NA
16	68	69	66	99	101	94	92	97	85
24	72	67	69	108	107	103	91	97	91
40	95	95	90	120	117	105	117	111	107

AR = air-rest, AB = air-blast, NA = non-aerated.

Analysis of variance for malt free amino nitrogen

Source of variation	Mean square	DF	F	P
Time	1030·57	2	208·82	0·000
Temperature	2146·78	2	435·00	0·000
Aeration	104·67	2	21·21	0·001
Time*Temperature	49·38	4	10·01	0·003
Time*Aeration	18·71	4	3·79	0·051
Temperature*Aeration	10·75	4	2·13	0·162
Residual	4·94	8		

DF = degrees of freedom, F = F-test, P = significance level.

conditions which may be compounded by microbial proliferation.

Analysis of variance indicated that steeping temperature also had a significant effect on the malt quality (Tables I, II and III). Malt DP was enhanced not only by an increase in the steeping time but also by an increase in the temperature of the steeping water ($P<0·001$). The maximum DP of 42·6 SDU/g was obtained in grains steeped for 40 h under AR, 30 °C conditions (Table I). With respect to malt FAN and extract content, it was apparent that 25 °C was optimum (Tables II and III). The highest values for malt FAN and extract (119·8 mg/100 g and 62·2%, respectively), were obtained from grain that had been steeped at 25 °C for 40 h under AR conditions for the former and for 24 h under aerated conditions for the latter. Steeping temperature has also been shown to be important in that, although cultivar related, steeping sorghum with a final warm water steep (40 °C for 6 h) improves the quality of the malt[12, 16]. In South Africa, few commercial malting operations have temperature-controlled steeping vessels. In winter the temperature of the steeping water can be as low as ±12°C and in summer as high as ±34 °C (unpub. data). These results show clearly the need for temperature control during steeping.

It has been stated that adequate oxygen is necessary for the formation of *alpha*-amylase and peptidase and that excessive carbon dioxide inhibits the formation of these enzymes even in the presence of sufficient oxygen[21]. In terms of FAN and to a lesser extent extract content, this would appear to hold true in that aeration during steeping improved the quality of the malt ($P<0·001$ and $P<0·05$, respectively) (Tables II and III). Analysis of variance, however, suggested that aeration during steeping did not significantly affect the DP of the malt ($P>0·05$; Table I). In other studies[13,15], however, even the length of the air rests employed during steeping were shown to significantly affect the quality of sorghum malt produced. The apparent discrepancy between these results may be explained by the fact that the FAN and extract assays measure the products of enzymic hydrolysis, whereas DP measures enzyme activity. During malting, DP has been shown to increase gradually and thereafter reach a plateau[3,25]. Therefore, it is probable that even if the enzyme activity of the

Table III Effect of steeping time, temperature and aeration on the extract content of sorghum malt (%)

Steeping time (h)	Temperature (°C)								
	20			25			30		
	Aeration								
	AR	AB	NA	AR	AB	NA	AR	AB	NA
16	48	49	47	58	59	56	57	54	54
24	53	51	48	62	62	58	57	58	55
40	56	56	52	61	61	59	54	61	58

AR = air-rest, AB = air-blast, NA = non-aerated.

Analysis of variance for malt extract

Source of variation	Mean square	DF	F	P
Time	33·00	2	12·02	0·001
Temperature	157·46	2	57·31	0·000
Aeration	19·12	2	6·96	0·018
Time*Temperature	5·75	4	2·10	0·173
Time*Aeration	2·27	4	0·83	0·541
Temperature*Aeration	1·79	4	0·65	0·641
Residual	2·75	8		

DF = degrees of freedom, F = F-test, P = significance level.

Table IV Effect of steeping time, temperature and aeration on malting losses (%)

Steeping time (h)	Temperature (°C)								
	20			25			30		
	Aeration								
	AR	AB	NA	AR	AB	NA	AR	AB	NA
16	16·2	16·5	16·1	17·7	17·3	17·2	18·0	17·5	17·4
24	16·2	16·1	16·1	17·5	18·2	17·1	17·1	17·5	17·1
40	16·5	16·6	15·8	17·1	17·1	15·5	18·3	17·7	16·3

AR = air-rest, AB = air-blast, NA = non-aerated.

Analysis of variance for malting losses

Source of variation	Mean square	DF	F	P
Time	0·29	2	4·11	0·058
Temperature	3·81	2	51·51	0·000
Aeration	1·06	2	15·07	0·002
Time*Temperature	0·30	4	4·26	0·039
Time*Aeration	0·54	4	7·61	0·008
Temperature*Aeration	0·15	4	2·18	0·161
Residual	0·07	8		

DF = degrees of freedom, F = F-test, P = significance level.

Table V Effect of steeping time, temperature and aeration on the steep-out moisture of sorghum grain (%)

Steeping time (h)	Temperature (°C)								
	20			25			30		
	Aeration								
	AR	AB	NA	AR	AB	NA	AR	AB	NA
16	30·5	30·9	30·6	31·6	31·9	31·8	33·2	32·8	34·1
24	nd[a]	31·1	30·4	31·8	33·5	32·5	34·6	34·2	34·4
40	33·5	32·9	32·6	34·4	35·0	33·6	35·9	36·5	35·2

[a] Not determined.
AR = air-rest, AB = air-blast, NA = non-aerated.

Analysis of variance for steep-out moisture

Source of variation	Mean square	DF	F	P
Time	11·54	2	66·60	0·000
Temperature	18·37	2	81·13	0·000
Aeration	0·38	2	1·75	0·242
Time*Temperature	0·16	4	0·74	0·592
Time*Aeration	0·43	4	1·96	0·206
Temperature*Aeration	0·24	4	1·03	0·434
Residual	0·22	7		

DF = degrees of freedom, F = F-test, P = significance level.

grains exposed to different levels of aeration had developed at different rates, this would not be reflected in the DP after 6 d germination.

Aeration and steeping temperature and the combined effect of time and aeration were found to have a significant effect on malting losses ($P<0·01$, $P<0·001$ and $P<0·01$, respectively; Table IV). Malting losses were generally retarded by steeping the grain for increasing periods in non-aerated water but the higher the steeping temperature the higher the losses accrued. These results are not unexpected as malting losses are an outcome of respiratory metabolism and, therefore, any condition that affects respiration and consequently malt quality will also affect the losses accrued.

Effect of steeping conditions on steep-out moisture

The steep-out moisture content of the grain was significantly affected by both steeping time and temperature ($P<0·001$). There was a general increase in steep-out moisture with increasing steeping time (16–40 h) and temperature (20–30 °C) (Table V). Analysis of variance indicated that aeration did not significantly affect the steep-out moisture of the grain ($P>0·05$).

It has been reported[3] that the moisture content of barley at the end of steeping (as long as air-rests are employed) is an effective way of selecting the steeping regime giving the highest hot water extract. In this study, a significant correlation was obtained between the steep-out moisture and malt DP ($R = 0·862$; accounting for 74·2% of the variation) (Fig. 1). Significant correlations were also found between steep-out moisture and FAN, and extract content ($R = 0·736$ and $R = 0·578$, accounting for 54·2% and 33·5% of the variation, respectively) (Fig. 1). It would appear, therefore, that steep-out moisture may similarly provide the sorghum maltster with a rough tool with which to select the steeping procedure that will maximise the malting quality of the grain. Although the absolute steep-out moisture may vary depending upon the grain size and cultivar, generally the higher the steep-out moisture the better the quality of the malt produced.

