SCIENTIFIC RESEARCH FOR THE INDUSTRIAL UTILIZATION OF SORGHUM IN AFRICA

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INTRODUCTION

Most of the most significant initial scientific research for the industrial utilization of sorghum in Africa started in South Africa in the early to mid 20th century mostly due to the then social policies of the then apartheid regimes to develop the so-called "Bantu beer" for the black population. The second wave of significant scientific research for the industrialization of sorghum in Africa started in the late 1970s and was driven entirely by economic realities – most importantly, the banning by the Nigerian Government of the importation of barley malt, wheat and other cereals such as maize as a means of conserving its foreign exchange and as a deliberate policy to instigate the use of local materials in the local production of cakes, biscuits, beer and non-alcoholic beverages (Palmer, 1992).

INDUSTRIAL MALTING AND BEER BREWING WITH SORGHUM

A significant part of the research and development work for the use of sorghum for industrial malting and brewing of clear beers (lagers and stouts) in Africa started in the mid-to-late 1980s (Dufour *et al.*, 1992; Owuama, 1999; Taylor *et al.*, 2006). Sorghum is rich in starch (≥ 70% with an approximately 75:25 amylopectin/amylose ratio) and should therefore constitute an optimum adjunct in industrial lager beer brewing. These brewing qualities are been further advanced by the gluten-free nature of sorghum protein (Taylor *et al.*, 2006). However, the use of unmalted sorghum in beer brewing is linked to problems of incomplete saccharification, low wort filterability and inadequacy of amino nitrogen (Taylor *et al.*, 2006). The Incomplete saccharification of sorghum starch and, consequently, its low fermentable extract recovery has been attributed to the low digestibility of its starch. Initially the low starch digestibility had been attributed solely to the high gelatinization temperature of sorghum starch (67 – 81^{o}C (or 7 – 30^{o}C higher than barley starch (51 – 60^{o}C)) (Dufour *et al.*, 1992; Okolo *et al.*, 1997). Results of more recent studies, in which sorghum starch digestibility remained low, even after cooking at up to 98 – 100^{o}C (Chandrashekar and Kirleis, 1988; Zhang and Hamaker, 1998; Ezeogu *et al.*, 2005a) showed that other factors, particularly the tendency by complex disulphide-linked kafirins polymer (Duodu *et al.*, 2003) to restrict amylase access to starch granules contributed significantly to the reduced α-amylase digestibility of sorghum starch. It appears that the use of waxy and heterowaxy cultivars can drastically reduce the problems of low starch digestibility in sorghum adjunct brewing. waxy and heterowaxy sorghums are susceptible to amylolysis, give shorter conversion times and very high wort filtration rates, have low wort glucose and fructose content and their beers resemble those brewed from barley more (Barredo-Moguel *et al.*, 2001; Del Pozo-Insfran *et al.*, 2004). On the other hand, Ezeogu *et al.* (2005a) and Ortega Villicaňa and Serna-Saldivar (2004) have shown that pressure cooking of sorghum flours could markedly improve sorghum starch amylase digestibility. Three-dimensional confocal microscopy

investigation has shown that this may have been achieved through the possibly forceful disruption of the sorghum protein matrix (Ezeogu *et al.*, (unpublished)).

Brewing with sorghum malt is associated with some shortcomings including its low β-amylase activity, its β-glucanase and proteolytic activities deficiencies (Palmer, 1992) as well as problems of inadequate extract recovery during mashing and the relatively high glucose content and reduced fermentability of its worts. Inadequate β-amylase activity results in insufficient maltogenesis and lower levels of fermentable extracts in brewing. Efforts have been made to improve sorghum β-amylase through the selection and breeding of cultivars with increased β-amylase activity and through the optimization of the malting conditions as well as by the adoption of so-called decantation mashing procedure whereby the enzymic mash is first separated from the starch then re-united with it after the latter has been gelatinized (Palmer, 1992). Some significant success has been attained with studies to improve sorghum malt β-amylase activity, although most has been concerned with the effects of malting conditions and involved only very few lines, mostly grown in Nigeria, India and South Africa (Ezeogu and Okolo 1994; Dewar *et al.*, 1997a, b; Okungbowa *et al.*, 2002). In a recent study from Botswana, Letsididi *et al.* (in press) observed that β-amylase constituted up to 80% of DP in at least four of the varieties studied. This challenges long-held views that α-amylase is the main source of DP in all sorghum (Dufour *et al.*, 1992; Taylor and Robbins, 1993; Okungbowa *et al.*, 2002) and highlights the need to investigate the sorghum germplasm further.

Sorghum malt proteases play important roles in seed structure modification during germination and the generation of free-amino acids during lager beer brewing. Sorghum malt proteinase and carboxypeptidase are markedly dependent on both grain variety and condition of malting (Evans and Taylor, 1990; Okolo and Ezeogu 1995). Macedo *et al.* (1999a,b) purified a metalloprotease as well as two pepstatin A-sensitive (aspartyl-) proteinases while Garg and Virupaksha (1970) purified a cysteine protease from sorghum. Beyond these, no attempt has been made to study the functions of sorghum proteases during malting or germination. On-going work in our lab in Botswana (Mokhawa *et al.*, unpublished) indicates that sorghum may contain from five to eleven or more endoprotease forms (including cysteine, aspartyl, metallo- and serine endoproteinases). These proteases differ in their activities on kafirin, gelatin and casein and their profiles vary by assay pH and grain variety. The work also looks at the effect of steeping condition and germination time on the appearance of the various protease isoforms as a means of further elucidating their individual contributions to sorghum endosperm modification during malting. A critical elucidation of the roles played by the enzymes in this cocktail of proteinases as well as that played by other enzymes in various other sorghum malt hydrolase groups (e.g. amylases, β-glucanases, hemicellulases etc.), followed by a clarification of how these functions are influenced by environmental conditions of malting is vital to the accelerated development of optimal sorghum malting processes.

