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Review: Brewing Conventional Beer with Sorghum Cultivars

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REVIEWBREWINGCONVENTIONALBEERWITHSORGHUMCULTIVARS

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Beer Brewing with Sorghum

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I. Introduction

Sorghum is the fifth most produced cereal in the world and belongs to the grass family, *Graminae* and tribe, *Andropogonae*. It was first used as a brewing adjunct in conventional lager beer production during the second World War (Owuama, 1999). There are two major groups of sorghum varieties viz., the non-sweet sorghum, *Sorghum vulgare* and the sweet sorghum, *Sorghum bicolor* [L] Moench), which is characterised by having sweet stalk (Owuama, 2019). Over 14,000 varieties or cultivars of sorghum exist and more new improved varieties of sorghum are being developed through continuous plant breeding research, aimed at selecting and concentrating desirable characteristics for industrial livestock feeds and food (Owuama, 1999). Among the improved varieties are those whose malts possess desirable qualities for beer brewing, such as good diastatic power, α- and β-amylase activities, proteinase activity and good extract recovery (Bekele et al., 2012; Owuama, 1999; Taylor & Daiber, 1988). The potential of sorghum as a viable alternative substrate for beer brewing, particularly in the tropics where barley does not thrive well, has been recognized (Hill & Stewart, 2019; Palmer et al., 1989; Owuama, 1999;). So far, the research on sorghum as substrate for conventional beer brewing has been going on for several decades (Hill & Stewart, 2019).

Remarkable progress has been made to date in investigating different factors that influence various stages of beer production with sorghum viz., malting, mashing, fermentation and aging (Agu & Palmer, 1996; Dale et al., 1990; Harry et al., 2019; Morall et al., 1986; Owuama, 1999). Innate enzymes in sorghum grain and those developed during malting are known to play remarkable roles in the hydrolyses of carbohydrates, proteins and lipids during mashing to yield fermentable wort (Dlamin, et al., 2015; Espinosa-Ramírez et al., 2013; Uvere & Orji, 2002). Variations in steeping, germination and kilning regimes have remarkable impact on sorghum malt quality. The mashing of sorghum malt alone or in combination with sorghum grit at varying proportions, with and without the addition of external enzymes, have also received adequate attention (Heredia-Olea et al., 2017, Hu et al., 2014). Several research results on extracts of sorghum malts and mashes (worts) reveal the presence of sugars, lipids, proteins, total soluble nitrogen and free amino nitrogen adequate to support yeast fermentation (Evans & Taylor, 1990a; Odibo et al., 2002; Okolo & Ezeogu, 1996b; Owuama, 2019; Pickerell, 1986; Taylor & Boyd, 1986;). Viscosity and fermentability of worts as well as character of sorghum beers, which include alcoholic content, specific gravity, bitterness and colour, and sensory properties (mouthfeel, appearance, bouquet, aroma and taste) have also been examined (Dale et al., 1990; Harry et al. 2019; Owuama & Okafor, 1987; Tailor & Daiber, 1988). Thus, this review, reappraises and updates the progress made so far in brewing conventional beer with sorghum

II. Sorghum Grains for Malting

Grain sorghum matures when the moisture in the grain drops to about 30 %, however, the seeds are usually too soft for harvesting when moisture content exceeds 25 % moisture. Usually, sorghum grains are harvested at optimal percentage moisture content of about 20 % so as to minimize losses and drying expense. Further drying and storage of sorghum

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however, decrease the moisture content to below 20% moisture (McNeil & Montros, 2003; Owuama, 2019). The percentage moisture content of sorghum grains for malting range from 12.5 to 20.5 % (Bekele et al., 2012; Owuama, 2019). The variations in moisture content of grains for malting may be attributable to differences in sorghum cultivar, storage conditions, maturity and age of grains (Owuama, 1999).

Sorghum grains have varying physical and biochemical characteristics within and between the two major different sorghum cultivars; *Sorghum vulgare* and *Sorghum bicolor* varieties. Sweet sorghum (*Sorghum bicolor*) varieties have larger granule size, higher water solubility index, lower amylose content and lower swelling power than grain sorghum (*Sorghum vulgare*) (Ahmed et al., 2016). Major differences between *Sorghum vulgare* and *Sorghum bicolor* is the presence of sugary stalk in sweet sorghum unlike the grain sorghum, and this may be a reflection of the physiological differences between the two cultivars (Regassa et al., 2014). Evaluation of sorghum *(Sorghum bicolor* (L.) Moench) accessions showed variations in total starch (31.01 to 64.88 %), amylose (14.05 to 23.0 %), the amylose/amylopectin ratio (0.31 to 0.73), total stalk sugar content (9.36 to 16.84 %) and crude protein (7.0 to 11.9%) (Bekele et al., 2012; Gerrano et al., 2014).

Grain characteristics usually considered for selecting sorghum variety for malting include, sorghum kernel shape and size (as reflected by thousand grain weight) (Rooney, 1973), germination energy [GE] *(measure of the percentage of grains expected to germinate fully at the time of test)*, germination capacity *(used to determine if seeds that did not germinate in the GE test are dormant or dead i.e. measures percentage of viable corns in a sample)* (Owuama, 2019), percentage moisture content and water sensitivity *(a reflection of a oxygen requirement for germination by the embryo)*. Unlike sorghum, barley contains husk, and a surface of film of water in the husk, has been shown to reduce the oxygen uptake, thereby causing embryos of water sensitive barleys to germinate to a lesser extent at low oxygen tension, thus the need for steep-aeration (air-rest or air sparging) during steeping (Crabb & Kirsop, 1969; Kelly & Briggs, 1992;). Water sensitivity of grains for malting is usually carried out to ascertain if the grains require air-rest period during steeping (Crabb & Kirsop, 1969). Thus, water sensitivity is apparently a reflection of a higher oxygen requirement for germination by the embryo. When the water sensitivity of grains for malting is less than 30 %, the grains are not water sensitive and so do not need air-rest time during steeping. Sorghum grains with water sensitivity values of 7.1 to 27.6 % have been used for malting (Anon, 1997; Davidson et al., 1976; Kelly & Briggs, 1992; Owuama, 2019). Nevertheless, no clear relationship has been established between grain moisture content and water sensitivity among different varieties of sorghum (Owuama, 2019).

Thousand grain weight of sorghum varieties used for malting differs and generally falls within 22.8 g and 58.7 g (Owuama, 2019; Subramanian et al., 1995), apparently due to varietal differences in grain sizes, storage period and conditions (Owuama, 1999; Svenson et al., 2011). The germination energy (GE) of some sorghum grains used for malting range from 96.3 to 100 % while the germination capacity (GC) falls between 99.7 and 100 % (Bekele, 2012; Dewar et al., 1995; Owuama, 2019). The recommended GE value required for sorghum to be considered suitable for malting is greater than 90% (Agu & Palmer, 2013).

III. Stages in Beer Brewing

There are fundamentally five stages in conventional beer brewing namely; malting, mashing, wort boiling, fermentation and aging. Except for wort boiling, all the other stages of the brewing process are further discussed below. Wort boiling has generally been reviewed elsewhere (Willaert Baron, 2001).

a) Malting

Malting of grains for brewing involves essentially steeping, germinating and limiting cereal seedling growth after the production of enzymes required for degradation of starch and proteins in cereal grain but before the exhaustion of polysaccharides, plus kilning or drying of green malt. Prior to malting, a small proportion of β-amylase in cereals such as wheat, rye, barley and sorghum is insoluble (Owuama, 1999; Owuama & Okafor, 1990). However, the percentage of soluble amylases in sorghum appears to be influenced by temperature and time of storage of the grains. Storing sorghum grains for 2 to 3 years at 12 to 23°C gives higher level of amylases (57 to 73%) while newly harvested grains contain about 25%. Lowering storage temperature to 7 °C reduces level of soluble amylases in the grains to about 31% after 3 years. But, storing malts for any period of time seems not to affect soluble amylase content (Owuama, 1999). Nevertheless, malting yields higher proportions of hydrolytic enzymes such as α-glucosidase, α- and β-amylases which may be either completely soluble or largely insoluble (Demuyakor & Ohta, 1992; Jayatissa et al., 1980; Taylor & Dewar, 1994). For example, insoluble amylases and α-glucosidase have been detected in malts from sweet sorghum and related variety. The insolubility of these enzymes is apparently due to their strong adhesion to insoluble malt solids (Taylor & Dewar, 1994).

Malting causes a decrease in density of caryopsis in sorghum grain (Beta et al., 1995), lowers the amount of lysine from 0.25% in unmalted sorghum to 0.18% in sorghum malt 84 and also reduces milling energy (Swanston et al., 1994). Sorghum endosperm contains both vitreous and mealy regions with the percentage of vitreous endosperm highly correlating

with grain hardness (Hallgren & Murty, 1983). Sorghum grains with intermediate endosperm texture are more suitable for malting than those with floury endosperm (Adeole, 2002). Also, waxy and hetero-waxy sorghum genotypes have soft endosperm texture which allows hydrolytic enzymes access to starch granules (with enhanced gelatinization vis-à-vis non-waxy genotypes), thus have better malting potential and consequently are more suited for beer brewing (Bekele et al., 2012; Beta et al., 2000; Taylor et al., 2006). The vitreous part of endosperm seems to contribute greatly to grain milling energy and also to malt milling energy since it is largely unmodified during malting (Owuama, 1999). Thus, there is a positive correlation between grain milling energy and malt milling energy (Swanston et al., 1992). The loss in milling energy due to starch granule modification during malting may be responsible for the highly significant correlation between diastatic power and malt milling energy. However, grain milling energy shows no significant correlation with percentage extract in sorghum (Swanston et al., 1992). Protein apparently plays a minor role in determining the quality of sorghum malt as high protein content in sorghum malt causes no brewing problems since most of the high molecular weight proteins are degraded into simpler compounds during mashing or coagulated during wort boiling and removed as protein sediment. As well, malting grains of some sorghum hybrids reduced the total phenolic content (TPC), flavan-4-ols, total flavonoid levels but more than doubled the total anthocyanin levels while the 3-deoxyanthocyanins in sorghum grains increased by about 8-fold in the malt (Khoddami et al., 2017; Owuama, 1999).

Nevertheless, malting quality of sorghum is determined by physical and biochemical factors such as temperature and time of steeping and germinating of grains with their inherent enzymic activities, kilning temperature regimes (Owuama, 1999; Owuama & Asheno, 1994), and the sorghum cultivar (Owuama & Okafor, 1987; Subramanian et al., 1995). Malt quality has been shown to influence the type and character of beer produced (Owuama, 1997). The impact of various physical and biochemical factors on various stages of malting are discussed below.

b) Steeping

Steeping involves soaking grains in water with or without air-rest until desirable moisture level (steep-out moisture) is attained. During steeping certain physical and biochemical changes occur, such as, swelling of grains, degradation of soluble carbohydrates and removal of some pigments, microorganisms and bitter substances from grains. Factors that affect the rate at which the grains absorb water include, grain structure (softer grains absorb more water than hard grains), and grain size (smaller grains absorb moisture more rapidly) (Pitz, 1989). Aeration during steeping has been shown to affect the rate at which the grain absorbs water (Olkku et al., 1991). Steeping is essentially regulated to achieve a suitable moisture level and avoid over-steeping or reaching a saturation point, which usually results in killing of seed germ. Suitable steep moisture varies with sorghum grain variety, steeping time and temperature (Owuama & Asheno, 1994; Owuama & Okafor, 1987), and steep moisture of grain directly affects sorghum malt quality (Dewar et al, 1997). Steep-out moisture contents of 32 to 35% have a positive correlation with free alpha amino nitrogen (FAN), total non-protein nitrogen (TNPN) and cold water soluble protein (CWS-P) (Ogbonna et al., 2003).