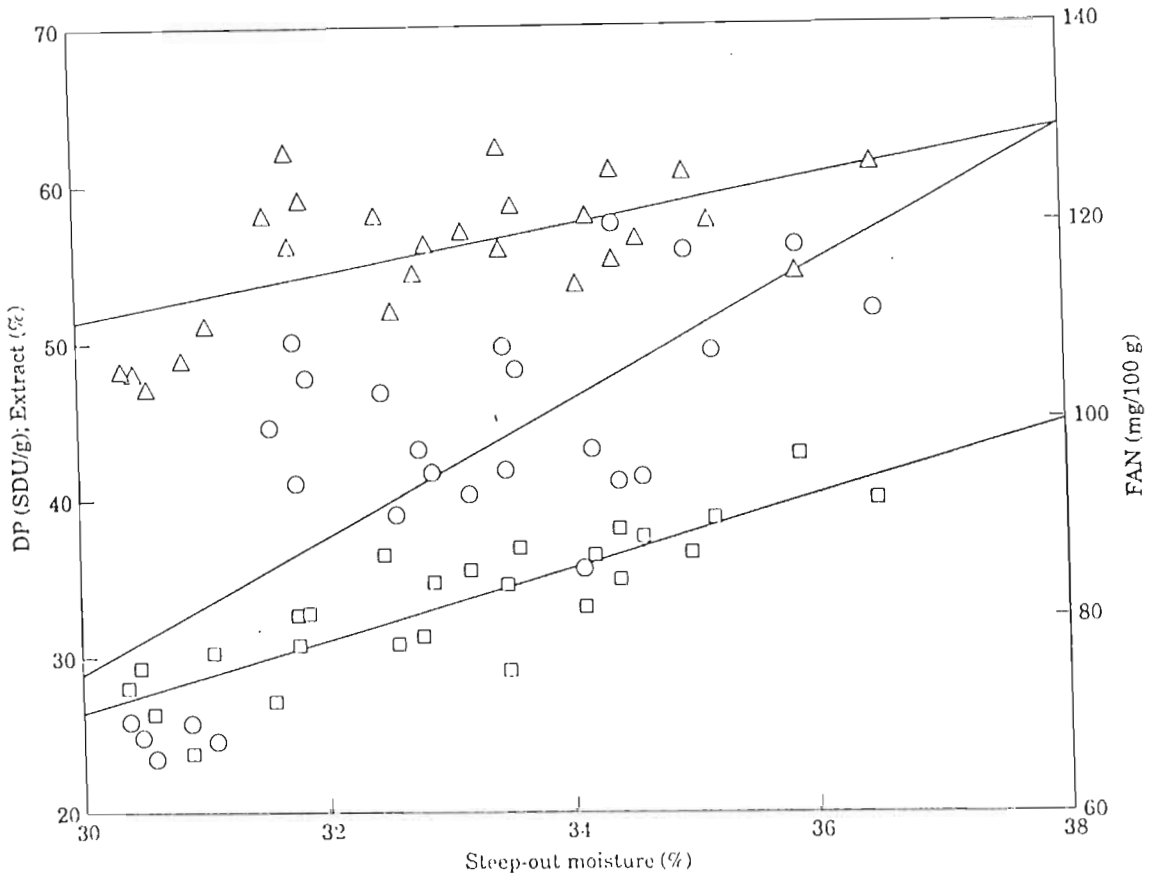

Figure 1 Relationship between steep-out moisture and sorghum malt quality (all data). □ = DP (SDU/g), ○ = FAN (mg/100 g), △ = Extract (%).

CONCLUSIONS

The findings of this study clearly indicate that in South Africa, commercial steeping practices are sub-optimal for sorghum. Sorghum malt quality is significantly affected by steeping time and temperature. Malt quality within the range of parameters studied, increases with steeping time (16–40 h) and the optimum steeping temperature is between 25 and 30 °C. Aeration during steeping was also shown to be necessary to maximise malt quality. The quality of the sorghum malt was found to be directly related to the steep-out moisture of the grain. Although further work is required, it is suggested that steep-out moisture may provide a means of estimating, at an early stage, the quality of finished malt. These findings have relevance for sorghum malting for both opaque and clear beer brewing.

Acknowledgement

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APPENDIX D

Dewar, J., Taylor, J.R.N. and Berjak, P. (1997). Effect of germination conditions, with optimised steeping, on sorghum malt quality - with particular reference to free amino nitrogen. *Journal of the Institute of Brewing*, **103**, 171-175.

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EFFECT OF GERMINATION CONDITIONS, WITH OPTIMISED STEEPING, ON SORGHUM MALT QUALITY—WITH PARTICULAR REFERENCE TO FREE AMINO NITROGEN

By J. Dewar

(CSIR Food Science and Technology, P.O. Box 395, Pretoria, 0001, South Africa)

J. R. N. Taylor

(University of Pretoria, Department of Food Science, Cereal Foods Research Unit, Pretoria, 0002, South Africa)

and P. Berjak

(University of Natal, Department of Biology, Plant Cell Biology Research Unit, Durban, 4041, South Africa)

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Using optimised steeping conditions for sorghum, the effect of various germination parameters (time, temperature and moisture) on the quality of sorghum malt for brewing purposes (in terms of diastatic power, free amino nitrogen and hot water extract) and on the associated malting losses, was investigated. Over the range studied (2, 4 and 6 days), the quality of the malt and the losses incurred during malting increased with increasing germination time. In general, the optimum germination temperature was between 25 and 30°C, and 18°C was found to be sub-optimal for the development of malt diastatic power. The quality of the finished malt and the associated malting losses were significantly correlated with the moisture content of the green malt. The root and shoot portion of the malt was found to be rich in free amino nitrogen (more than four times richer than the berry portion). Although a relatively small proportion of the total weight of the whole malt, the roots and shoots were found to contribute a substantial amount (as much as 61% under certain circumstances) to the whole-malt free amino nitrogen.

Key Words: *Sorghum, germination conditions, malting, diastatic power, free amino nitrogen*

Abbreviations: *DP: Diastatic power; FAN: free amino nitrogen; TKW: thousand kernel weight*

Introduction

Malted sorghum is widely used in southern Africa to brew the traditional alcoholic opaque sorghum beer[4,10,22]. There is also growing interest in the use of malted sorghum in the brewing of clear lager type beers[1].

The malting process comprises the operations of steeping, germination and drying. A number of factors, such as germination temperature, time and moisture content, are known to have an effect on the proper development of the enzymes synthesised during germination[16] and thus to affect the quality of the finished malt[14,26].

A number of workers have investigated the effects of malting conditions (i.e. germination conditions) on sorghum malt quality[11,13,16,19]. However, until recently[5,8,9,23–25,29], there has been little research into the effect of different conditions of steeping on subsequent sorghum malt quality. We[5] identified that steeping time, temperature and aeration significantly affect the quality of sorghum malt produced in terms of diastatic power (DP), free amino nitrogen (FAN) and extract content. Because the optimum conditions for sorghum germination may be dependent on the prior steeping conditions, the work reported here, utilises the optimised steeping conditions for sorghum[5] to investigate the effect of various germination conditions (*viz.* germination time, temperature and moisture) on sorghum malt quality.

Morrall *et al.*[14] found that 24 and 28°C were equally good for the development of sorghum malt DP, FAN and extract, and that higher temperatures were progressively worse. The effect of malting sorghum at temperatures lower than 24°C, however, has not been addressed, despite the fact that barley is generally malted at much lower germination temperatures[3]. In South Africa, during the winter months, sorghum is often malted at temperatures far below the 24°C investigated by Morrall *et al.*[14]. An additional aim of this study was, therefore, to investigate the effect of germinating sorghum at a temperature considered suitable for barley.

A major objective of sorghum malting for sorghum beer brewing is to produce malt with a high FAN content. FAN is produced during malting by the action of the endogenous proteinase and peptidase enzymes on the protein reserves of the grain[6]. The breakdown products of the proteolytic action are amino acids and small peptides and are collectively referred to as FAN. During the brewing process, FAN is required by yeast as a source of nutrition in fermentation[2,28]. FAN can be particularly limiting in sorghum beer brewing, where there is a low ratio of malt to adjunct[20,21] and also in lager beer brewing with unmalted grain where only small amounts of sorghum malt are added to supply yeast nutrients[15]. In this study, particular emphasis is given to the development and location of FAN in the malt, which despite the acknowledgment of its importance in brewing[2], has received little attention.

Materials

Grain

Sorghum (*Sorghum bicolor* (L.) Moench) grain, cultivar Barnard Red, was used. This grain had good germinability (germinative energy > 95%).

Germination vessels

The germination apparatus comprised six perspex germination vessels (each of 2 kg capacity) and a 600×900×230 mm stainless steel box (in which the vessels were placed). Each of

TABLE I. Effect of low and high watering levels on the green malt moisture content of sorghum malt (%)

Germination time (days)	Low	High
0	30.4[1]	30.4
2	43.5±1.2[2]	49.2±2.7[2]
4	56.1±1.8[2]	66.2±3.8[2]
6	67.3±2.4[2]	73.8±4.5[2]

[1] = mean of three germination temperatures
[2] = standard deviation

the malt. The temperature of the germinating grain was continually controlled and monitored in each of the vessels by computer.

Methods

Steeping

The steeping conditions utilised were those found previously to be optimal[5]. Samples of grain (2 kg) were placed into 600×600 mm nylon bags and steeped for 24 hours at 25°C in steeping vessels (of two kg capacity). The vessels were drained of water every three hours and refilled with fresh 25°C tap water after one hour of air-rest. At the end of the steeping period, excess surface-held water was removed from the grain by centrifugation in a domestic spin drier (one minute at 200×g).

The conditions of steep were controlled and monitored by computer.

Germination

Samples of steeped sorghum grain (1.2 kg) were germinated at a pre-determined temperature (18, 25 and 30°C), for one of three different times (i.e. 2, 4 and 6 days), under either high or low watering conditions. A measured volume of distilled water was sprayed onto the malt by means of an atomiser spray and the grain was turned. For the "low" watering treatment, the grain was watered once daily with a set 300 ml of water. For the "high" watering treatment the grain was watered twice daily with an excess of distilled water. Table I shows the moisture content of the green malt for the low and high watering treatments.

Drying

After the pre-determined malting time, germination was arrested by drying the malt for 24 hours in a forced-draft oven set at 50°C.

Analyses

Contribution of roots and shoots

A 100 g sample of each of the malts was polished as described by Morrall *et al.*[14] (i.e. the roots and shoots were removed from the berries) and the contribution by weight, of roots and shoots to the whole malt determined.

Malting loss—thousand kernel weight (TKW)

Malting loss was estimated by calculating the thousand kernel weight (TKW) of the dried, polished malt and comparing this with the TKW of the dry grain.

Diastatic power (DP)

DP was determined according to the South African Bureau of Standards method 235[30], except that water was used as the extractant, and 5 g of malt used. The volume of the extractant was reduced accordingly. The results were expressed as sorghum diastatic units (SDU)/g dry weight.