SORGHUM AND BIO-ENERGY

Properties that make sorghum an ideal crop for bio-energy generation include its high levels of carbohydrates including starch (60 – 78% of the grain) (Shelton and Lee, 2000) free sugars, cellulose and hemicelluloses (abundant in the stalk) (Reddy and Yang, 2004). According to Kim and Dale (2004), bio-energy processes from wasted sorghum grain and straw could, if properly harnessed, generate 4.9 Gl of bioethanol globally, replacing 3.5 Gl of gasoline or about 0.3% of annual global gasoline consumption. Unfortunately the conversion of sorghum to bioethanol has

received little attention compared to maize (Taylor *et al.*, 2006), owing mostly to its low starch digestibility and poor wet-milling properties (Zhan *et al.*, 2006). Zhan *et al.* (2006) employed supercritical-fluid-extrusion cooking to increase sorghum starch gelatinization and digestibility and as a result enhanced the amount of ethanol produced from sorghum by >5%. It is conceivable that the use of similarly disruptive cooking methods such as pressure-cooking (Ezeogu *et al.*, 2005a) would also improve sorghum starch digestibility and bioethanol production. The sorghum stem (61% total carbohydrates with 27-31% cellulose and 25-30% hemicelluloses and 11-15% lignin) (Reddy and Yang, 2004) is an excellent substrate for bioethanologenesis (Mamma *et al.*, 1995; Ballesteros *et al.*, 2004; Kim and Dale, 2004). The conventional process for sorghum stem conversion to ethanol was by sequential hydrolysis and fermentation, involving the initial acid or enzyme hydrolysis of the biopolymer to fermentable sugars or oligosaccharides followed by fermentation to ethanol, although alternative processes have been developed which employ simultaneous hydrolysis and fermentation (Mamma *et al.*, 1995). Sweet sorghums contain very high levels of fermentable sugars (mostly sucrose) and have traditionally been used for the production of edible sugar syrups, although their sugar can be extracted and used as feedstock for industrial bioethanologenesis (Gnansounou *et al.*, 2005). Under appropriate conditions, sweet sorghums can generate far more ethanol than cassava and sugar cane (Nguyen, 1984). Sorghum can be converted to other bioenergy forms including biomethane and biohydrogen (Antonopoulou *et al.*, 2007; Gunaseelan, 2007). Sweet sorghum bagasse has been employed successfully as an immobilization matrix for yeast *Saccharomyces cerevisiae* in a system for ethanol production from sucrose (Yu *et al.*, 2007) to generate a 2.24 fold increase in productivity. The immobilized cell reactor was stable even after 20 days of use and showed a very high yeast cell retention rate.

Sprouts from malted sorghum are rich sources of organic nitrogen and minerals for microbial fermentation (Ezeogu *et al.*, 2001; Ezeogu and Ogbonna, 2005). Employing various acid and tryptic digests of sorghum malt sprouts Ezeogu *et al.* (2001, 2005b) showed enhanced ethanol fermentation by yeast in very high gravity ethanol fermentation. Sorghum steep liquor as well as a water extract of sorghum malt sprout is currently being studied for a similar process in Botswana. A koji-like sorghum malt product has been developed as source of enzyme to hydrolyse cassava starch in ethanol fermentation (Ugwuanyi and Ezeogu, unpublished). The bio-industrial production of fine chemicals such as lactic acid (Zhan et al, 2006), furfural and its derivatives (Vazquez *et al.*, 2007), polyhydroxyalkanoates for biodegrable plastics (Koutinas *et al.*, 2007) and edible biopolymer film coatings (Taylor *et al.*, 2006) is also possible with sorghum. Sorghum contains ß-linked carbohydrates like ß-glucan and arabinoxylan (hemicelluloses) (Verbruggen *et al.*, 1998) and should be a good candidate for the production of prebiotics.

BREEDING (RESEARCH) FOR INDUSTRIAL UTILIZATION

Sorghum improvement has relied heavily on conventional breeding processes with only limited improvement (Galili and Larkins, 1999). Transgenic sorghum presents an opportunity for the accelerated development of sorghum grains with excellent industrial quality (O'Kennedy *et al.*, 2006). Sorghum, transformed for enhanced ß-amylase activity and thermostability, better ß-glucanase and hemicellulases activities, enhanced protease levels and mold resistance, and higher protein and starch digestibility could become industrially successful in the brewing, fermentation and other industry. Recombinant DNA technology can also help develop fast

molecular screening methods for the selection from the sorghum germplasm of cultivars with desirable industrial qualities, as has been done for barley. Basic tools have been developed for sorghum improvement. These include the development of reliable and efficient *in vitro* protocols for plant regeneration, molecular markers, and gene transfer (O'Kennedy *et al.*, 2006).

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