Steeping sorghum grains at temperatures of 10 to 30°C causes an increase in steep-out moisture with apparently no appreciably effect on diastatic power of malts (Owuama, 1999). Also, steeping temperature (up to 30°C) increase malt diastatic power while free amino nitrogen and extract content peak at a steeping temperature of 25°C (Oikku et al., 1991). Steep moisture affects extract yield, reducing sugar, diastatic power of malt and level of amino acids in wort. Steeping sorghum at 30°C for 18 to 22 h results in steep moisture of 44-48% which is optimal for enzymic activity (Morall et al., 1986; Owuama & Asheno, 1994; Ratnavathi & Ravi, 1991) while steep moisture of 35-40% seems to encourage rapid germination at a temperature of 22°C, in the dark (Aisien & Ghosh, 1978). Apparently, increase in steep moisture with steeping time from 12 to 20 h at 30°C is directly proportional to diastatic power of malt and consequently an increase in reducing sugar, cold and hot water extracts (Owuama & Asheno, 1994). However, steep moisture levels beyond the optimum, leads to a decrease in extract and diastatic power of malt (Owuama, 1999).

Steeping methods (i.e. with or without change of water) have virtually no effect on sorghum malt (Owuama, 1999). Steeping sorghum with increasing air rest periods of 1 to 4 h at 30°C for 48 h to attain steep moisture of 40-42%, germinating for 4 d and kilning at 50°C result in (a) a decrease in average main rootlet length (b) decrease in malting loss from 14.1-18.1% to 9.5-13.6% and (c) an increase in malt diastatic power (including α- and β-amylases) up to 3 h air-rest period followed by a decrease after 4 h. However, variations occur among sorghum cultivars e.g. the optima for α- and β-amylase activities in cultivar KSV 400 occur at air rest periods of 3 h and 1 h respectively but at 2 h and 3 h air rest periods for cultivar KSV 8 (Ezeogu & Okolo, 1995). β-Amylase activity constitutes 36-50% of total diastatic activity in cultivar KSV 400 but 27-49% in cultivar KSV 8 while cold and hot water extracts give highest values for KSV 400 and KSV 8 after air rest of 3 and 4 h respectively (Ezeogu & Okolo, 1995). Increase in steeping time plus aeration and steep water temperature enhance diastatic power. Steeping grains

plus aeration at 30°C for 40 h yield maximum diastatic power of 42.6 SDU/g. Steeping at 25°C for 40 h under air rest condition produce maximum malt FAN (119.8 mg/100g) while 24 h steeping with aeration yield highest malt extract (62.5%) (Dewar et al., 1997b). And aeration during steeping appears to enhance the extract and free amino nitrogen content of the finished malt (Dewar et al., 1997a).

Varying the duration of final warm water steep at 40°C between 1.5 h to 7.5 h and germinating for 4 d at 30°C cause (a) malting loss and a decrease in average main root length with increase in the duration of final warm water steep and (b) increase in diastatic activity, α- and β-amylolytic activities, and extract yield as the final warm water steep period increases up to 3 h and thereafter declines. However, these observations vary with sorghum cultivars (Okolo & Ezeogu, 1995b). The highest α-amylolytic activity occurs at relatively shorter duration of final warm water steep e.g. 3 h for KSV 8 and 1.5 h for KSV 400 while peak β-amylases activity result after 3 h and 7.5 h final warm water periods for KSV 400 and KSV 8 respectively. Nevertheless, diastatic activity for KSV 8 attains another peak, albeit smaller, after 7.5 h of final warm water steep, thus suggesting the involvement of at least another β-amylase component. A marked reduction in average main root length of 53% and 25% occur after 1.5 h and 3 h final warm water steep for KSV 400 and KSV 8 respectively (Okolo & Ezeogu, 1995a).

Steeping solution (i.e. water with or without amendments), time and temperature have highly significant effects on sorghum malt quality. Steeping in dilute sodium hydroxide solution enhances water uptake by sorghum grains. A positive linear relationship exists between increase in NaOH concentrations (0.1-0.6% w/v) and steep-out moisture content of grains. Steeping in 0.6% NaOH (w/v) for 48 h results in the highest steep-out moisture content of grain (Bekele et al., 2012; Beta et al., 2000). Steeping grain in NaOH (ca 0.2% v/v) and dilute formaldehyde (ca 0.05% v/v) has been shown to improve sorghum malt quality, by suppressing inhibitory effects on the malt enzymes, particularly in cultivars with high levels of condensed tannin (Beta et al., 2000; Taylor et al., 2006). Malt from grains steeped in NaOH solution vis-à-vis control malt (not steeped in NaOH), show enhanced diastatic power, free α-amino nitrogen and hot water extract (Ukwuru, 2007). In contrast, repression of carbohydrate modification occurs when sorghum grains are steeped in dilute calcium hydroxide solution (Okolo et al., 2010). Steeping sorghum continuously in alkaline liquor (0.1% NaOH) and germinating for 4 d at 30°C repress germinability (by 3-34%), root length and malting loss. However, steeping sorghum cultivar SK 5912 continuously in alkaline liquor plus a final warm water steep enhances malt diastatic activity (50-250%) and α- and β-amylase activities. β-Amylase activity constitutes over 70% of the total diastatic activity in alkaline steeped cultivar ICSV 400 malts (Okolo & Ezeogu, 1996a). In contrast, alkaline steeping of ICSV 400 with air rest and final warm water treatment repress diastatic activity by 9% although similar treatment significantly enhance diastatic power and α-amylase development in cultivars KSV 8 and SK 5912 (Okolo & Ezeogu, 1995a). Nevertheless, cultivar SK 5912 produces relatively low HWE although it has improved amylolytic activity (Okolo & Ezeogu, 1996a). As well, steeping sorghum in 0.1N ammonia solution (NH_4OH) up to 18 h increasingly reduces enzyme development, cold and hot water extracts, and malting losses (by suppressing the growth), but does not prevent mouldiness (Ilori & Adewusi, 1991).

However, soaking white sorghum grains with 1 or 2% (w/w) koji *(Aspergillus oryzae)* and germinating for 4 d yield malt with diastatic power comparable to barley malt. The addition of 1% (w/w) *A. oryzae* to sorghum grains before germination does not affect germination capacity (97.3%), whereas inoculation with 2% (w/w) reduces germination capacity by about 5%. The sorghum malts from five d of germination show similar malting losses. Addition of *A. oryzae* during malting enhances the α-amylase activity of malts but has no effect on the β-amylase activity. Addition of 1% koji during malting enhance amyloglucosidase activity (AMG) of malt while 2% koji, causes a reduction in AMG activity of the malt (Heredia-Olea et al., 2017).

c) Germination Stage

Germination basically involves outgrowth of plumule and radicle of the seedling until the production of adequate enzymes for the malt but prior to the exhaustion of seed nutrients. During seed germination, storage proteins within endosperm are hydrolysed by enzymes to provide nitrogenous compounds for grain outgrowth. Small peptides and products of partial protein hydrolysis in endosperm are translocated across scutellum to embryo where peptides are degraded by peptidases to release amino acids for plant structure and enzyme synthesis. The radicle usually grows out first before the plumule during germination. The lengths of the radicles (rootlets) and plumules (acrospires) increase with d of germination. Sorghum grains germinated for 4 d produce seedlings with radicles 2 to 5-fold longer than the plumules. Nevertheless, vegetative outgrowths in seedlings apparently have no clear relationship with the size of sorghum grains (as reflected by 1000 grain weight) (Owuama, 1999; Owuama, 2019).

Both germination period (3 to 4 d) and sorghum variety remarkably affect malt quality (Bekele et al., 2012; Owuama, 2019). Increase in germination period (2 to 4 d) show direct correlation to sorghum malt diastatic power (DP, 18.96 to 31.39 L), hot water extract (HWE, 41.85 to 85.08 %), malting loss (8.68 to 27.56 %) and

free amino nitrogen (FAN, 185.67 to 343.29 mg L^{-1}) (Bekele et al., 2012). Varietal differences and the malting processes, particularly steeping and germination influence quality of sorghum malt (Abuajah et al., 2016; Ogu et al., 2006; Svenson *et al*., 2011; Taylor et al., 2006). Germination significantly affects increase in amylase activity, malting loss, soluble solids yield and protein content (Abuajah et al., 2016; Claver et al., 2010; Svenson *et al*., 2011). As the germination period increases up to 5 d, quality of sorghum malt increases with increase in wort filtration rate, fermentable sugars, the specific gravity and wort extract but a marginal decrease in the specific viscosity (Abuajah et al., 2016).

Germinating sorghum grains at optimal temperatures of 25 to 30°C for 3 to 7 d, depending on the grain variety, leads to rapid growth of radicle, a reduction in adequate germination period and the production of well modified malts (i.e. where horny grain endosperm has completely changed to powdery, chalky state) with high diastatic power (Demuyakor & Ohta, 1992; Lasekan et al., 1995; Owuama & Okafor, 1991; Palmer et al., 1989; Ratnavathi & Ravi, 1991), hot water extract, sugar contents (Lasekan et al., 1995) and free amino nitrogen (Morrall et al., 1986). The optimal germination period varies with sorghum grain varieties and germination conditions such as, illumination and steep moisture. Three days of germination of sorghum grains steeped in the dark for 18 h, produce malts with higher diastatic power than those steeped for 32 h. As well, increasing germination period from 2 to 6 d at 30°C results in an increase in diastatic power, reducing sugar, cold water and hot water extracts (Demuyakor & Ohta, 1992; Lasekan et al., 1995; Palmer et al., 1989), as well as protein content of sorghum malt (Okoh et al., 1989). The DP increases as the germination period increased from 48 to 96 h, but no remarkable difference between 96 and 144 h. Considering the excessive malting loss and marginal increase in HWE beyond 96 h, the optimum malting period is about 96 h (Bekele et al., 2012). In contrast, germinating sorghum at relatively higher temperature of 35°C or lower temperatures of between 15 and 20°C, slows down amylase formation and invariably reduces diastatic power (Owuama, 1999; Morrall et al., 1986).

Diastatic power, which largely measures the combined activity of α- and β-amylases, is of a greater importance in sorghum malt than extract (Raschke et al., 1995) and seems to be directly proportional to its reducing sugar content (Lasekan et al., 1995). Generally, diastatic power, free α-amino nitrogen, extract and malting loss increase with germination time (Morrall et al., 1986). High moisture level in the early stages i.e. within 8 d of germination, usually results in a high diastatic power and consequently early enzymatic hydrolysis and transfer of solubilised products to embryo. The diastatic power subsequently slows down but may in some cases, increase slowly to the end of the germination period (Aisien & Ghosh, 1978; Owuama, 1999). Diastatic activity of malts range from 32.3 to 150.0 SDU/g (Subramanian et al., 1995) and over 50% of β-glucan is digested by enzymes after 2 d of germination (Ogbonna & Egonwu, 1994). However, diastatic power of 60 to 80 KDU/g is recommended for sorghum grain to be considered for commercial malting (Owuama, 1999).