Free amino nitrogen (FAN)

FAN analysis was conducted on: whole malt, roots and shoots, and berries. The FAN content was determined according except that 1 g of the sample was used. The results were expressed as mg FAN/100 g dry weight.

A second set of malt samples (malted previously in our laboratories and stored in airtight containers at 4°C), which had been germinated for various germination times, at 28°C and under the "high" moisture level of Morrall *et al.*[14] was also analysed.

Hot water (60°C) extract

Samples of malt (7.78 g) were placed in 80 ml plastic tubes containing 62.22 g of distilled water. Extraction was conducted at 60°C and the specific gravity of the extract determined as described by Morrall *et al.*[14]. The results were expressed as a percentage dry weight.

Results and Discussion

Diastatic power

A primary objective of malting is to promote the development of hydrolytic enzymes which are not present in the ungerminated grain. The development of the amylase enzymes during malting is of critical importance. These enzymes are required to hydrolyse the malt and adjunct starch to fermentable sugars when the malt is used to brew beer[1]. Sorghum malt quality is assessed primarily in terms of DP, which is a measure of the joint activity of α- and β-amylase. Malt DP was significantly affected by germination time ($p \leq 0.001$), temperature ($p \leq 0.001$), watering level ($p < 0.05$), the combined effect of time and temperature ($p < 0.05$), and time and watering level ($p < 0.05$) (Table II). At 18°C, malt DP increased almost linearly with germination time (Table II). However, at 25°C (no significant difference was found between 25 and 30°C malts) the increase in the DP was more marked over the first four days (Table II). Indeed, it took the 18°C germinated grains at least six days to attain a DP approaching that obtained by a 25 or a 30°C malt in just four days (Table II). Extending the germination time beyond four days, at germination temperatures of 25 and 30°C, did not produce an improvement, and when germination was conducted under the high watering condition, actually occasioned a decline in malt DP (Table II). Of the six day, 25°C germinated malts, those that received the high watering treatment had a green malt moisture content of 77% and a DP of 29 SDU/g, whereas those that received the low watering treatment had a 55% higher DP and a lower green malt moisture content of 69%. The high moisture content late in germination, may have indirectly had a negative effect on the malt DP, as has

TABLE II. Effect of germination time, temperature and watering level (low and high) on the diastatic power of sorghum malt (SDU/g)

Germination time (days)	Germination conditions					
	18°C		25°C		30°C	
	Low	High	Low	High	Low	High
2	15	12	27	29	31	32
4	30	23	45	46	43	45
6	43	31	45	29	45	39

Analysis of variance table for diastatic power

Source of variation	Mean square	DF	F	P
Time	821.78	2	61.89	0.001
Temperature	624.78	2	47.05	0.001
Watering	80.22	1	12.08	0.025
Time * Temperature	219.22	4	8.26	0.032
Time * Watering	115.11	2	8.67	0.035
Temperature * Watering	30.11	2	2.27	0.219

TABLE III. Effect of germination time, temperature and watering level (low and high) on the free amino nitrogen content of sorghum malt (mg/100 g)

Germination time (days)	Germination conditions					
	18°C		25°C		30°C	
	Low	High	Low	High	Low	High
2	110	96	72	92	102	110
4	176	159	133	165	141	106
6	167	159	177	230	131	213

Analysis of variance table for free amino nitrogen

Source of variation	Mean square	DF	F	P
Time	10372.69	2	22.73	0.006
Temperature	235.44	2	0.52	0.631
Watering	821.88	1	1.80	0.250
Time * Temperature	993.71	4	2.18	0.234
Time * Watering	990.64	2	2.17	0.229
Temperature * Watering	904.98	2	1.98	0.252
Residual	456.30	4		

been suggested by Novellie[18-19] and Morrall *et al.*[14]. A more likely explanation is that even the relatively low drying temperature of 50°C may have been inappropriate for such high moisture content malts, damaging the amylase enzymes in the process. This is supported by the fact that the high moisture continued to have a beneficial effect on FAN, the products of protease activity, late in germination (see below). Irrespective of the reason, it would appear that when sorghum is malted at relatively high germination temperatures it is necessary to control the length of the germination period critically.

Free amino nitrogen

The FAN content of the sorghum grain was significantly enhanced, as much as eight fold, by malting (i.e. from 28 mg 100 g^{-1} for the ungerminated grain to 230 mg 100 g^{-1} when malted at 25°C under the high watering conditions) (Table III). Malt FAN increased significantly with increasing germination time ($p<0.01$). These findings support those of Nout and Davies[17] and Evans and Taylor[7] who reported that the proteolytic activity of sorghum malt increased with germination time. Similarly, Morrall *et al.*[14] reported an increase in malt FAN up to six days of germination. In general, malt FAN was not statistically significantly affected by germination temperature, nor the watering treatment administered (Table III). However, when germination was conducted at 25 or 30°C, the high watering treatment did appear to benefit the final FAN content of the malt (Table III). Similarly, Morrall *et al.*[14] reported that FAN was greatest in malts germinated at high moistures when high germination temperatures were employed.

In South Africa, sorghum malt, unlike the normal practice with barley malt, is traded and brewed complete with external roots and shoots. It is known that the roots and shoots are rich in nitrogenous compounds[31]. However, until now the effect of germination conditions on the FAN content of the component parts of the malt has not been studied. Although analysis of variance indicated that neither germination temperature nor the watering regime administered affected the FAN content of the whole malt significantly (Table III), these variables did appear to affect significantly the FAN contributed by the component parts of the malt, particularly the root and shoot portion (Table IV). The FAN contributed by the roots and shoots was significantly affected not only by germination time ($p<0.001$), but also by germination temperature ($p<0.01$), especially from 18 to 25°C (25 and 30°C were not significantly different). With respect to the level of watering, it appeared that the high watering level improved the FAN content of the roots

TABLE IV. Effect of germination time, temperature and watering level (low and high) on the contribution of the root and shoot component to the free amino nitrogen content of whole sorghum malt (mg/100 g)

Germination time (days)	Germination conditions					
	18°C		25°C		30°C	
	Low	High	Low	High	Low	High
2	19 (17)[1]	25 (26)	19 (26)	36 (39)	25 (25)	34 (31)
4	55 (31)	60 (38)	56 (42)	79 (48)	61 (43)	68 (64)
6	81 (49)	74 (47)	79 (45)	95 (41)	87 (66)	90 (42)

[1] = Figures in parentheses represent the percentage contribution of the roots and shoots to the whole malt FAN

Analysis of variance table for free amino nitrogen—roots and shoots

Source of variation	Mean square	DF	F	P
Time	5153.44	2	955.72	0.000
Temperature	140.29	2	26.02	0.005
Watering	339.74	1	63.01	0.001
Time * Temperature	5.27	4	0.98	0.509
Time * Watering	27.56	2	5.11	0.079
Temperature * Watering	119.78	2	22.21	0.007
Residual	5.39	4		

The roots and shoots contained a far higher concentration of FAN than the berries (between 4 and 7 times more) (Table V). Also, the relative contribution of the roots and shoots to the total malt FAN increased with germination time (Table IV). Thus, although the roots and shoots represented only a relatively small proportion of the total mass of the malt (Table V), after six days of germination at 25°C, under the high watering condition, 95 mg FAN was contributed by the roots and shoots (41% of the total malt FAN) (Table IV), which accounted for only 21% of the total weight of the malt (dry weight basis). The dramatic increase in FAN in the roots and shoots can be explained as a result of translocation of the products of storage protein breakdown from the kernel[31].

Analysis of the FAN content of a second and independent batch of malt samples, germinated at 28°C under the "high" watering conditions of Morrall *et al.*[14], support these findings. With respect to the contribution that the roots and shoots made to the FAN content of the whole malt, this initially increased with germination time with a peak of 62% being reached at day four (Figure 1). This is particularly significant when it is realised that at day four the roots and shoots represented only 17.5% of the dry weight of the whole malt.

These findings are of importance to sorghum beer brewing where malt of a high FAN content is required. It is clear that even a relatively small loss of roots and shoots from the malt may represent a relatively large loss of FAN. Therefore, care should be exercised during malt processing to minimise root

TABLE V. Free amino nitrogen content and relative weight of the component parts of sorghum malt after various germination times (mg/100 g material)

Germination time (days)	Roots and shoots	Berries
2	585[1] a[2] (4.6)[3]	79 x (95.4)
4	553 b (12.0)	106 y (88.0)
6	500 c (17.1)	127 z (82.9)

[1] = Mean of six values
[2] = Dissimilar letters denote a statistically significant difference ($p < 0.05$)

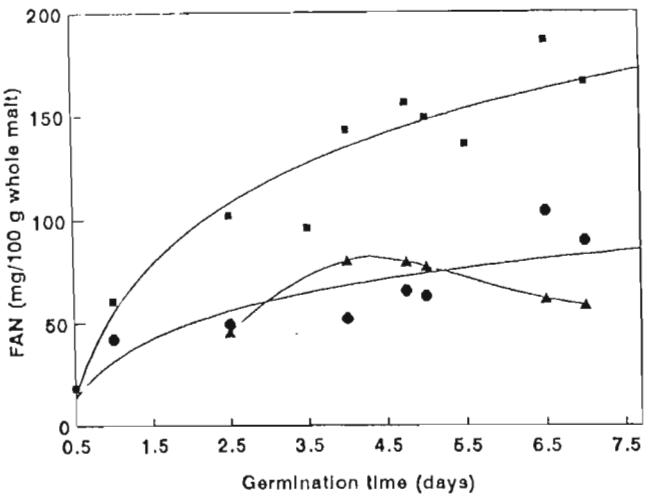

FIG. 1. Contribution of the berries and roots and shoots to the FAN content of whole sorghum malt at 28°C, high moisture (Morrall *et al.*[14]). ■, Whole malt; ▲, roots and shoots; ●, berries.

TABLE VI. Effect of germination time, temperature and watering level (low and high) on the extract content of sorghum malt (%)

Germination time (days)	Germination conditions					
	18°C		25°C		30°C	
	Low	High	Low	High	Low	High
2	38	36	41	41	54	59
4	68	62	65	61	54	78
6	75	77	70	60	70	77

Analysis of variance table for extract

Source of variation	Mean square	DF	F	P
Time	1163.17	2	33.596	0.003
Temperature	124.78	2	3.604	0.127
Watering	10.89	1	0.315	0.610
Time * Temperature	80.93	4	2.338	0.215
Time * Watering	9.36	2	0.270	0.776
Temperature * Watering	120.67	2	3.485	0.132
Residual	34.62	4		

and shoot losses. In addition, if sorghum malt and large quantities of unmalted cereal adjunct are to be used for conventional clear beer brewing, where the roots and shoots of the malt are routinely removed and discarded, attention should be given to the supply of a source of FAN in the wort. Failure to consider this could result in fermentation problems due to limited yeast growth[28].

Extract

Malt extract, as with DP and FAN, was significantly affected by germination time ($p<0.01$) increasing especially over the first four days of germination (Table VI). This result agrees with the findings of Jayatissa *et al.*[12] and Morrall *et al.*[14] who showed that hot water extract increased with germination time.

Morrall *et al.*[14] reported that there was no significant difference in sorghum malt extract when germination was conducted at either 24 or 28°C. However, at higher germination temperatures significantly less was produced. Under the conditions of the present study, the amount of extract obtainable from the malt was not affected significantly by germination temperature over the range 18, 25 and 30°C. It would appear that unlike the higher germination temperatures investigated by Morrall *et al.*, a lower germination temperature of 18°C is not detri-

TABLE VII. Effect of germination time, temperature and watering level (low and high) on the thousand kernel weight (g) of sorghum malt (dry weight basis)

Germination time (days)	Germination conditions					
	18°C		25°C		30°C	
	Low	High	Low	High	Low	High
2	29.3	28.0	23.2	22.0	24.0	22.6
4	29.2	30.3	19.6	17.3	20.9	20.5
6	25.3	21.7	16.9	15.1	18.3	17.6

Analysis of variance table for thousand kernel weight

Source of variation	Mean square	DF	F	P
Time	50.25	2	45.27	0.001
Temperature	115.54	2	104.09	0.000
Watering	7.69	1	6.93	0.058
Time * Temperature	4.26	4	3.83	0.110
Time * Watering	0.84	2	0.76	0.525
Temperature * Watering	0.35	2	0.31	0.746
Residual	0.11	4		

rate of sugar utilisation could be slower at the lower temperature.

Malting losses

Malting loss, as indicated by the thousand kernel weight (TKW) of the malt, was significantly affected by germination time ($p\leq0.001$) and temperature ($p<0.001$) (Table VII). As Pathirana, Sivayogasundaram and Jayatissa[27] have reported, the malting losses (as indicated by a reduction in TKW) were proportional to the numbers of days allowed for germination. The TKW decreased, and consequently the malting losses increased, quite markedly when the temperature was raised from 18 to 25°C (no significant difference was found when the temperature was raised from 25 to 30°C) (Table VII).

Malt moisture

Recently, we have shown that the higher the steep-out moisture, the higher the quality of the resulting malt in terms of DP, FAN and extract[5]. Similarly, in this study highly significant correlations were found between the moisture content of the green malt and DP ($p<0.01$), FAN and extract ($p<0.001$) (Figure 2). It would appear, therefore, that moisture content, whether it is at the end of steeping[5] or at the end of the malting process itself, is an important indicator of sorghum

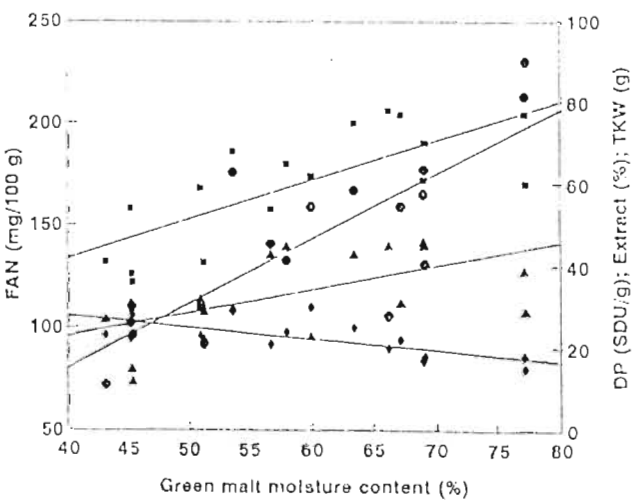

FIG. 2. Relationship between green malt moisture content and sorghum malt quality (all data). (DP, SDU/g; FAN, mg/100 g; Extract

malt quality for brewing purposes. It should be noted that in South Africa, the moisture content of sorghum malted under commercial sorghum malting conditions is often far less than 50% (fresh weight basis, unpublished data). This is considerably lower than that obtained at six days of germination under even the low watering condition (Table I). It would appear that a useful way of enhancing the quality of sorghum malt produced commercially in South Africa, would be to enhance the moisture content of the germinating grain during malting. However, malting losses, as indicated by TKW, were also significantly correlated with green malt moisture ($p<0.01$) (Figure 2). Therefore, in maximising the quality of the malt care should be taken not to accrue excessive malting losses.

Conclusions

Under optimum steeping conditions, the optimum germination temperature for sorghum malting is between 25 and 30°C. The findings indicate clearly that where germination is conducted at lower temperatures (18°C), as is sometimes done in South Africa during the winter months, this would be sub-optimal for the development of malt DP. The quality of the malt and the associated malting losses have been found to be directly related to the moisture content of the green malt. The root and shoot portion of the malt was found to be an extremely rich source of FAN, and to contribute a substantial amount to the malt FAN. Thus, where sorghum malt is being used as a critical source of FAN care should be exercised during malt processing to minimise the loss of roots and shoots.

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APPENDIX E Dewar, J., Orovan, E. and Taylor, J.R.N. (1997). Effect of alkaline steeping on water uptake and malt quality in sorghum. *Journal of the Institute of Brewing*, **103**, 283-285.

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EFFECT OF ALKALINE STEEPING ON WATER UPTAKE AND MALT QUALITY IN SORGHUM

BY J. DEWAR AND E. OROVAN

(CSIR Food Science and Technology, P.O. Box 395, Pretoria 0001, South Africa)

AND J. R. N. TAYLOR

(Cereal Foods Research Unit, Department of Food Science, University of Pretoria, Pretoria 0002, South Africa)

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Condensed tannin-free and high-tannin sorghum grain were steeped in dilute alkali and the effects on water uptake during steeping and final malt quality were determined. With the condensed tannin-free sorghum, steeping in dilute alkali led to increased water uptake during steeping and an improvement in malt quality in terms of diastatic power and free amino nitrogen. These effects are presumed to be due to the alkali opening up the pericarp cell wall structure of the grain, allowing more rapid water uptake. Best results were obtained by application of alkali early in steeping, presumably because the grain is less susceptible to alkali toxicity than later in steeping. Steeping high-tannin sorghum in dilute alkali did not improve malt quality. This appeared to be because water uptake was not increased to the same extent as with condensed tannin-free sorghum, as the alkali reacted with the tannins.

Key Words: *Sorghum, alkaline steep, malting, diastatic power, free amino nitrogen, water uptake.*

Abbreviations: DP: Diastatic power; FAN: free amino nitrogen.

INTRODUCTION

Recent research has shown that the quality of sorghum malt, with respect to diastatic power (DP), free amino nitrogen (FAN) and hot water extract, is highly significantly correlated with the steep-out moisture content of the grain[1]. Other recently published research has indicated that steeping sorghum in dilute alkali can increase the DP[5] and FAN[6] content of the malt, although the effect appeared to be cultivar dependent. These latter publications[5,6] offered no explanation as to the mechanism by which alkali steeping affected sorghum malt quality. However, in view of the fact that alkali is known to disrupt the molecular structure of the non-starch polysaccharides which make up the structure of sorghum cell walls[10], the relationship between alkali steeping, water uptake and sorghum malt quality was investigated.

EXPERIMENTAL

Grain

Two sorghum cultivars of good germinability (Germinative Energy > 95%) were used: NK 283, a condensed tannin-free hybrid (South African sorghum class GM) and a high-tannin hybrid (1.3% polyphenols) (South African sorghum class GH) (cultivar unknown).