Germination of sorghum grains steeped with air rest at 25-26°C for 6 d, produce malt whose percentage extract has highly significant correlation with the diastatic power (Swanston et al., 1992). Germination temperatures of 24 and 28°C are both equally good for the development of diastatic power, FAN and extract but higher temperatures are progressively worse. Germination of sorghum grains for 6 d under high (77%), medium (60.8%) and low (42.8%) moisture conditions affect the diastatic power, FAN, extract and malting loss and moisture content of green malt (Morrall et al., 1986). For example, high moisture during germination causes increases in diastatic power, FAN, extract and malting loss. However, towards the end of germination, high moisture negatively affects diastatic power (Morrall et al., 1986). A maximum diastatic power of 46.6 SDU/g occur within 5 d of germination at 24°C under medium moisture. Maximum FAN of 180mg FAN/100g malt is produced under high moisture after 6 d germination at 32°C (Morrall et al., 1986). Treatment of sorghum with thiram (0.2%) plus carbendazim (0.1%) improves seed germination by 8 to 40% and reduces seed mycoflora (Ingle et al., 1994). Sorghum grains heavily infected with mould produce malts with slightly higher amylase activity (Kumar et al., 1992), thus suggesting that fungi contribute towards the increase in amylase activity. Seed mycoflora of sorghum species include *Aspergillus flavus, Curvularia lunata, Cladosporium cladosporoides, Fusarium moniliforme, Rhizopus* sp., *Alternaria* sp., *Penicillium* sp., *Dreschlera* sp., and *Neurospora* sp. (Kumar et al., 1992; Owuama, 1991).

d) Kilning

Kilning involves the drying of green (wet and growing) malt in a kiln or oven at a relatively high temperature until the vegetative out growths become friable or brittle, desirable colour develops while the required hydrolytic enzymes for mashing remain intact. Kilning contributes to colour development which is influenced by the extent of modification, duration and levels of temperature-time sequence of kilning cycle and moisture content of green malt at different stages of the cycle (Briggs et al., 1981; Owuama & Asheno, 1994). Sorghum malts are kilned at elevated temperatures of 45 to 100°C (Owuama, 1999; Owuama & Asheno 1994), essentially to remove raw flavour of green malt and promote chemical reactions for the formation of

components which impart characteristic flavour to malt (Briggs et al., 1981). Commercially produced sorghum malts for brewing are usually dried at moderate temperatures up to 50°C (Abuajah, 2013). Kilning green sorghum malt above 50 ℃ can lead to loss of volatiles, reduced enzyme activities but enhanced malt flavour (Bekele, 2012; Dewar et al., 1997b).

Storage period of sorghum malts apparently affects the enzyme activity and the malt constituents and extracts (Etokakpan, 2004a). The diastatic power of freshly kilned sorghum malt (68.1°WK) decreases by 29% after six months of storage. Freshly kilned sorghum malt shows high wort turbidity (4.9 EBC) which drops to 0.95 EBC and 1 EBC after storage for 2 and 6 months respectively. Colour of worts derived from the malt diminishes slightly over six month-period from 7.6 EBC in freshly kilned malt to 6.8 EBC. Wort extract remains virtually unchanged throughout the six month-period probably due to the use of external amylolytic enzymes during mashing. The protein in wort extract (46.6%) decreases to 43.2% after six months. The apparent wort extract after final attenuation (AEFA) indicates more fermentability starting from two months after storage. Free-amino nitrogen (FAN) decreases from 238 mg/L to 194 mg/L after six months of storage while mash filtration period (86-93 min) using a micro-mash filter was virtually the same throughout the six months of storage (Etokakpan, 2004b).

Temperature, moisture content of green malt and duration of kilning influence amylase activity of sorghum malts (Malleshi & Desikachar, 1986). Kilning green malts with moisture contents over 10% at elevated temperature accelerates the inactivation of enzymes (Andriotis et al., 2016), but kilning sorghum green malt with less than 10% moisture at 100°C for 3 to 4 h has little effect on the amount of hydrolytic enzymes and diastatic power (Owuama, 1999). Varying kilning process produces malts of differing characteristics. Kilning malts in two stages i.e. exposing green malt initially to 55°C and subsequently to 65°C, produce malts with higher sugar content than kilning at a single temperature of 65°C (Owuama & Asheno, 1994). In two-stage treatment, initial exposure to 55°C for sometimes, considerably reduces moisture content of green malt before final temperature (65°C) treatment (Owuama & Asheno, 1994), a process which apparently encourages greater survival of hydrolytic enzymes while malt acquires characteristic flavour. Higher kilning temperature causes a relatively smaller decrease in reducing sugar and diastatic power of malts than on hot water extract and liquefying power. This is apparently due to inactivation of saccharifying amylase, β-amylase to a greater extent than liquefying amylase, α-amylase (Owuama, 1999). During kilning, reducing sugars decrease in quantity while sucrose level often increases (Owuama & Asheno, 1994) possibly because of a reversal in the action of hydrolytic enzymes (Andriotis et al., 2016) that appears not to have a direct relationship with amylase content in sorghum malt (Owuama, 1999; Owuama & Asheno, 1994) suggesting the involvement of other enzymes, with varying contributions in different sorghum varieties (Briggs et al., 1981; Owuama & Asheno, 1994).

Diastatic power and extract yield of the sorghum malt show a linear decrease with increase in kilning temperature while the total soluble nitrogen (TSN), permanently soluble nitrogen (PSN), Kolbach index and free amino nitrogen (FAN) show parabolic variation (to an optimum temperature range of 50 to 60°C) with increase in kilning temperature (Abuajah, 2013). But the colour of the worts produced from the malts dried at different temperatures show a linear increase with increase in kilning temperatures. However, the pH values of the worts did not show any significant change with increase in kilning temperature. Apparently, a temperature range of 50 to 60°C for kilning sorghum malt is suitable for producing good quality malt (Abuajah, 2013). Percentage moisture content of kilned malts from different sorghum varieties have been shown to fall between 5.2 to 13.8 % (Bekele, 2012; Etokakpan, 2004a; Ogu et al., 2006; Owuama, 2019).

IV. Enzymes in Malting

A variety of enzymes are present in sorghum grains and some are developed or activated during malting. These enzymes include; carbohydrases (α-, β- and γ-amylases), proteinases, lipases and peroxidases. Some of these enzymes present in malt are examined in greater details below.

a) Diastatic Power

Diastatic power (DP) refers to the combination of activities of enzymes (carbohydrases) in malt that hydrolyse starch into fermentable sugars. Thus, diastatic power correlates with sugar content in wort derived from mashing (Etokakpan & Palmer, 1990). Diastatic power of malt increases with steeping temperature up to 30°C and germination period up to 5 d (Dewar et al., 1996; Subramanian et al., 1995; Swanston et al., 1994) after which a plateau is reached (Okon E.U. & Uwaifo, 1985). However, brewing with sorghum *(Sorghum vulgare)* malt is apparently challenging due to low diastatic activity inadequate for complete saccharification, high starch gelatinization temperature and low FAN content (Taylor et al. 2013). Sorghum malt has a low β-amylase activity, but a higher α-amylase activity than barley malt. This leads to production of low fermentable sugars and a high dextrins content, causing an increase of viscosity (Espinosa-Ramírez et al. 2013; Owuama, 2019).

Diastatic power in sorghum malt differs with sorghum cultivars and usually comprise α-amylase and β-amylase (Mouria et al., 1998), but *Sorghum bicolor* (sweet sorghum) malt additionally contains

amyloglucosidase, thus the DP of *S. bicolor* comprises α-amylase, β-amylase and amyloglucosidase (γ amylase) (Owuama, 2019). Amyloglucosidase (γ-amylase or glucoamylase) encompasses α-glucosidase and limit dextrinase, which act synergistically with α-amylase and β-amylase respectively (Evans et al., 2010; MacGregor et al., 1999; Owuama, 2019; Preseˇki et al., 2013; Zhang et al., 2013). Generally, *S. bicolor* and *S. vulgare* varieties have virtually similar α-amylase and β-amylase activities but *S. bicolor* varieties show higher DP (Owuama, 2019; Subramanian et al., 1995). Concisely, *Sorghum vulgare* malt DP = α- + β-amylases activities while *Sorghum bicolor* malt DP > α- + β- + γ-amylases activities (MacGregor et al., 1999; Owuama, 2019; Preseˇki et al., 2013; Zhang et al., 2013;), apparently because of AMG synergism with α- and β-amylases. Malts with high levels of diastatic power are known to yield increased reducing sugar levels in wort and enhance its fermentability. Addition of AMG in mash increases diastatic power, wort glucose and total fermentable sugars equivalents (Pozo-Insfran et al., 2004) apparently due to the synergistic activity between α-glucosidase and α-amylases (Wong et al., 2007), and between limit dextrinase and β-amylases (MacGregor et al.; 1999). Diastatic power of quality sorghum malt suitable for brewing should be greater than 28 SDU/g of malt (i.e. ca 45-49 degrees Litner [°L]) (Beta et al., 1995; Taylor, 1992). Presently, malts of some sorghum cultivars have with high DP up to 136.7 °L (Beta et al., 1995; Morall et al., 1986; Owuama, 2019). Differences exist in DP of *Sorghum vulgare* cultivars and range from 112.6 to 117° while malts of *Sorghum bicolor* cultivars have DP of 123.7 to 136.7° (Owuama, 2019).

Diastatic power is measured in a variety of units viz., SABS (South African Bureau of Standards) DP assay in SDU/g (sorghum diastatic units per gram), the IoB (Institute of Brewing) in degree Lintner (°L) and EBC (European Brewing Convention) in Windisch-Kolbach (°WK) (Bajomo and Young, 1990; Etokakpan, 2004a, 1990; Owuama, 2019). SDU methods are considered suitable for sorghum DP measurements. However, in a bid to use appropriate DP unit when sorghum and barley malts are combined in one mash, it is desirable to convert SDU to °WK and °L, which are usually applied in measuring DP of barley malts, thus the need to employ the following relationships in equations, 1 to 4 (Etokakpan, 2004a)

$$SDU = 0.741°WK + 0.8272 \quad \text{Eqn. 1;}$$

$$SDU = 0.559°WK + 15.677 \quad \text{Eqn. 2}$$

$$SDU = 1.6397°L - 1.0506 \quad \text{Eqn. 3;}$$

$$SDU = 1.06°L + 19.748 \quad \text{Eqn. 4}$$

As well, DP in °WK can be converted to °L by using the equation below (Hopkins et al., 1934);

$$°L = (°WK+16)/3.5 \text{ i.e. } °WK = 3.5 \times °L - 16 \quad \text{Eqn. 5}$$

Diastatic power of sorghum varieties determined in different units show a range of 56 to 132 °WK corresponding with 29 to 67°L and 47 to 87 SDU (Etokakpan, 2004a). Equations 1 to 4 are applicable under appropriate conditions (Etokakpan, 2004a). The IoB and EBC methods are considered suitable for sorghum DP measurements.

b) Alpha Amylase

Alpha-amylase (endo-acting) randomly hydrolyses starch chains at α(1,4) glucosidic linkages distant from the ends of the chains and from α(1,6) linked branches in the chains yielding dextrins, oligosaccharides, maltose and glucose (Briggs et al., 1981; de Souza & Magalhães, 2010). During malting significant quantity of α-amylase is produced in embryos of sorghum (Palmer, 1989). α-Amylase in sorghum malt may be either completely soluble or largely insoluble depending on variety of sorghum (Demuyakor & Ohta, 1992; Jayatissa et al., 1980). The formation of α-amylase requires adequate oxygen, however this can be prevented in the presence of excess carbon dioxide (Owuama, 1999). α-Amylase activity in sorghum malt is 25 to 183 SDU/g depending on sorghum variety (Aisien & Ghosh, 1978) and increases with sorghum diastatic power in cultivars with SDU values greater than 30 (Lasekan et al., 1995; Ratnavathi & Ravi, 1991). Differences exist in α-amylase activities of malts between sorghum cultivars *S. bicolor* ([sweet sorghum] and *S. vulgare* [non-sweet sorghum], and within the various sorghum cultivars (Owuama, 2019; Subramanian et al., 1995). Generally, α-amylase activities of different *S. bicolor* cultivars (71.8-83.2°) are slightly lower than those of *S. vulgare* malts (78.8- 85.2°). α-Amylase activities in *S. vulgare* varieties are 70-75 % of the diastatic power (DP) and substantially higher than the 56 - 61 % of DP in *S. bicolor*. However, α-Amylases activities in both *S. bicolor* and *S. vulgare* malts are 2 to 4-fold those of β-amylases. In *S. bicolor*, α-amylases activities are 3.6 to 5-fold those of amyloglucosidase (AMG) (Owuama, 2019; Subramanian et al., 1995).

Steeping sweet sorghum grains at three different time intervals of 8, 12 and 16h and germinating subsequently for 2 and 3 d show the highest amylase activity (1266.10 μg of protein/15 min/g) and highest reducing sugars (33.85 mg/g) in 16h steeped grains, germinated for 3 d. Similarly, addition of different concentrations (0.1, 0.5 and 1%) of commercial α-amylase (Palkozyme), show the highest reducing sugar value (78.83 mg/g) at 1% enzyme concentration at 70°C for 24h (Mesta et al., 2018). However, alkaline steeping with final warm water steep improves substantially α-amylase activity in sorghum malt in sorghum cultivar SK 5912 but represses it in cultivars ICSV 400 and KSV 8. The reason for this variation with different cultivars is unclear but may be attributable to α-amylase polymorphism. It is known that steeping or germinating

conditions influence the inhibition or enhancement of the synthesis of particular isoforms detectable in cereal grains during malting (Jones & Jacobsen, 1983; Owuama, 1999). The inhibition of a specific dominant α-amylase isotype by native proteinaceous α-amylase inhibitor in sorghum (Macgregor & Daussant, 1981) invariably depresses total amylase activity while inactivation of the inhibitor during alkaline steeping enhances total amylase activity (Okolo & Ezeogu, 1996a). Alternatively, enhancement of alkaline α-amylase activity in one cultivar but not in another may be attributable to the capacity of alkaline steep liquor to influence protein-binding properties of tannins/ polyphenols which vary in concentration and distribution in various sorghum cultivars (Chavan et al., 1981). Tannins (located mainly in pericarp and testa) and other polyphenols can bind to proteins including enzymes, and are therefore likely to inactivate enzymes involved in hydrolysis of endosperm materials (Chavan et al., 1981; Owuama, 1999).

c) Beta Amylase

Beta-amylases (exo-acting) hydrolyse penultimate α-1,4 glucan linkages from the non-reducing end of starch yielding maltose and beta-limit dextrins. Non-germinated sorghum grain show virtually no β-amylase activity (Taylor & Robbins, 1993). Sorghum β-amylase develops during germination by transforming from a latent bound form to a free or active form in starchy endosperm (Aisien & Palmer, 1983; Owuama, 1999),. β-Amylase may be either completely soluble or largely insoluble in malt depending on the variety of sorghum (Agu & Palmer, 1997; Demuyakor & Ohta, 1992; Jayatissa et al., 1980; Owuama, 1997). Malts made from sweet sorghum and related variety, birdproof kaffircorn usually contain insoluble amylases which appear to adsorb tenaciously to insoluble substances, thus making aqueous extraction impossible (Owuama, 1999). Thus, peptone solutions have been used to liberate the bound β-amylase, resulting in higher DP of the sorghum malts in coloured and bird-proof varieties (Agu & Palmer, 1996; Kumar et al., 1992; Owuama, 1999). However, a contrary report indicates that β-amylase is not bound since neither reducing agents nor papain treatment affects its activity (Taylor & Robbins, 1993). Apparently, the difference in observations reflect variation in physiological activities of the sorghum cultivars. Beta amylase activities in malts vary with sorghum cultivars and in *S. vulgare cultivars range from* 22 – 25 % of DP and slightly higher than 19 – 22 % of DP in *S. bicolor* (Owuama, 2019). β-Amylase activity in sorghum malt range from 11 to 41 SDU/g (Beta et al., 1995; Taylor & Robbins, 1993) and constitutes 27 to 49% of total diastatic activity in sorghum (Ezeogu & Okolo, 1995).

β-Amylase is more labile than α-amylase and is influenced by germination time and temperature. A rapid increase in β-amylase activity occurs within the first 2 d of germination and subsequently declines in rate of increase up to 6.5 d. β-amylase activity is inversely related to temperature, giving the highest activity at 24°C over a range of 24 to 32°C (Taylor & Robbins, 1993). More maltose producing enzyme, β-amylase is present in sorghum malts made at 25°C and 30°C, producing 66% more maltose during mashing than malts made at 20°C (Owuama, 1999). There is a wide variations regarding β-amylase activity of sorghum malt and this may be due to the assumptions that β-amylase activity is the difference between total amylase activity and α-amylase activity. An assumption which ignores activities of other starch degradation enzymes such as α-glucosidase and limit dextrinase.

β-Amylase activity also shows significant correlation with malt diastatic power and is completely inactivated in 15 min at 68°C (Taylor & Robbins, 1993). However, alkaline steeping with final warm water steep treatment and air rest result in a decrease in β-amylolytic activity in cultivar ICSV 400 but an increase in both cultivars KSV 8 and SK 5912 (Okolo & Ezeogu, 1996a). The reduction in β-amylase activity in cultivar ICSV 400 may reflect repression of the synthesis of a major β-amylase isotype. Isoelectric focussing indicates that sorghum β-amylase has a major and a minor isoenzyme of approximate pl 4.4-4.5 (Taylor & Robbins, 1993). β-Amylase heterogeneity is influenced by malting stage and conditions (Laberge & Marchylo, 1986; Macgregor & Matsuo, 1982). The activity of β-amylase in sorghum malt significantly increases when a combination of final warm water and air rest cycles are employed during malting. β-amylase activity of malt is known to be prominently affected by steep regime, alkaline steep liquor, and kilning conditions as well as their various interactions. Steeping in $Ca(OH)_2$ enhances malt β-amylase activity at higher kilning temperature (50°C) unlike steeping in KOH that shows a reduced effect. Nevertheless, the extent of β-amylase activity enhancement is cultivar dependent (Okungbowa et al., 2002).

d) Amyloglucosidase or Glucoamylase (γ-Amylase)

Amyloglucosidase or glucoamylase [γ-amylases] is exo-acting and hydrolyses both α-1,4 and branching α-1,6-linkages to yield glucose. Amyloglucosidase comprises α-glucosidase and limit dextrinase. α-Glucosidase and limit dextrinase have been shown to act synergistically with α-amylase and β-amylase respectively, in starch hydrolysis, yielding glucose (Evans *et al*., 2010; MacGregor *et al*., 1999; Owuama, 2019; Presečki *et al*., 2013; Zhang *et al*., 2013). Evaluation of sorghum cultivars reveals that while amyloglucosidase is present in *S. bicolor* cultivars, it is not detectable in *S vulgare* varieties (Owuama, 2019). Malts of *S. bicolor* cultivars show

amyloglucosidase (AMG) activities ranging from 14.5 – 21.3°. The α-amylase activities in *S. bicolor* cultivars are 3.6 to 5-fold those of AMG, while the β-amylase activities are 1.2 to 1.9 fold those of AMG. AMG activities in *S. bicolor* malts are 12-16 % of their diastatic power (DP) (Owuama, 2019). Generally, DP in *Sorghum vulgare* malts are equal to α-amylase plus β-amylase activities, but in *Sorghum bicolor*, DP is greater than the sum of α-amylase, β-amylase and AMG activities, thus suggesting synergism among the amylases (Owuama, 2019). See below for discussion on α-glucosidase and limit dextrinase.

e) Alpha-Glucosidase

Alpha glucosidase or maltase is one of the enzymes involved in starch degradation during cereal seed germination (Sun & Henson, 1992). α-Glucosidase in germinating grains catalyses hydrolysis of terminal, non-reducing α-(1, 4) glucosidic linkages in both oligosaccharides and α-glucans yielding glucose (Andriotis et al., 2016; Owuama, 1999;). α-Glucosidase in sorghum malt contributes to glucose production in wort by hydrolysing terminal α-1,4 linked D-glucose residues to release glucose (Agu & Palmer, 1997). Purified alpha-glucosidase is quite thermolabile (less than 50°C), cleaves a single glucose from a starch chain or splits maltose to produce two glucose units, thus reducing the level of maltose in the fermentable sugar profile (Fox, 2018). Although, α-glucosidase in sorghum is soluble in water, it is also active in insoluble state while adhering strongly to insoluble malt solids (Taylor & Dewar, 1994; Watson & Novellie, 1974). α-Glucosidase development in sorghum in influenced by germination period and temperature. Limited α-glucosidase extracted with sodium chloride under alkaline conditions is enhanced by adding papain (Owuama, 1997). Sorghum malt from 5 d germination at 30°C, show highest α-glucosidase activity in extract with sodium phosphate pH 8 containing L-cysteine at pH 3.75 compared to those of 1 to 4 d (Agu & Palmer, 1997; Taylor & Dewar, 1994). The sorghum malt with the highest α-glucosidase activity however produces the lowest glucose levels in wort, suggesting that α-glucosidase is not the dominant glucose-producing enzyme during mashing of sorghum malts (Agu & Palmer, 1997). Malts from germinating sorghum at 30°C show the highest levels of α-glucosidase, β-amylase and α-amylase as well as the highest maltose to glucose ratio, relative to 20°C and 25°C germinated sorghum malt. However, the role of each enzyme in the sugar ratios is unknown (Agu & Palmer, 1997). Nevertheless, the sorghum malts produced at 20°C and 25°C yield worts which contain more glucose than worts from malts produced at 30°C. The individual activities of α-glucosidase, α-amylase and β-amylase of sorghum malts apparently do not correlate with the sugar profile of the worts (Agu & Palmer, 1997). However, α-glucosidase is known to have synergistic activity with α-amylase in solubilizing starch (MacGregor et al., 1999).