Steeping

Samples of pre-washed, spin-dried (one min at 300×g) sorghum grain (100 g), were steeped in a still solution of NaOH (0 (control), 0.1, 0.3 and 0.5% w/v) for a period of 8 h during either the first 8 h (0–8 h) or during the last 8 h (16–24 h) of a 24 h steeping period. After the alkali steep, the grain was rinsed thoroughly in fresh tap water. For the remainder of the steep, the grain was steeped in continuously changing tap water (±16°C). At the end of steeping, the grain was spin-dried (one

Germination

The grain was germinated in a water-jacketed incubator (Forma Scientific, Marrietta, USA) set at 25°C and 100% relative humidity. Twice daily, the bags were removed from the incubator, the grain turned (to avoid meshing of the roots and shoots) and immersed for 10 min in tap water. Following the short steep, the grain was spin-dried (one min at 300×g) to remove the excess surface-held water and returned to the incubator.

Drying

After six days from the beginning of steeping, germination was arrested by drying the malt for 24 h in a forced draft oven set at 50°C.

Analyses

DP was measured according to the standard method for sorghum malt[8], except that 5 g malt was used and the quantity of extractant reduced accordingly. Both 2% peptone and distilled water were used as extractants. FAN was determined as described[3], except that 1 g of malt was used and the amount of extractant increased accordingly.

The amount of water taken up during steeping was determined and expressed as a percentage of the pre-washed, non-steeped grain weight.

RESULTS AND DISCUSSION

Figures 1 and 2 show the effect of steeping the condensed tannin-free sorghum in different concentrations of NaOH on water uptake during steeping and on DP and FAN in the final malt. It can be seen that water uptake increased almost linearly with increasing NaOH concentration, presumably as a consequence of disruption of the pericarp non-starch polysaccharide cell wall material by the NaOH. Specifically, alkali is known to saponify acetyl groups and other ester linkages, cause cellulose to swell and disrupt the hydrogen bonds between hemicelluloses, resulting in the solubilisation of hemicelluloses[10]. Both malt DP and FAN were increased substantially by steeping in 0.1% NaOH, by 34% and 33%, respectively when NaOH was administered during the first 8 h of steeping (Fig. 1) and by 21% and 22%, respectively when

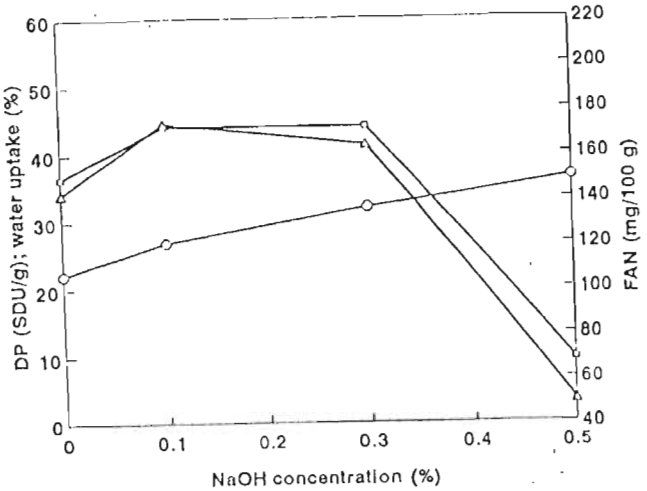

FIG. 1. Effect of steeping (0–8 h) in different concentrations of NaOH on water uptake and sorghum malt quality—condensed tannin-free sorghum. –□–, DP (water extract), –△–, FAN, –○–, water uptake.

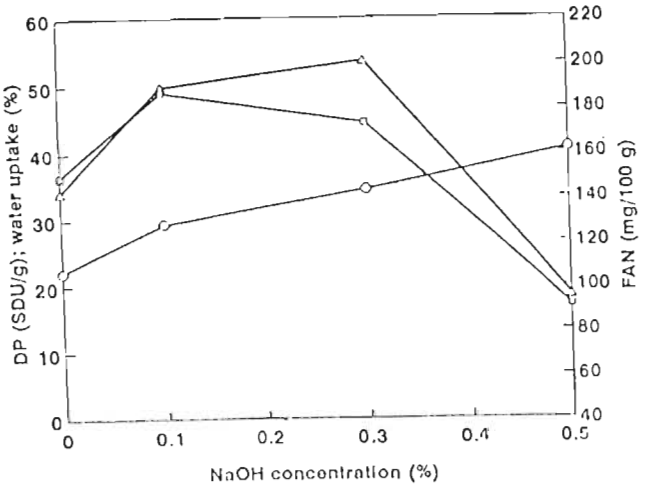

FIG. 2. Effect of steeping (16–24 h) in different concentrations of NaOH on water uptake and sorghum malt quality—condensed tannin-free sorghum. –□–, DP (water extract), –△–, FAN, –○–, water uptake.

further. Steeping in 0.5% NaOH resulted in levels of malt DP and FAN which were far lower than when the grain had been steeped in water only, presumably as a result of the phytotoxicity of the high concentration of NaOH.

The fact that DP, a measure of joint alpha- and beta-amylase enzymic activity[4], and FAN, a measure of the products of proteolysis[2], were similarly increased by steeping in 0.1% NaOH suggests that the effect was due to increased metabolic activity in the malt. Such an effect can be attributed to the more rapid hydration of the grain brought about by the NaOH. The lower increase in DP and FAN obtained when NaOH was administered during the last 8 h of steeping (Fig. 2), as opposed to the first 8 h (Fig. 1), is possibly due to the later administration bringing about a smaller increase in hydration. Alternatively or additionally, it could be due to the fact that as the germinating grain became more metabolically active it became more susceptible to the toxic effects of the NaOH. This latter explanation is supported by the fact that the administration of 0.5% NaOH during the first 8 h of steeping gave levels of DP and FAN of 47% and 67%, respectively of the water control, whereas NaOH administration during the last 8 h gave only 26% and 35%, respectively.

With the high-tannin sorghum, the administration of NaOH was examined only over the first 8 h of steeping, in view of the

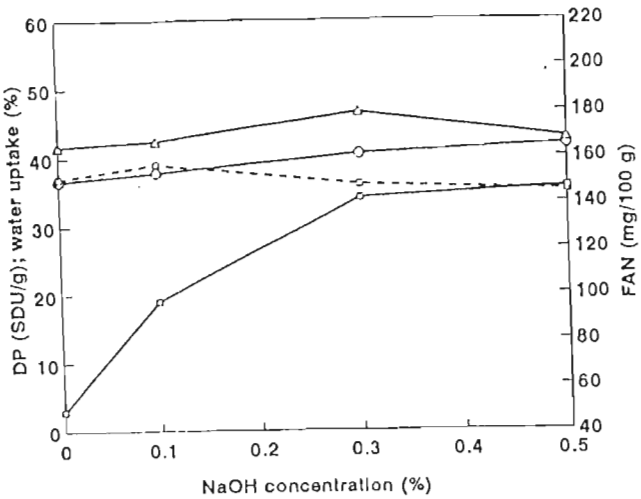

FIG. 3. Effect of steeping (0–8 h) in different concentrations of NaOH on water uptake and sorghum malt quality—high-tannin sorghum. –□–, DP (water extract), - - -□- - -, DP (peptone extract), –△–, FAN, –○–, water uptake.

as with the condensed tannin-free sorghum, water uptake increased with increasing NaOH concentration, although the increase in water uptake was much lower. The water uptake of the high-tannin sorghum control was, however, considerably greater than that of the condensed tannin-free sorghum control (Fig. 1). It has been observed that high-tannin sorghums take up more water than condensed tannin-free types (pers. comm. Dr K. H. Daiber, Sorghum Beer Unit, CSIR (retired)). The probable explanation for this is that high-tannin sorghums normally have a softer endosperm texture[11]. This is borne-out by the fact that this high-tannin sorghum had both a lower 1000 kernel weight and hectolitre weight (21.6 g and 70.7 kg versus 24.7 g and 73.4 kg for the condensed tannin-free sorghum).

Unlike the situation with the condensed tannin-free sorghum (Fig. 1), increasing concentrations of NaOH had little effect on malt FAN and DP (as measured by peptone extraction) (Fig. 3). Low concentrations of NaOH did not increase malt DP and FAN, and they were not reduced by high NaOH concentrations. It should be noted that when determining the DP of malts made from high-tannin sorghum it is necessary to extract the amylases in the presence of peptone, otherwise they will be inactivated by the tannins, giving an artificially low value for DP[9].