Mashing at pH 4, near optimum for α-glucosidase yields relatively higher proportion of glucose than at usual mash pH 5-5.5, which is optimal for β-amylase (Taylor & Dewar, 1994). Although, sorghum malt α-glucosidase activity is highest at pH 3.75, it is still quite active at pH 5.4 employed in mashing sorghum malt (Agu & Palmer, 1997). However, at pH 5-5.5, both total fermentable sugars and free glucose increase with mashing temperature to a maximum at 70°C but the proportion of glucose declines with increasing mashing temperature from 58.6% at 60°C to 23.1% at 80°C. In contrast, mashing at pH4 produces less amount of total fermentable sugars and free glucose at 70°C than at 60°C (Taylor & Dewar, 1994). Maltose in sorghum worts produced at 65°C is limited because of inadequate gelatinization of starch and not β-amylase and α-amylase activities since gelatinization of the starch granules of sorghum malt occurs between 68-72°C (Taylor and Taylor, 2018). Hence, the decantation mashing method yielded sorghum worts with high levels of maltose, particularly when sorghum malt is produced at 30°C (Agu and Palmer, 1997). Higher amount of glucose is observed in wort from EBC conventionally mashed malt as against using pre-cooked malt insoluble solids where α-glucosidase inactivation occurs preventing hydrolysis of maltose to glucose and resulting in high maltose levels in sorghum worts (Taylor & Dewar, 1994).

f) Limit dextrinase

The activities of starch degrading enzymes (including α-amylase, β-amylase, alpha glucosidase and limit dextrinase) result in the production of a mixture of low molecular weight dextrins (Aisien et al., 1983; Etokakpan & Palmer, 1990; Okon & Uwaifo, 1985; Taylor & Robbins, 1993). Limit dextrinase (LD) also called R-enzyme, pullulanase, isoamylase or amylopectin 6-glucanohydrolase, is a debranching enzyme that hydrolyses α-(1 → 6) linkages in amylopectin or in branched dextrins derived from the actions of α- or β-amylases (Yang et al., 2009). LD cleaves the α-1,6 branches on amylopectin, producing linear α-(1 → 4)-linked chains for α- and β-amylases to further hydrolyse to glucose and maltose. The degree of branching on amylopectin and amylose in any cereal used either as malt or as an adjunct source, could impact on the residual dextrins which are not fermentable (Denyer et al., 1999). Purified limit dextrinase from malted sorghum flour readily hydrolyses alpha-limit dextrins which have maltosyl or maltotriosyl side-chains, pullulan, amylopectin and beta-limit dextrin (Haedi et al, 1976). Though, LD is quite temperature sensitive, it can survive for a reasonable time in mash, where it cleaves α-1,6 linkages and thereby contributes remarkably to

fermentable sugars (Fox, 2018; Hu et al., 2014; Izydorczyk & Edney, 2003). The initial temperature of the brewing process influences LD activity, and with highly branched amylopectin, more non-fermentable solubilized residual dextrins are produced that affect beer flavour and contribute to mouthfeel (Langstaff & Lewis, 1993). Maintaining optimum temperature of 60-62°C for malt limit dextrinase as opposed to 50°C of purified LD, and lowering pH from 5.8 to 5.4 increase wort fermentability due to increased LD activity. However, wort fermentability is more strongly correlated to free LD activity of malt than to α- and β-amylase activities (Stenholm & Home, 1999). Nevertheless, limit dextrinase has been shown to have synergistic activity with β-amylase in solubilizing starch (MacGregor et al., 1999).

Dextrins containing from 4 to 10 glucose units have been observed in sorghum malt, wort and beer. During 10 d malting period, about 5% fermentable sugars and trace amounts of dextrins are detectable. Using maize adjunct during mashing at pH 4, produce a wide range of dextrins which greatly diminish towards the final stages of mashing. Both sorghum and barley beer contain similar amounts of dextrins, majority of which are branched, and the activity of LD largely reduce their concentration (Glennie & Wigh, 1986).

g) Carboxypeptidases and Proteinases

Carboxypeptidases (exopeptidases) and proteinases (endopeptidases) are important in protein mobilisation during grain germination. Peptidase formation requires adequate oxygen but is prevented in the presence of excess carbon dioxide (Owuama, 1997). Carboxypeptidases specifically hydrolyse solubilised proteins to free alpha amino nitrogen (FAN) [proteolytic breakdown products of endosperm proteins comprising amino acids and small peptides], which is the source of nitrogen essential for anabolic functions of germinating seedling and as nutrients for yeast metabolism in wort (Baxter, 1981; Enari & Sopanen, 1986). Germination conditions and sorghum cultivar influence carboxypeptidase activity. For example, carboxypeptidase activity increases with germination time up to 4 d showing 4 times the activity in resting grains (Evans & Taylor, 1990a). Also moisture, temperature and germination time significantly affect carboxypeptidase activity with the highest activity occurring in malt from 4 d germination under medium moisture at 24°C, and yielding maximum FAN value of 275μg FAN/5h/g dry malt (Evans & Taylor, 1990a; Morrall et al., 1986). Sorghum malts resulting from different final warm steep treatment periods show poor correlation between the period of final warm steep treatment and carboxypeptidase activity, whose levels vary with sorghum cultivars. Also, correlation between sorghum malt FAN and carboxypeptidase activity can be poor or strong depending on cultivar (Okolo & Ezeogu, 1996b). Proteolytic enzyme activity in sorghum is influenced by both cultivar and malting conditions but steeping does not significantly affect proteinase or carboxypeptidase activity. However, different sorghum cultivars grown and malted under similar conditions differ significantly in proteinase (endopeptidase) and carboxypeptidase activities (Evans & Taylor, 1990b).

Germination temperature (24-32°C) and moisture have little or no effect on proteinase activity (Evans & Taylor, 1990b). Germinating sorghum for 36 h or 48 h causes a considerable increase in protease activity in embryo or endopeptidase activity in both embryo and endosperm (Morrall et al. 1986). Increase in germination time up to 4 d moderately increase proteinase activity with a maximal yield of 1604μgN/5h/g dry malt. The highest proteinase activity differs with sorghum malts resulting from different final warm steep period and also with various cultivars (Okolo & Ezeogu, 1996b). Proteinase activity in cultivar ICSV 400 rises from 1224 to 1469μgN/3h/g dry malt as final warm steep period increases from 1.5 to 3.0 h. However, proteinase activity declines with increase in final warm steep period beyond 3.0 h suggesting an optimum final warm steep period similar to that for carboxypeptidase activity. Nevertheless, sorghum cultivar, KSV 8 attains highest proteinase and carboxypeptidase activities at 6 h final warm steep period (Okolo & Ezeogu, 1996b).

Optimal proteinase and carboxypeptidase activities occur after 3 h final warm water steep period in cultivar ICSV 400 but after 6 h final warm water steep in cultivar KSV 8 (Okolo & Ezeogu, 1996b). However, higher proteinase activity occurs in cultivar KSV 8 in relation to cultivar ICSV 400, although with lower CWS-protein in KSV 8. This apparent contradiction can be attributed to qualitative differences in complexity and structure of endosperm proteins of various sorghum cultivars and/or differences in the nature of the major proteinase isoforms in grains (Okolo & Ezeogu, 1996b; Riggs et al., 1983). Apparently, the highest proteinase and carboxypeptidase activities occur in the same final warm water treatment period for given sorghum cultivars (Okolo & Ezeogu, 1996b). Varying sorghum cultivars and air rest periods from 1 to 4 h during steeping with 6 h final warm water (40°C) steep, greatly influence CWS-protein, total cold water soluble, cold water soluble protein modification index, total free alpha amino acid nitrogen, and carboxypeptidase and proteinase activities of malt (Okolo & Ezeogu, 1995b).

Evaluation of the effects of calcium ion in steep liquor, on sorghum endosperm reserve protein mobilization of two sorghum cultivars, ICSV 400 and KSV 8, reveal remarkable enhancement of total non-protein nitrogen (TNPN) accumulation in ICSV 400 malt, but 23 to 69% repression in KSV8 malt. Likewise, Ca^{2+} ion treatment effectively stimulates peptide accumulation in ICSV 400 indicating that it largely

enhances TNPN accumulation in this cultivar unlike in KSV 8 where peptide accumulation is highly repressed. Protein solubilisation, soluble protein accumulation and cold water soluble protein modification in ICSV 400 and KSV 8 cultivars were highly repressed by Ca^{2+} treatment. Ca^{2+} treatment remarkably stimulates carboxypeptidase development in both cultivars, slightly enhances proteinase development in KSV 8 but causes reduced proteinase development in ICSV 400 (Okolo et al., 2011).

White non-tannin sorghum grain produces substantially higher levels of FAN than white type II tannin sorghum, due to the presence of tannin. Incubating sorghum grains with combined exogenous neutral proteinase and amino-peptidase, improve FAN production. However, malts from the white non-tannin and tannin sorghum types produce similar FAN levels when incubated in the absence of the exogenous proteases. Malts of both tannin and non-tannin sorghums incubated with neutral proteinase alone yield substantially more FAN (124-126 mg 100 g^{-1}) than the grains (61-84 mg 100 g^{-1}). The combination of amino-peptidase and proteinase do not improve on FAN yield. Also, malting does not influence wort free amino acid profile. Nevertheless, group B amino acids constitute the highest percentage (42-47%) (Dlamin et al., 2015).

h) Lipases

Lipase (triacylglycerol acyhydrolase) catalyses the hydrolysis of triacylglycerides to free fatty acids and glycerol (Lin et al., 1983). Malt lipoxidase catalyses peroxidative reaction that converts free fatty acids to hydroperoxides and aldehydes which have detrimental effects on beer such as poor acceptability and reduced shelf-life (Kobayashi et al., 1993). A higher level of fatty acid is present in sorghum relative to barley, wheat and millet (Osagie, 1987). Sorghum grains contain detectable lipase activity which varies slightly during 24 h steeping period at 30°C and increases during germination to about 4-fold after 96 h. However, lipase activity varies among different sorghum (red and white) cultivars, but peaked in malts derived from 4 d of germination, though the red showed higher activity (Nwanguma et al. 1996, Uvere & Orji, 2002). Differences in lipase activity apparently suggest variations in lipase synthesis or differences in endogenous regulators of lipase activity (Chapman, 1987). The lipase activity in plumule, endosperm and radicle are 68%, 29% and 3 % respectively in 72 h old malt. Sorghum malt lipase apparently consists of three isoforms, two of which have their highest activity optima within the acidic pH range (Uvere & Orji, 2002). The optimal pH for sorghum lipase is 7 although the activity range is between pH 5.5 and 9. The percentage lipase activity at pH 5.5, 6, 8 and 9, relative to that at pH 7 are 50%, 95%, 88% and 60% respectively (Nwanguma et al. 1996; Uvere & Orji, 2002). Because of the wide pH range, sorghum lipase activity occurs during steeping, malting and mashing (Gram, 1982, Uvere & Orji, 2002). Lipase activity decreases in sorghum malt after kilning at 48°C for 24 h to between 24% and 66% of total lipase activity in green malt depending on sorghum variety, however mashing at 65°C yields wort with no detectable lipase activity (Uvere & Orji, 2002). Exposing malt crude water extract for 10 min to temperatures of 50°C, 60°C and 65°C reduce lipase activity to 57%, 43% and 14% respectively, of the original activity and total loss of lipase activity result from heating extract for 30 min at 50°C (Nwanguma et al. 1996).

i) Peroxidases

Plant peroxidases are heme-proteins that utilise hydrogen peroxide ($H_2O_{2)}$ to oxidise various hydrogen donors including phenolic substances, amines, ascorbic acid, indole and particular inorganic ions (Diao et al., 2011; Dicko et al., 2006; Dunford, 2010; Murphy et al., 2012). Peroxidase catalyses the reductive destruction of hydrogen peroxide and invariably contributes to the defence system of living organism against peroxidation of unsaturated lipids involving oxygen radicals (Floyd, 1990; Nwanguma & Eze, 1995). Lipid peroxidation causes reduction in quality and shelf life of most cereal products. Peroxidase activity in different sorghum varieties differs with malting regimes. Various sorghum varieties differed in their expression of peroxidase over different germination periods. The least peroxidase activity was ≤ 0.6 peroxidase units in the different varieties, occur at the end of 24 h steeping period. The highest peroxidase activity (above 6 peroxidase units) occur between 72 and 96 h of germination. Generally, the size of the sorghum grain affects peroxidase expression. Most of the sorghum varieties that show remarkable differences in peroxidase expression between the raw grains and the green malt at the end of germination period, are among the smallest sized varieties (Nnamchi et al., 2013).

Lipid peroxidation is undesirable in malting and brewing (Bamforth et al., 1993; Kobayashi et al., 1993). During malting, aldehydes and other lipid peroxidation products are released that affect the availability of wort nutrients, interfere with yeast metabolism, cause flavour deterioration and affect colloidal stability of beer (Bamforth et al., 1993; Nnamchi et al., 2013). Peroxidase activity increases by about 14-fold during the germination of sorghum grains steeped at 30°C for 24 h, however the levels present vary with sorghum varieties (Nwanguma & Eze, 1995). Peroxidase activity of 39-40% is detectable in endosperm while a combined activity of 56-61% occur in the acrospire and rootlet. The optimal pH for sorghum peroxidase is 5.5 and kilning at 48°C for 24 h shows no depressing effect on the peroxidase activity (Nwanguma & Eze, 1995). In crude extract, sorghum peroxidase activity decreases from 77% to 7.5% after 15 min exposure to temperatures of 60°C to

80°C respectively. Nevertheless, peroxidase activity declines to 5% in 5 min at 85°C and is completely absent at higher temperatures. Sorghum peroxidase survives better in wort than crude extract and about 50% of peroxidase activity is retained in wort after mashing for 1 h at 65°C (Nwanguma & Eze, 1995). Since remarkable amounts of lipid oxidation products form during mashing (Meersche et al., 1983), it is therefore important that sorghum peroxidase remains active in wort to remove oxygen radicals at the later stages of brewing.

V. Malting Loss

Malting loss is the summation of leaching/steeping, metabolic/respiration and vegetative/sprout losses (Malleshi & Desikachar, 1986; Owuama, 1999). Basically, it is the loss in weight of grains after malting. However, malting loss in commercial kaffircorn malts are only due to metabolic and leaching losses, since roots and shoots are not usually removed but milled in with the berry (Owuama, 1997). Factors which influence malting losses include germination period, germination temperature, steep moisture, kilning temperature and sorghum variety. Malting losses, generally vary with germination temperature and increase with germination period. Percentage malting loss increases with germination period among sorghum varieties and range from 8.68% to 27.56% (Bekele, 2012). Malting loss is higher at 25°C (8.4%) and 30°C (10.9%) than at 20°C (6.5%) and malts produced at 30°C over 1 to 6 d show losses of 3 to 31% depending on sorghum variety (Owuama, 1999; Beta et al., 1995; Owuama & Asheno, 1994). Germination temperatures of 25 to 30°C are optimal for amylase and diastatic power development in sorghum malt, and encourage vigorous respiration and high malting losses (Owuama, 1999). High steep-out moisture of grains and watering during malting, enhance the rate of germination and malting loss while reducing malting loss by lowering temperature or moisture level causes a marked decrease in diastatic power (Beta et al., 1995; Owuama, 1999). Thus, the attainment of a good diastatic power in sorghum malt may be linked to high malting loss. Percentage malting loss has also been shown to differ among sorghum varieties and generally lower among cultivars of *Sorghum bicolor* (16.3 and 17.8 %) than those of *Sorghum vulgare* (16.4 to 26.0 %) (Owuama, 2019). A respiration/metabolic loss of 10 to 15% and percentage vegetative loss for *S. bicolor* cultivar (8.9 - 10.1%) and *S. vulgare* varieties (7.2 - 13.3 %) are expected in well-malted sorghum with good diastatic power (Owuama, 2019). Minimizing malting loss, while achieving sufficient grain modification during malting is desirable to produce malt for brewing (Aisien et al., 1983; Bekele, 2012; Ezeogu & Okolo, 1996).

VI. Proteins in Sorghum Grains and Malt

Amorphous storage proteins associate with starch granules within endosperm of barley and sorghum, and during grain germination, malt proteolytic enzymes initiate the modification of grain reserve in endosperm by hydrolysing proteins associated with starch granules, thereby exposing the starch and increasing its susceptibility to amylolysis (Holmes, 1992; Palmer, 1989). The hydrolysis of insoluble reserve protein in germinating grain provides amino acids necessary for the synthesis of hydrolytic enzymes and grain structural materials in growing tissues of seedling (Owuama, 1999). Nevertheless, malts show lower protein than unmalted grains and malts from sorghum cultivars with high diastatic activity exhibit high levels of albumin-globulin fraction (Subramanian et al., 1995). Crude protein contents of grains differ with sorghum varieties and range from 7.0 to 12.3% (Bekele et al., 2012; Owuama, 2019).

During malting, FAN is mainly derived from the hydrolysis of proteins in the endosperm and comprises free amino acids and small peptides, produced by proteinases and carboxypeptidases activities of the malt, and remarkable portion of the nitrogen in the kernel is transferred to the roots and shoots. Proteolytic activity increases with germination time during malting (Evans and Taylor, 1990a). FAN increases in wort with germination period (48-144 h) is partly due to the inclusion of dried roots and shoots (which are rich in FAN) during mashing. The addition of dried roots and shoots of sorghum malt during mashing to ensure adequate FAN level in the wort is necessary particularly for cultivars with minimal FAN content (Dewar, et al., 1997a). Unlike barley malt which is much richer in proline, sorghum malt has asparagine and glutamine as its two most important free amino acids. Also, sorghum malt has higher percentage of amino acids readily assimilated by yeast than barley malt and other cereals such as wheat (Hill and Stewart, 2019). However, percentage malt total nitrogen in sorghum malts vary considerably between 2.0 and 3.1 % while their protein contents range from 12.2 to 19.5 % (Owuama, 2019).

Sorghum malts obtained by steeping grains for 22 h followed with 4 h air rest and further 24 h wet steep at 20°C (giving steep moisture of 34-35%) and subsequently germinated for 5 d at 20°C, 25°C and 30°C show more effective hydrolysis of endosperm proteins at 20°C than at 25°C and 30°C. Malting at 30°C transfers larger quantities of nitrogen from endosperm to embryos (axes and scutella) than malting at 20°C and 25°C, but less amino acids and peptides are transferred to root during malting at 30°C than at 20°C and 25°C. Nitrogen may also move from root to embryo by physiological mechanisms (Agu & Palmer, 1996).

Steeping regime and sorghum cultivar significantly influence FAN values. Generally, exposing sorghum grains to a steep regime incorporating air rest cycles and final warm water steep result in the highest FAN level in ICSV 400 and KSV 8 varieties while continuous steep regime without final warm water steep produce the lowest FAN values. Cultivar and duration of final warm water (40°C) steep highly influence protein modification indices viz., soluble protein of cold water extract (CWS-protein), total non-protein nitrogen (TNPN), a small peptide accumulation, free alpha amino nitrogen, carboxypeptidase and proteinase activities (Okolo & Ezeogu, 1996b). The application of final warm water steep without air rest stimulates FAN development in cultivars ICSV 400 and KSV 8 but significantly represses FAN development in SK 5912. Nevertheless, significant improvement of FAN values occurs in all sorghum varieties after the application of air rest cycles during steeping although the FAN levels vary with cultivar (Ezeogu & Okolo, 1996). Apparently, these differences reflect variations in grain protein structure and degradability (Riggs et al., 1983), amino acid transport processes, and probably differences in enzyme characteristics (Owuama, 1999).

Generally, ICSV 400 shows higher FAN, CWS-protein solubilising activity and accumulation, and better protein modification potential than KSV 8. However, lower TNPN and TNPN-FAN difference in ICSV 400 contrasts with its high FAN, thus suggesting superior anabolic protein turnover apparently from efficient peptide translocation process. Nevertheless, the levels of nitrogenous substances are inconsistent with the proteolytic activities suggesting the involvement of factors other than proteolysis in protein modification (Okolo & Ezeogu, 1996b). Remarkably, KSV 8 records lower FAN although it generally expresses higher carboxypeptidase activity in relation to ICSV 400. This suggests a variation in the rate of protein synthesis from FAN and thus a possible higher rate of anabolic protein turnover in KSV 8 and lower FAN accumulation (Okolo & Ezeogu, 1996b).

Four days of germination of sorghum cultivars steeped in alkaline liquor (0.1% NaOH solution) for 48 h at 30°C under different steeping regimes, reveal that steep regime, steep liquor and sorghum cultivar highly and significantly influence the protein modification indicators viz., CWS-protein, CWS-protein modification index, TNPN, peptide accumulation, FAN, endo- and exo-protease activities. Alkaline steeping causes a highly significant increase in sorghum malt FAN (Okolo & Ezeogu, 1996b). FAN in malt is a net balance of amino acids and peptides resulting from storage protein degradation and those utilised for synthesising new proteins in roots and shoots of growing plant (Morrall et al., 1986; Taylor & Boyd, 1986). FAN development vary among cultivars probably because of differences in major enzyme characteristics and rate of protein metabolism during sorghum grain malting as well as variations in grain protein structure and degradability (Riggs et al., 1983), amino acid and peptide transport processes (Owuama, 1999). Nevertheless, other miscellaneous cultivar-dependent factors also play a role in the control and modulation of protein degradation and synthesis in germinating plant seeds (Shutov & Vaintraub, 1987). Free alpha amino nitrogen development in malt is important in brewing as it constitutes about 70% of total FAN in wort (Pickerell et al., 1986; Taylor & Boyd, 1986).

In general sorghum malts from grains steeped with air rest period and steepout moisture of 33-35% reveal increase in diastatic power, FAN, extract and malting loss with germination time. Germination temperatures of 24 and 28°C are equally good for the development of diastatic power, FAN and extract. Diastatic power, FAN, and extract and malting loss increase with high moisture during germination (Morrall et al., 1986). Germination at 32°C under high moisture shows similar FAN level in malt at 3.0-4.5 d, possibly a period of catabolic and anabolic equilibrium, before increasing further to a maximum of 180 mg FAN/100 g malt after 6 d (Morrall et al., 1986).

FAN levels in sorghum grain wort mashed with commercial enzymes are considerably lower than those obtained with sorghum malt (Dale et al., 1989; Goode et al., 2003). FAN levels of 130–150 mg/L are considered adequate to support optimal yeast growth and fermentation efficiency (Dhamija & Singh, 1978; O'Connor-Cox & Ingledew, 1989), thus to overcome the very low FAN levels when brewing with sorghum, high levels of proteolytic enzymes are required. Use of reducing agents such as 2-mercaptoethanol (Dale et al., 1990; Hamaker et al., 1987), sodium bisulphite and ascorbic acid (Aisien & Palmer, 1983; Arbab and El Tinay, 1997) have been shown improve sorghum protein hydrolysis. Addition of reducing agents such as KMS (potassium metabisulphite), when mashing sorghum grain with exogenous protease also improves FAN production. The rate of sorghum protein hydrolysis is significantly increased by KMS which reduces intermolecular molecular disulphide bonds in the kafirin polymers and oligomers, and apparently allows better access of protease to the kafirin (Ng'andwe et al., 2008). Presumably, reducing agents can reduce the stabilizing inter- and intra-molecular disulphide bonds, which influence the conformation of kafirin before and after exposure to wet cooking (Enari & Sopanen, 1986; Ng'andwe et al., 2008).

VII. Water Extracts of Malts

Hot water extracts (HWE) and Cold water extracts (CWE) (which *are soluble products from enzyme hydrolysis within endosperm during the malting process*

that include sugars and amino acids) vary with sorghum cultivars. However, there are substantial differences between CWE and HWE of malts among various sorghum cultivars (Holmes, 1991; Owuama, 2019). HWE values have been shown to be about 1.5 to 3 fold higher than CWE in both *Sorghum bicolor* and *S. vulgare* varieties. CWE apparently correlate with total nitrogen and protein contents in malts from *S. bicolor* but not with those from *S. vulgare* (Owuama, 2019). CWE and HWE are influenced by cultivar, steeping conditions and steep liquor. CWE is generally enhanced in certain cultivars by alkaline steep with final warm water steep but depressed in others apparently due to alkaline steep repression of certain malt properties like diastatic power and α-amylase activity (Okolo & Ezeogu, 1996a). A combination of air resting and final warm water steep at 40°C reduces kernel growth and malting loss but significantly improves CWE, HWE, diastatic power, α- and β-amylase activities. But final warm water steep without air resting causes a decrease in extract recovery and enzyme activity (Ezeogu & Okolo, 1994). Generally, sorghum malt produced at 25°C and 30°C show depressed HWE yield and total soluble nitrogen development during mashing in contrast to that produced at 20°C (Agu & Palmer, 1996). Steeping sorghum grains in alkaline liquor generally enhances HWE of malts in cultivar ICSV 400 but reduce HWE in cultivar SK 5912 albeit with an increase in α- and β-amylolytic activities. This suggests possible inhibition of other enzymes contributing to endosperm cell wall structure solubilisation such as exo-and endo-proteases and β-glucanase, and consequent prevention of amylase access to starch granules for efficient conversion (Okolo & Ezeogu, 1996a).