The lack of a positive or negative effect of NaOH steeping with the high-tannin sorghum can be attributed to the NaOH reacting preferentially with the tannins. Treatment with alkali is a well-described method of inactivating the tannins in sorghum[7]. This reaction effect is borne-out by the fact that the pH of the high-tannin sorghum steep liquors at the end of steeping from all three alkali steeps (0.1, 0.3 and 0.5% NaOH) were considerably lower than those of the corresponding steep liquors from the condensed tannin-free sorghum (Table I). In fact, at the end of the 8 h steep, the pH of the high-tannin sorghum 0.5% NaOH steep liquor (pH 11.1) was lower than

TABLE I. pH of the steep liquor of the condensed tannin-free (GM) and the high-tannin (GH) sorghums at the start and the end of 8 h steeping in various concentrations of NaOH

NaOH concentration (%)	pH at start of 8 h treatment		pH at end of 8 h treatment	
	GM	GH	GM	GH
0	7.4	7.3	7.3	7.6
0.1	12.0	12.0	10.1	8.9
0.3	12.3	12.3	11.0	10.2

that of the 0.3% NaOH steep liquor for the condensed tannin-free sorghum (pH 11.9). The results of the reaction between the NaOH with the condensed tannins can be seen clearly from the fact that when the grain was steeped in high concentrations of NaOH, the water and peptone extract DPs were virtually the same (34 and 36 SDU/g, respectively for 0.3% NaOH and 35.5 and 35 SDU/g, respectively for 0.5% NaOH) (Fig. 3). In contrast, when the grain was steeped in water only, the water extract DP was only 3 SDU/g whereas the peptone extract DP was 37 SDU/g. In other words, at the higher NaOH concentrations the reaction between the NaOH and the tannins prevented reaction between the tannins and the malt amylase enzymes. It is suggested that the preferential reaction between the alkali and the tannins reduced the effect of the alkali on the sorghum cell walls. Hence, the increase in water uptake due to alkali steeping high-tannin sorghum (Fig. 3) was lower than for alkali steeping condensed tannin-free sorghum (Fig. 1).

Conclusions

It appears that the improvement in sorghum malt quality brought about by steeping in dilute alkali is due to increased water uptake during steeping. This is presumed to be as a result of the alkali disrupting the sorghum pericarp cell wall structure. The improvement in malt quality appears only with condensed tannin-free sorghum, as in high-tannin sorghum the alkali reacts with the tannins. This difference in the effect of alkali between condensed tannin-free and high-tannin sorghum may account for some of the cultivar effect differences reported in previous work on alkali steeping of sorghum[5,6].

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APPENDIX F Dewar, J., Taylor, J.R.N and Berjak, P. (1998). Changes in selected plant growth regulators during germination in sorghum. *Seed Science Research*, **8**, 1-8.

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Changes in selected plant growth regulators during germination in sorghum

J. Dewar*[1], J. R. N. Taylor[2] and P. Berjak[3]

[1]CSIR Food Science and Technology, PO Box 395, Pretoria, 0001, South Africa
[2]Department of Food Science, University of Pretoria, Cereal Foods Research Unit, Pretoria, 0002, South Africa
[3]Department of Biology, University of Natal, Plant Cell Biology Research Unit, Durban, 4000, South Africa

Abstract

The technique of radioimmunoassay following sample resolution by HPLC was used to assay the amounts of the cytokinins zeatin (Z), zeatin riboside (ZR) and isopentenyladenine (IPA), the combined amounts of gibberellins$_{1,3}$ (GA$_{1,3}$), and the amounts of indole acetic acid (IAA) and abscisic acid (ABA) during germination in grains of sorghum. Concentrations of GA$_{1,3}$ were low throughout germination and did not appear to be related to the time of germination. In the mature, non-germinated grain, the concentration of each of the other plant growth regulators was much higher in the smaller component comprised of the embryonic axis and scutellum than in the much larger endosperm tissue. During the germination period studied (64 h), these concentrations declined, with a peak in the amount of the cytokinin IPA and a small peak in Z+ZR (24 h) in the embryo following the first visible signs of root protrusion and coincident with a large enhancement in amylase activity. The high concentration of ABA in the embryo tissue prior to germination was noteworthy. It is suggested that the interaction of ABA and the cytokinins IPA and Z+ZR may play a significant role in controlling sorghum germination.

Keywords: germination, malting, plant growth regulators, sorghum.

Introduction

A variety of internal and external factors is known to be important in controlling germination in seeds. Paleg (1960) and MacLeod *et al.* (1964) demonstrated that on germination gibberellic acid (GA$_3$) induces barley aleurone cells to produce several endosperm-degrading enzymes.

During the process of malting (i.e. the germination of cereal grains in moist air under controlled conditions) a number of hydrolytic enzymes develop and degrade the reserve food materials of the endosperm (Bewley and Black, 1978). Barley malting practices have taken advantage of the knowledge that the application of the plant growth regulator (PGR) GA$_3$ can dramatically enhance the synthesis of the hydrolytic enzyme, α-amylase. The action of α-amylase is of critical importance in malting as it initiates the breakdown of starch granules. This so-called modification is essential to hydrolyse the starch into fermentable sugars when malt is used by the brewing industry for beer making.

In Africa, sorghum malt is used for the brewing of traditional opaque-type beers (Novellie and De Schaepdrijver, 1986; Haggblade and Holzapfel, 1989; Daiber and Taylor, 1995). Recently, in tropical countries which are not suitable for the cultivation of barley, there has been interest in substituting sorghum malt for barley malt for the brewing of 'clear' beers (Ajerio *et al.*, 1993). Compared with barley malt, sorghum malt has low β-amylase activity, the α-amylase activity being similar (Aniche and Palmer, 1990), and therefore worts (unfermented malt infusions) produced using sorghum malt generally have lower fermentability than barley malt. There have been reports that the application of GA$_3$ can promote shoot growth in sorghum (Morgan *et al.*, 1977; Wright *et al.*, 1983; Rood, 1995). However, unlike the situation for barley, there is contradictory evidence as to whether the application of GA$_3$ to germinating sorghum grain increases amylase activity. Daiber and Novellie (1968) and Asien *et al.* (1983) found little stimulation of amylase activity. However, other studies have indicated that application of GA$_3$ can be used to improve the amylase activity of sorghum malt

*Correspondence
Fax: 00 27 12 841 2386
E-mail: jdewar@csir.co.za
Abbreviations: plant growth regulator (PGR), radioimmunoassay (RIA), zeatin (Z), zeatin riboside (ZR), isopentenyladenine (IPA), gibberellin$_{1,3}$ (GA$_{1,3}$), indole acetic acid (IAA) and abscisic acid (ABA).

(Agu *et al.*, 1993; Nzelibe and Nwashike, 1995). The stimulatory effect, however, appears to be somewhat variety-dependent and in some cases, the application of GA_3 actually reduced amylase activity (Nzelibe and Nwashike, 1995). Hence, current sorghum malting practices tend to rely almost entirely on the provision of suitable environmental conditions to initiate germination and promote the development of the essential malt hydrolytic enzymes (Morrall *et al.*, 1986; Dewar *et al.*, 1997a,b).

This study was initiated in an attempt to elucidate the possible control mechanisms involved in sorghum germination. A combined HPLC-radioimmunoassay (RIA) technique was used to determine the amounts of selected endogenous PGRs from the groups auxins, cytokinins, gibberellins and abscisic acid in sorghum at various stages of germination.

Materials and methods

Material

Sorghum cv. NK 283 grain, a cultivar widely grown in South Africa and recommended for malting purposes, was used. To minimize fungal contamination during germination, the grain was given a hot-water treatment (immersed in 55°C water for 10 min) (Berjak *et al.*, 1992; Erdey *et al.*, 1997) prior to being used. This treatment had no effect on subsequent germination itself.

Germination conditions

Samples of hot-water-treated grains were plated out immediately and germinated (in triplicate) for different lengths of time (0, 8, 16, 24, 32, 40, 48, 56 or 64 h) under controlled conditions. The grains were germinated in Petri dishes (130 per 9-cm-diameter dish) on two black filter-paper discs (Whatman No. 29; 9-cm-diameter) which had been evenly wetted with 4 ml distilled water, and maintained in an incubator set at 25°C and 100% relative humidity.

Analyses

Germination and amylase activity

Upon sampling, the percentage of germinated grains (i.e. those grains that showed a protrusion of the root and/or shoot) was determined.

The joint α- and β-amylase activity of the samples was determined according to South African Bureau of Standards method 235 (1970), except that water was used as the extractant and 5 g of malt was used and the extraction volume reduced accordingly. This method involves making an aqueous extract containing the enzymes and then incubating the extract with soluble starch under standardized conditions. The products of joint α- and β-amylase activity are then measured in terms of reducing power. The results were expressed as sorghum diastatic units (SDU)/g dry mass.

Extraction, purification and HPLC separation of PGRs

Each grain (130 grains per sample) was separated into three parts [the embryo (including the scutellum and the roots and shoots), the proximal endosperm and the distal endosperm (including the pericarp and the aleurone layer)]. (Financial constraints made it impossible to assay more than one pooled sample per PGR). The samples were freeze-dried and maintained at −70°C until required. The freeze-dried sorghum samples were finely ground with a mortar and pestle and extracted in 10 ml 90% methanol in water (containing 50 mg butylhydroxytoluene and 100 mg sodium ascorbate/litre). Extraction was done in the dark at 4°C for 16 h. The extracted samples were centrifuged at 20 000 × *g* for 10 min. The supernatant was collected and reduced to dryness in a Savant vacuum concentrator. The dried extracted samples were stored at −4°C until required.

Each of the dried extracts was dissolved in 1 ml 90% HPLC-grade methanol. Internal standards (i.e. 100 µl ^{14}C-ABA and [^{3}H]dihydrozeatin) (10 000 dpm each) were added to each of the samples (so as to determine recoveries), and the samples were passed through a 0.45-µm polytetrafluoroethylene disposable filter and the filtrate was injected onto the HPLC column. The PGRs were separated on a Waters gradient HPLC instrument fitted with a 10 × 250-mm Zorbax 5-µm semiprep. ODS column (Dupont, Wilmington, USA) and a U6K variable volume injector. The column was eluted with a gradient of methanol in 0.1-M acetic acid (buffered to pH 3.5 with triethylamine) starting at 10% and changing to 50% methanol over 120 min at a flow rate of 1 ml min^{-1}. Immunohistograms were obtained from the series of 2-min fractions collected from 20 to 80 min. Retention times of zeatin (Z), zeatin riboside (ZR), isopentenyladenine (IPA), indole acetic acid (IAA), abscisic acid (ABA) and gibberellic acid (GA_1 + GA_3) were determined using authentic standards as described by Farrant *et al.* (1993). Fractions corresponding to the elution times of each of the PGRs were collected and dried in a Savant concentrator and used for the quantification of the PGR by radioimmunoassay (RIA).

Quantification of PGRs by RIA

The HPLC-separated, dried, samples were reconstituted in 2 ml 100% methanol and subjected to RIA according to verified protocols for each of the PGRs tested (Cutting *et al.*, 1983, 1986; Hofman *et al.*,

1985; Cutting and Bower, 1989). All RIA quantifications were done in triplicate and the Securia data reduction radioimmunoassay computer package (Packard Instrument Company, 1986, publication no. 169-3016) was used to analyse the raw data and correct for cross-reactivity and recoveries.

Results and discussion

Germination and amylase activity

The first sign of germination (embryo protrusion) occurred after 16 h, at which time approximately 89% of the grains had germinated (Fig. 1). Germination increased to 96% over the remaining germination period. Virtually no amylase activity was detected in mature, non-germinated grains (Fig. 1). This is in agreement with the findings of other workers (Daiber and Novellie, 1968; review by Palmer, 1989). Indeed, little amylase activity was detectable in the grain for up to 24 h of germination. Thereafter, the amylase activity of the grain increased more or less linearly up to 64 h, supporting the observation that in sorghum, α- and β-amylases develop following germination (Dufour *et al.*, 1992).

Cytokinins

Note that for the sake of clarity the quantities of Z and ZR present in the samples have been combined and will be referred to as a combined total. The concentration of IPA (Fig. 2) and Z+ZR (Fig. 3) was highest in the embryo and very much lower in both the proximal and distal endosperm tissues. In the mature sorghum grain the combined amount of the cytokinins IPA and Z+ZR (836 ng.g^{-1} dry mass) was high compared with the 57 ng.g^{-1} dry mass reported by Hocart *et al.* (1988) for maize. Both IPA and Z+ZR declined with germination time. There was a small peak in the concentration of Z+ZR and a larger peak in IPA concentration at 24 h, following the onset of the first sign of root protrusion and coinciding with the initiation of the more-or-less linear increase in amylase activity (Fig. 1). At 24 h of germination, the embryo represented a mere 6.8% of the grain mass yet it contributed 76% and 77% of the IPA and Z+ZR

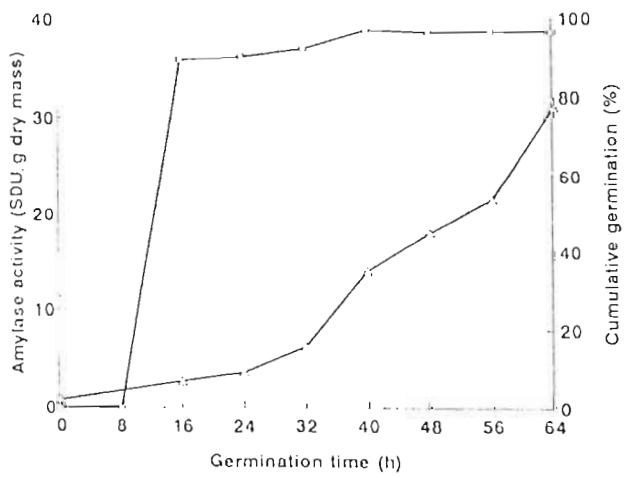

Figure 1. Effect of germination time on the cumulative germination (□) and amylase activity (Δ) of sorghum.

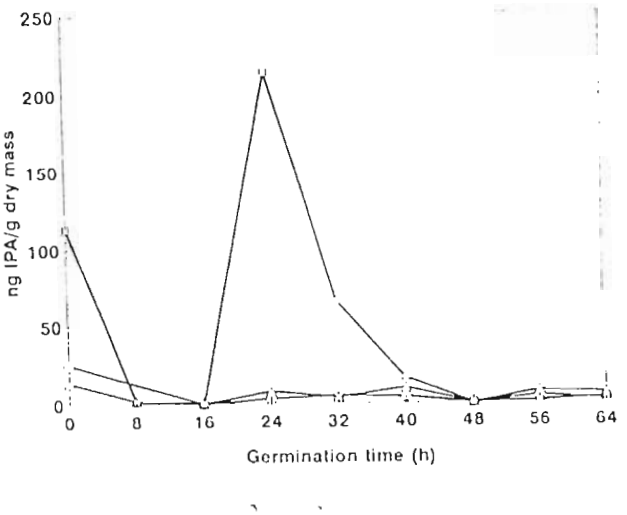

Figure 2. Changes in the concentration of isopentenyladenine (IPA) with germination time in the grain parts of sorghum (embryo (□); proximal (Δ) and distal (○) endosperm).

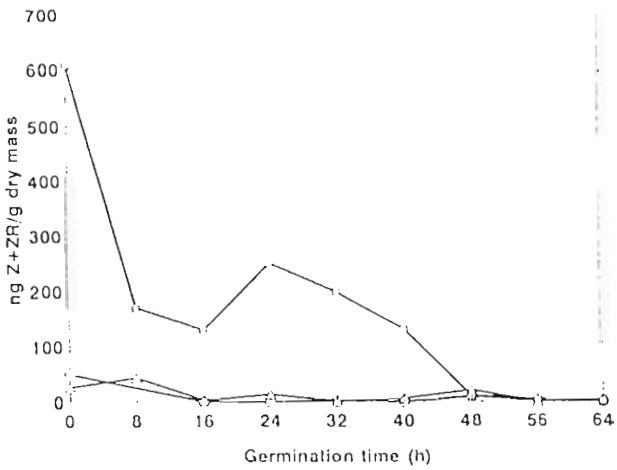

Figure 3. Changes in the concentration of zeatin and zeatin riboside (Z+ZR) with germination time in the grain parts of sorghum (embryo (□); proximal (Δ) and distal (○) endosperm).

amounts of the grain, respectively. After further time, the amounts of these PGRs declined again.

Cytokinins have been implicated in radicle growth and seedling establishment following germination (Hocart and Letham, 1990). The peaks in the amounts of IPA and Z+ZR following the onset of root protrusion suggest that these cytokinins may play a role in the cell division and elongation processes affecting root growth in sorghum. There have been reports that suggest that cytokinins are implicated in mobilization of storage reserves for utilization during germination (Fincher, 1989; Hocart *et al.*, 1990). When expressed on a per-grain-part basis there was a decline in cytokinin concentration in the endosperm and a subsequent increase in the embryo tissue at around the time of root protrusion in sorghum. This might aid in the formation of an embryo sink for nutrients and facilitate the polarized movement of reserve breakdown products from the endosperm to the embryo. It is possible that the cytokinins are re-distributed within the embryo to regions (e.g. the root meristematic region) where they effectively concentrate and direct root growth (see review by Letham, 1978). This would obviate the necessity for much overall increase in the amounts of the cytokinins IPA and Z+ZR.

Auxin

As was the case for the cytokinins, the concentration of IAA in the mature, non-germinated, sorghum grain was much higher in the embryo (343 ng.g^{-1} dry mass) than in the proximal or distal endosperm (69 and 6 ng.g^{-1} dry mass, respectively) (Fig. 4). The concentrations of IAA in both the embryo and the endosperm declined dramatically upon the grain being set out to germinate. When sampled after 8 h (i.e. the first sampling point), before the first sign of root protrusion (Fig. 1), virtually no IAA was detected in the component parts of the sorghum grain (Fig. 4). Concentrations of IAA in mature seeds appear to vary among species from approximately 7 and 1572 ng.g^{-1} dry mass in French bean and maize, respectively (Tillberg, 1977), to approximately 1700 ng.g^{-1} dry mass in rice (Bandurski and Schilze, 1977). The IAA concentration in sorghum appears somewhat low when compared with rice which is similar to sorghum in that they are both tropical cereals. Most reports of IAA concentrations in mature orthodox seeds indicate that the amounts are low relative to earlier developmental stages (Cohen and Bandurski, 1982; Bialek and Cohen, 1989). Likewise, it is possible that the amount of IAA in sorghum could have been higher during the developmental phase.

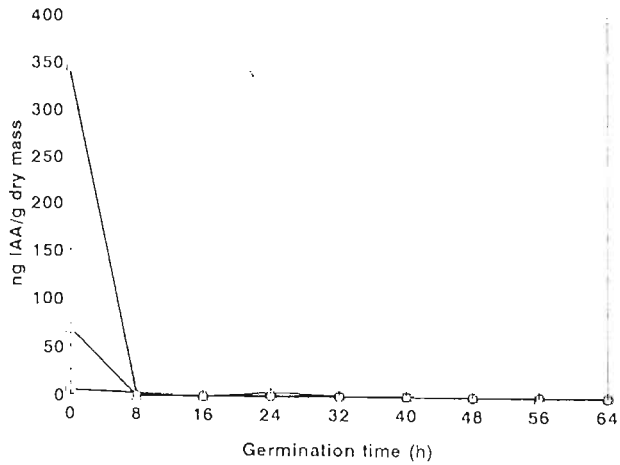

Figure 4. Changes in the concentration of indole acetic acid (IAA) with germination time in the grain parts of sorghum (embryo (□); proximal (△) and distal (○) endosperm).