The α-amylase development in sorghum malt is better enhanced during germination at 30°C than at 28°C. Using infusion mashing, hot water extract (HWE) show remarkable difference within germination time over 3–6 d, but not influenced by germination temperature. However, using the decantation mashing method, no appreciable change in HWE occurred over the germination period. Relatively, low HWE obtained from sorghum malt in the infusion mashing process indicate that it is unsuitable for optimal extract production from malted sorghum. Sorghum malt from germination at 28°C releases more FAN products into the worts than the malt from 30°C, using both the infusion and decantation methods (Ijasan, et al., 2011).

Generally, malting increases water extract (WE), water extractable protein (WEP), HWE, and hot water extractable protein (HWEP) of sorghum grains by 3.0-, 3.4-, 2.3- and 2.0-fold respectively (Subramanian et al., 1995). Diastatic activity correlates significantly and positively with WEP and water-extractable contents of malt produced at 30°C. Percentage WEP as a proportion of total protein vary between 11.0 and 36.0% and HWEP range from 19.3 to 44.1% (Subramanian et al., 1995). CWS-protein in grains steeped with aeration at 30° and final warm water steep at 40°C for 6 d is significantly higher than those steeped without air cycle. This may be due to an increase in protein solubilisation in response to improved enzyme synthesis or better hydration of endosperm and enzymes mobility (Ezeogu & Okolo, 1996). The CWS-protein yield varies with sorghum cultivar in both protein solubilisation activity and CWS-protein accumulation. For example, CWS-protein value from cultivar SK5912 (1680 mg % dry malt) is significantly higher than those for ICSV 400 (1030 mg % dry malt) and KSV 8 (1280 mg % dry malt) (Ezeogu & Okolo, 1996).

VIII. Mashing

Mashing in conventional brewing is basically by two methods, viz., decoction and infusion processes (Briggs et al., 1981). During mashing, water soluble substances dissolve, enzymes hydrolyse solubilised starch and proteins and to a lesser extent other higher molecular weight substances essential for the type and character of beer, and finally dissolved substances are separated. Hydrolyses of substances involve enzymes such as amylases, proteases, peptidases, transglucosidases and phosphorylases which are regulated by factors like temperature, pH, time and concentration of the wort. Mashing extracts about 80% of the dry matter from the malt while cold water extracts about 15% (Briggs et al., 1981; Mandl & Wagner, 1978).

Mashing sorghum malt by decoction process and infusion methods are influenced by temperature-time regimes and sorghum variety, and produce worts of varying composition (Owuama & Okafor, 1987). In three-stage decoction, about 70% of mash is boiled to gelatinise starch for greater amylolytic activity while creating plenty of opportunity for proteolytic enzyme action and minimising scope for the development of lactic acid bacteria (Owuama, 1999). Sorghum starch gelatinization temperature (68-72℃) is influenced by kafirin (sorghum prolamin protein) (Taylor and Taylor, 2018). Kafirin resistance to protease digestion (mainly due to intermolecular disulphide bonding), affects the digestibility of starch. (Elkonin, et al., 2013), resulting in partial starch hydrolysis into fermentable sugars (Heredia-Olea, 2017). Thus, starch digestion by amylolytic enzymes increase the quantity of protein in individual kafirin fractions (α, β and γ kafirin) and reduce the amount of high molecular weight proteins. And consequently, kafirin digestion by pepsin results in the formation of polypeptide (Elkonin, et al., 2013). Mashing of sorghum malt at 65°C and 70°C for 30 min each, at second and third stages respectively, of three stage-decoction process, provides wort with complete hydrolysis (Owuama, 1999; Solomon et al., 1994). A longer incubation time at saccharifying temperature

(65°C) than dextrinising temperature (70°C) gives wort with higher reducing sugar levels (Owuama & Okafor, 1987). However, maintaining mash for 60 min at second stage and 70°C for 60 min in third stage produce more fermentable sugars (Owuama & Okafor, 1987). Reducing sugars and proteins in wort increase as concentration of sorghum malt rises from 15 to 25% (Owuama & Okafor, 1987), apparently because of a simple increase in mash concentration and stability of enzymes. Infusion mashing at 65°C releases higher levels of peptides but lower quantities of α-amino nitrogen and total soluble nitrogen than decantation mash in which decanted enzymatically active wort is used to mash gelatinised sorghum starch at 65°C (Mandl & Wagner, 1978; Owuama, 1999).

Mashing sorghum malt by the European Brewing Convention (EBC) congress procedure (EBC, 1987), which involves hydrolysis of pre-cooked malt insoluble solids using an enzymatic malt extract, yield wort with approximate maltose to glucose ratio of 4:1. But mashing malt extract without pre-cooking of malt insoluble solids produce worts containing approximately equal amounts of maltose and glucose (Taylor & Dewar, 1994). Nevertheless, both treatments give the same quantity of total fermentable sugars and wort extract. Infusion mashing of 13.8 dry weight of total cereal content, {composed of 21% sorghum malt (diastatic power ca 38 SDU/g) with cooked adjunct of 70% maize grit and 8% sorghum malt}, at 60°C, pH 4 for 2 h in the presence of about 200 ppm calcium ions results in almost complete conservation of diastatic activity, increase in extract, maximum yield of reducing sugar in wort, and the detection of α-amylase activity which appears to be lacking in the absence of calcium ions (Taylor & Daiber, 1988).

A relatively high level of starch extracts and low level of fermentable extracts have been obtained by using a non-conventional mashing procedure i.e. decanting active enzyme wort after mashing sorghum malt at 45°C for 30 min, and gelatinising starchy grist residue at 80-100°C before mixing with wort, to achieve a saccharifying temperature of 65°C (Palmer, 1989). Palmer (1989), attributed the result to smaller quantities of β-amylase in the wort. Lower wort filtration volume is produced in mashes containing raw sorghum than in all malt mashes. Adding external enzyme during mashing of sorghum malt increases extract yields and free amino nitrogen in wort (Agu et al., 1995; Bamforth et al., 1993). Introducing industrial enzyme preparations containing α-amylase and β-glucanase to mashes with raw sorghum yield higher values of extract recovery in relation to untreated mashes. Addition of amyloglucosidase (AMG) to sorghum during mashing results in an improved wort yield, filtration rate, and a higher percentage ethanol after fermentation (Urias-Lugo and Saldivar 2005, Espinosa-Ramírez, 2014). Moreover, adding enzyme preparations containing a neutral proteinase increases wort total nitrogen and free amino nitrogen while enzyme preparations with β-glucanase or cellulase decrease wort viscosity relative to untreated mashes (Dale et al., 1990). Also a 20% (w/v) sweet potato flour substitution for sorghum malt increases maltose level in wort, apparently because of the presence of β-glucanase (limiting in sorghum) in sweet potato (Etim & Etokakpan, 1992). Mashes composed of 50% malt and 50% raw sorghum and supplemented with enzyme preparations show an increase in wort filtration volume relative to similar mashes without enzyme supplements (Dale et al., 1990). Mashing 50% malt and 50% polished (whole) sorghum by single decoction mashing regime produce wort with filtration behaviour (lautering) comparable to that from control mash (70% malt and 30% maize grits) while wort produced by double mashing regime from 20% malt and 80% raw sorghum supplemented with industrial enzyme show slow filtration and result in sweet and turbid wort. Apparently, this reflects low malt content of grist and lack of suitable material to form mash filter bed (Dale et al., 1990).

a) Wort and Wort Extracts

Worts are usually produced from mashing malts plus adjuncts and contain a variety of fermentable extracts. Worts from two varieties of sorghum malts mashed using commercial brewing enzymes reveal sorghum wort and evaporated wort (extract), containing sufficient sugars and amino acids required for yeast growth and alcohol production during fermentation (Odibo et al., 2002). Mashing different varieties of sorghum malts with exogenous enzyme extracts from sweet sorghum (*Ipomoea batatas*) and yellow yam (*Discorea cayensis*) yield worts containing higher reducing sugars than the untreated malts. However, worts from malts mashed with *Discorea cayensis* show remarkably higher reducing sugars than those mashed with *Ipomoea batatas* (Owuama & Adeyemo, 2009). Worts from barley malt and waxy sorghum grits are comparable to commercial wort and provide adequate substrates for *Saccharomyces cerevisiae* fermentation (Barredo Moguel et al., 2012). Sugar profile of wort from sorghum malt, barley malt, sorghum and barley grains mashed with commercial enzyme show that wort of barley malt and sorghum malt have similar ratios (1:7) of glucose to maltose. However, mashing barley or sorghum grains with commercial enzymes alter the glucose to maltose ratio in both worts, although a greater change is observed in wort from sorghum grains. Nevertheless, hydrolysis with commercial enzymes yield more glucose in sorghum wort, but have more maltose in barley wort. Adding barley malt to sorghum grains mashed with commercial enzymes, re-establish the glucose to maltose ratio in sorghum mash (Okolo et al., 2020).

Worts from grists containing raw sorghum are of higher fermentability and show lower levels of total nitrogen and free amino nitrogen compared to control worts. Worts from mashes containing raw sorghum and malt comprising 20% malt and 80% raw sorghum possess higher levels of total nitrogen and free amino nitrogen than is expected from the reduction of malt content of mash, consistent with the release of nitrogenous components (polypeptides, peptides and amino acids) from sorghum in wort. Wort from 20% malt and 80% raw sorghum has greatly reduced total nitrogen and free amino nitrogen compared to that of all malt wort (Dale et al., 1990). However, levels of both total nitrogen and free amino nitrogen in wort from 20% malt and 80% raw sorghum are not reduced in proportion to malt content of mash, thus suggesting that nitrogenous materials from sorghum are released during mashing into wort. The wort from 20% malt and 80% raw sorghum contains higher proportions of aspartic acid, serine, asparagine, glutamic acid, alanine and histidine but lower proportions of proline, leucine and phenylalanine than control wort (Dale et al., 1990). Worts derived from sorghum malt-1% koji (sorghum grains steeped with 1% *Aspergillus oryzae* and germinated for 4 d) using double mashing procedure generated 27% more fermentable sugars and 24% more FAN. Remarkably, wort from sorghum-1% koji malt contains 8.8% less fermentable sugars compared to the barley malt. However, barley wort has higher maltose concentration than the sorghum worts. The sorghum-2% koji malt does not yield more fermentable sugars than sorghum-1% koji malt. Sorghum malt and sorghum malt-1% koji produced 12°P worts with 40% and 21% less fermentable sugars respectively, compared to the control wort from barley malt (Heredia-Olea et al., 2017).