Gibberellic acid

Note that the quantities of GA_1 + GA_3 have been combined and will be discussed as one (i.e. $GA_{1,3}$). Gibberellic acid was detected in the embryo and the endosperm components of sorghum grain after 8 h of germination and at several stages during germination (Table 1). There was no obvious trend and there did not appear to be a relationship between the amount of $GA_{1,3}$ detected and either germination time or amylase activity (Fig. 1). Compared with reports on other seed types, where the concentrations of endogenous GAs have been represented on a mass basis, the $GA_{1,3}$ concentrations detected in germinating sorghum (Table 1) appear to be relatively

Table 1. Changes in the concentration of gibberellins$_{1,3}$ during germination in the grain parts of sorghum (cv. NK 283)[1]

Germination time (h)	Embryo	Endosperm	
		Proximal	Distal
0	–	–	–
8	–	4.2	N/A
16	18.3	2.8	–
24	–	22.2	1.2
32	–	3.4	1.5
40	4.4	–	–
48	7.8	N/A	3.4
56	1.0	–	–
64	–	–	–

[1] Results are for a single analysis per sample and are expressed as ng $GA_{1,3}$.g^{-1} dry mass
– Indicates below detection level
N/A No data available

low. For example, a peak in GA-like activity of 500 μg.g^{-1} fresh mass has been reported for pea seeds (Eeuwens and Schwabe, 1975) and Piagessi *et al.* (1989) have reported 100 μg.g^{-1} fresh mass of bioactive $GA_{1+3+5+11}$ in suspensor cells of *Phaseolus coccineus*. Other forms of gibberellic acid, not presently assayed, may also occur in sorghum grains. However, the forms GA_1 and GA_3 are considered to be highly active in seeds (Pharis and King, 1985; Sponsel, 1987). It is possible that in germinating sorghum the gibberellins GA_1 and GA_4 occur in predominantly conjugated forms as has been proposed by Rood (1995) for GA_8 in sorghum shoots. This may offer an explanation as to why free GA_{1+3} was detected in only a few instances during sorghum germination.

In germinating barley, gibberellins are thought to be transported from the scutellum or embryonic axis to the aleurone where they induce synthesis of specific enzyme proteins (e.g. α-amylase) which are active during germination (MacLeod *et al.*, 1964; Palmer, 1982). Good evidence exists that the enzyme distribution in sorghum is not compatible with the concept of *de novo* α-amylase formation in the aleurone layer (Daiber and Novellie, 1968; see review by Palmer, 1989). Those authors postulated that in sorghum, amylases are synthesized in the embryo and then diffuse to the endosperm, unlike barley where the aleurone is predominantly responsible for α-amylase production. Although endogenous GA_{1+3} was detected in the germinating sorghum grains (Table 1), the results of the present study do not give an indication as to the role gibberellins may play in the production of enzymes during sorghum germination.

Abscisic acid

The amount of ABA in the embryo tissue of the mature, non-germinated, sorghum grain was high (1399 ng.g^{-1} dry mass) compared with the proximal and distal endosperm tissues (219 and 79 ng.g^{-1} dry mass, respectively) (Fig. 5). Indeed, even though relatively small in proportion to the whole grain (8.1% by mass), the embryo contributed a high proportion (38%) of the ABA content of the whole sorghum grain at maturity. During the first 32 h of germination the concentration of ABA in the embryo declined sharply, approaching concentrations comparable to those of the endosperm parts. This decline in embryo ABA to apparently negligible amounts coincided with a marked increased in amylase activity following the onset of germination (Fig. 1).

The ABA content of seeds is usually highest in developing seeds and low or even absent at maturity (Black, 1983). Although the embryo ABA concentration of mature sorghum grains reported in this study appears somewhat high, it is similar to

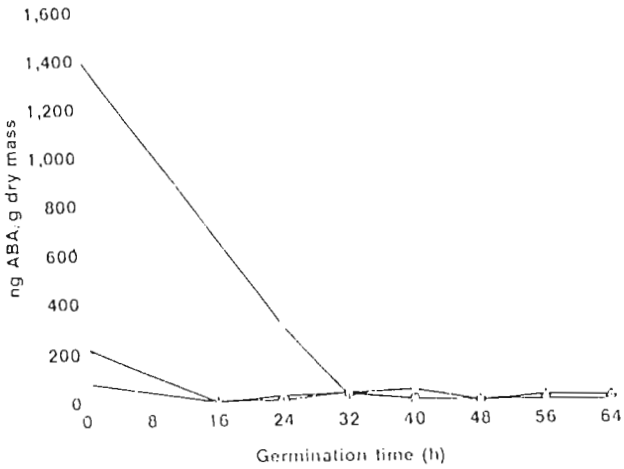

Figure 5. Changes in the concentration of abscisic acid (ABA) with germination time in the grain parts of sorghum (embryo (▲); proximal (△) and distal (○) endosperm)

embryonic ABA contents reported by Steinbach *et al.* (1995) for developing caryopses of sorghum. Physiologically mature grain from one season was reported to have an embryonic ABA content of more than 600 ng.g^{-1} dry mass and more than 1200 ng.g^{-1} dry mass in a second season. ABA prevents precocious germination (Bewley and Black, 1985; Quatrano, 1986; Kermode, 1990). Studies of developing rape seeds have shown that *in planta* the embryos are held in embryogenetic growth by the native ABA. However, when isolated they lose the ABA and germinative growth occurs (see review by Black, 1991). Similarly, for developing caryopses of sorghum, ABA has been shown to inhibit embryonic germination (Steinbach *et al.*, 1995) and when ABA synthesis is inhibited by fluridone precocious germination occurs (Steinbach *et al.*, 1997). The extent of inhibition, however, appears to be related to the embryonic sensitivity to ABA (Steinbach *et al.*, 1995).

We suggest that although a high concentration of ABA in the embryo tissue of mature sorghum grains does not completely inhibit germination (i.e. impose dormancy) it may constitute an endogenous mechanism that will delay germination until the ABA concentration has declined significantly. Similarly, studies of several seed species (rape, soybean and dwarf French bean), have shown that the lag phase prior to axial extension is related to the native concentration of ABA at the time the seed is removed from the parent plant; the higher the initial ABA concentration the longer it takes in culture before germination is initiated (see review by Black, 1991). If the lag period prior to germination in sorghum is likewise related to the ABA content, malting quality

could perhaps be enhanced by reducing the concentration of endogenous ABA at maturity. Steinbach *et al.* (1995) have suggested, from their study on immature caryopses of sorghum, that differences in pre-harvest germinative behaviour may not be related simply to the endogenous ABA concentration, but rather to the rate at which the ABA concentration decreases. For example, embryo ABA decreased faster and germination was initiated sooner in a pre-harvest sprouting-susceptible sorghum variety than in more resistant varieties.

The production of hydrolytic enzymes, such as the amylases, is one of the main objectives of malting. Evidence exists that ABA is involved in the suppression of α-amylase activity in developing seeds of some species e.g. triticale (King *et al.*, 1979) and wheat (King, 1976, 1982; Napier *et al.*, 1989), presumably by arresting enzyme synthesis. Oxygenated metabolites of ABA, 7'-hydroxy-ABA and phaseic acid, have also been shown to be effective in suppressing α-amylase activity (Nolan and Ho, 1988; Hill *et al.*, 1992, 1995; Todoroki *et al.*, 1995; Walker-Simmons *et al.*, 1997). It would appear that constraints, one of which is thought to be ABA (Garcia-Maya *et al.*, 1990), operate on the embryo *in planta* to suppress amylase production. The present evidence suggests that ABA could play a similar role in sorghum, taking into account the high concentration of ABA in the embryo tissue (Fig. 5) and the absence of amylase activity in the mature, non-germinated sorghum grain (Fig. 1). Thus, the sooner the endogenous ABA content is reduced, the faster germination would occur (Steinbach *et al.*, 1995) and the greater the potential would be to produce the required malt hydrolytic enzymes.

It has been proposed that during germination, cytokinins may play a role in countering the effect of germination inhibitors (Khan, 1975). It is thus possibly of significance that the increase in the amounts of the cytokinins IPA and Z+ZR (Figs 2 and 3) coincided with the decrease in the concentration of ABA (Fig. 5) and the concomitant increase in amylase activity (Fig. 1).

Conclusions

Although in this study a direct causal relationship between germination, amylase activity and PGRs was not investigated, from the data we suggest that germination in sorghum is controlled within the embryo, as the concentration of the PGRs in the mature grain (with the exception of $GA_{1,3}$) is much higher in the relatively small embryo tissue than in the larger mass of endosperm tissue. Furthermore, it appears that the high concentration of ABA at maturity, and the interaction of ABA and the cytokinins IPA and Z+ZR, may play a significant role in amylase production during sorghum germination. These possible causal relationships require further investigation.

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