Worts from upward infusion mashing contain more reducing sugars and proteins than those from downward infusion process. Perhaps, initial high temperature (70°C) of downward infusion method inactivates some saccharifying and proteolytic enzymes (Owuama & Okafor, 1987). Worts from three-step decoction and upward infusion mashing processes contain virtually the same quantities of reducing sugars and proteins although mashing malt of different sorghum varieties with three mashing processes, yield worts with little variation in the types of sugars present (Owuama & Okafor, 1987). Mashes with grists containing high proportions of raw sorghum (50-80% malt replacement) yield high values of extract and produce worts of lower nitrogen, free amino nitrogen, viscosity and colour but higher pH values than in worts from all malt mashes (Dale et al., 1990). Increase in the proportion of raw sorghum in grist relative to malt results in decline in extract recovery, wort total nitrogen, free amino nitrogen but increase in pH. Also, worts from mashes containing raw sorghum have lower viscosity than those from all malt worts (Dale et al., 1990). Mashing of grists containing 50% extruded whole sorghum produces worts of high yield and low viscosity. Increasing the proportion of extruded sorghum in grist causes a decrease in wort filtration volume, total nitrogen and FAN (Dale et al., 1989). The wort filtration behaviour of mashes containing sorghum extruded at 175°C compare favourably with all malt control and is superior to those of mashes containing sorghum extruded at 165°C or 185°C. The results are comparable to those with extruded barley and extruded wheat as brewing adjuncts (Dale et al., 1989).

Generally, mashing sorghum malt, with three-step decoction, upward and downward infusion mashing methods yield worts with similar amino acids. The amino acid, tryptophan which seems to be absent in sorghum grain (Aisien et al.,1983) is present in worts from sorghum malt (Owuama & Okafor, 1987). Except proline, amino acids in wort are assimilated by yeast during fermentation and preferentially provide nitrogen for yeast growth while their metabolic products affect beer flavour and stability (Owuama, 1999). However, yeasts can also utilise some small peptides which only permit slow growth (Bamforth, 2001) thus emphasising the importance of high level of free α-amino nitrogen (FAN) in wort to support rapid and proper fermentation (Owuama, 1999). Mashing at 51°C and pH 4.6 yield approximately 30% free amino nitrogen (FAN) essential for yeast growth during fermentation while the rest 70% is pre-formed in malt and adjunct (Taylor & Boyd, 1986). And, sorghum beer contains low percentage of proline indicating good quality FAN (Taylor & Boyd, 1986). In infusion mashing at 60°C, pH 4.0 for 2 h, very high (VH) or high medium (HM) FAN worts promote almost complete attenuation of sugars in 48 h while low FAN worts require 72-96 h. High FAN worts promote more rapid fermentation of available sugar by yeasts than low FAN worts and a highly significant correlation exist between total brewing time and total soluble nitrogen in wort (Agu et al., 1995; Pickerell, 1986;). The higher the initial FAN concentration, the greater the rate of uptake by yeast (Jones & Pierce, 1969). Further, wort sugar level which influences overall demand for FAN seems not to affect FAN uptake rate (Pickerell, 1986). FAN in wort is higher after 120 h than after 24 h, particularly in high FAN wort. This may be attributable to lysis of aging or dead yeast cells and nitrogenous substances excreted by yeast cells during fermentation (Pickerell, 1986). Higher initial FAN level encourages greater rate of ethanol production, thus, in very high FAN wort, ethanol production is slightly faster than in medium high FAN wort, indicating possible FAN optimum for sorghum beer fermentation. Furthermore, in very low FAN wort, fermentation is protracted and sugar utilisation by yeast is poor and invariably alcohol yield is low. However, sugar uptake depends on its level in wort i.e. high wort sugar is taken up faster than low wort sugar (Pickerell, 1986).

Proteolytic activity during infusion mashing at 60°C and pH 4.0 for 2 h produces about 30% of wort FAN while 70% is pre-formed in malt and adjunct. FAN in sorghum beer wort is good as it contains a low percentage (ca 10%) of proline (Taylor & Boyd, 1986). Optimum mashing conditions for FAN production are 51°C and pH 4.6. Raising the ratio of sorghum malt to adjunct leads to a proportional increase in wort FAN while raising ratio of adjunct to malt results in a decrease in wort FAN. However, wort FAN is directly proportional to malt FAN and the addition of microbial proteolytic enzyme to mash increases wort FAN (Taylor & Boyd, 1986).

IX. Fermentation and Beer Characteristics

Yeast is usually pitched into wort, which consists mainly of fermentable sugars, including glucose, fructose, sucrose, maltose and maltotriose, as well as dextrins, nitrogenous materials, vitamins, ions, mineral salts, and trace elements (Bamforth, 2001). During fermentation, brewing yeasts adapt quickly to the wort environment, utilizing available nitrogen for the synthesis of cellular proteins and other cell components (Hill & Stewart, 2019). Wort encourages the growth of new yeast cells which ferment the medium to produce ethanol, carbon dioxide and other metabolic products, many of which contribute to the flavour of the beer (Ferreira & Guido, 2018). Beer brewed from the normal wort of sorghum is lighter in colour than that brewed from the re-dissolved sorghum extract (evaporated wort). The lower alcohol values or higher colour of beer brewed with sorghum extract was linked to the Maillard reaction, which occurs during the process of evaporating the wort to produce the extract. However, organoleptic assessment showed that beer brewed using the extract was generally acceptable. (Odibo et al., 2002).

Fermentations of lager worts from waxy sorghum grits inoculated with either yeast cultured in wort or yeast grown in yeast-malt media produce levels of alpha amino nitrogen (AAN) and fusel alcohols comparable to that of commercial wort. The oxygen concentration decrease from 20% at the start of fermentation to below 1% after 72 h fermentation reflecting a gradual change from aerobic to anaerobic condition. The utilization of AAN from waxy sorghum grits wort for production of amyl-isoamyl alcohol, propanol and isobutanol is comparable to the control barley wort, over 144 h of fermentation. The isobutanol produced has the least concentration. Propanol production started after 24 h fermentation of worts inoculated with yeast cultured in wort, and after 36 h with yeast cultured in yeast-malt media. The concentration of ethanol and fusel alcohols in sorghum beer falls within the commercial beer range (Barredo Moguel et al., 2012).

Worts from grist containing extruded sorghum ferment more quickly than all malt wort and attain lower final gravity values (Dale et al., 1989). Worts and beers produced under isothermal infusion mashing conditions from grists comprising 70% malt plus 30% extruded sorghum and 100% malt filter without difficulty. Beers from grists containing extruded sorghum contain lower levels of total nitrogen and FAN compared to all malt beer, an observation which is consistent with extruded sorghum contributing little or no nitrogenous material to wort and beer (Dale et al., 1989). Beers from grists containing extruded sorghum are of sound flavour and show reasonable foam stability behaviour (Dale et al., 1989). Fermentation of normal brewing sorghum wort produced slightly higher levels of alcohol than evaporated sorghum wort (extract) (Odibo et al., 2002). However, the non-fermentable residual dextrins are solubilized during brewing and remain in beer and contribute to mouthfeel (Langstaff and Lewis, 1993).

Beers produced from 50% malt and 50% polished sorghum, and 20% malt and 80% raw sorghum filter without difficulty and have sound flavour (Dale et al., 1990). Beers produced from 50% malt and 50% polished sorghum contain lower levels of isobutanol, 2-methylbutanol, dimethylsulphide and higher level of n propanol and diacetyl in relation to control beers. The post-fermentation gravity, colour and pH are comparable to control beers (Dale et al., 1990). Carbohydrate composition of beer brewed from 20% malt and 80% raw sorghum compare favourably with those from all malt beer as well as that from commercial beer brewed from 60% malt and 40% sorghum grits. However, form stability behaviour of beer brewed from 20% malt and 80% raw sorghum is poor relative to that from all malt beer (Dale et al., 1990).

The polypeptide content of beer influences foam stability behaviour and susceptibility to non-biological haze development (Dale & Young, 1987). The low total nitrogen content of beer from 20% malt and 80% raw sorghum is responsible for high resistance to non-biological haze formation but low head retention. Beer susceptibility to microbial spoilage may be influenced by level of free amino nitrogen present (Owuama, 1999). Thus, low levels of total nitrogen and free amino nitrogen in beer from 20% malt and 80% raw sorghum may confer good storage properties against non-biological and microbial spoilage (Dale et al., 1990).

Supplementation of sorghum mash comprising sorghum malt plus adjunct (regular or waxy sorghum) with β-amylase or amyloglucosidase and using a double-mashing procedure yield sorghum malt worts with increased amount of fermentable sugars. Addition of amyloglucosidase during mashing increases total sugar content by 20% and glucose content by five-fold

vis-à-vis worts without exogenous enzymes. Worts from barley malt and sorghum malt contain adequate quantity of free amino nitrogen. Fermentation of worts by typical lager brewing conditions yield barley malt beer containing approximately 1% more ethanol relative to the sorghum malt beers that are not supplemented with exogenous amylolytic enzymes. Fermentation of worts from AMG supplemented mash produce beers with ethanol increase by 1.1% units, and comparable contents regardless of the type of malt. Fusel alcohol concentrations do not differ with mash treatments. Addition of amyloglucosidase to mash is known to give higher yields of alcohol in 100% gluten-free sorghum beers (Espinosa-Ramirez et al., 2013). Addition of β-amylase or amyloglucosidase (AMG) (Urias-Lugo and Saldivar, 2005), during mashing of sorghum malt, results in improved wort yield and filtration rate, as well as a higher percentage of ethanol production in beer. However, alcohol content of sorghum beer is approximately 1.1% less than barley malt beer. Introduction of AMG during mashing has no effect on colour, pH and FAN content of wort (Cela et al., 2020)

European beers brewed with sorghum generally yields beer with lower alcohol contents than barley beers. Lager beers produced using worts adjusted to 15° Plato from sorghum malt and inoculated with 1% *Aspergillus oryzae* yield 21.5% more volume than sorghum malt wort and 5% more than wort from barley malt. The major fermentable sugar in all worts is maltose. Higher amounts of glucose are present in both sorghum worts vis-à-vis barley malt worts (Rubio-Hores et al., 2020). Beer from sorghum malt–*A. oryzae* wort has similar specific gravity and alcohol content compared to the barley malt beer. Sorghum malt–*A. oryzae* beer contained lesser amounts of hydrogen sulphide, methanethiol, butanedione, and pentanedione relative to barley malt beer. Sorghum malt–*A. oryzae* lager beer shows similar yield for wort extract and alcohol content compared to the barley malt beer but varies in key volatiles, colour and aromatic compounds (Rubio-Flores et al., 2020).

Gluten is a protein found in most grains commonly used in brewing beer including barley, wheat, rye and oats. Barley malt contains traces of hordein (gluten), thus, barley beer contains gluten too high to be safely consumed by those suffering from coeliac disease (Tanner et al., 2013). Therefore, grains which lack gluten such as, corn, rice, sorghum, buckwheat, millet and quinoa, are suitable for brewing gluten–free beer. Presently, sorghum malt which lacks gluten has proven to be an excellent substrate and is currently used to produce gluten-free beers acceptable to sufferers of celiac disease (allergy/intolerance to gluten) (Hager et al., 2014).

x. Sorghum as Adjunct

Sorghum was recognised as an important adjunct in brewing during World War II (Owuama, 1997). Brewing adjuncts are essentially starchy materials with little or no protein content. They are a potential source of additional alcohol and may add to the colour, taste, aroma, vitamin, protein content and head retention of beer (Briggs et al., 1981; Dhamija & Singh, 1978). Other unmalted materials such as bajra, tapioca (*Manihot esculentum*), soy beans, wheat, maize and barley flours have also been added to grists as adjuncts (Agu, 2002; Dale et al., 1989; Dhamija & Singh, 1978).

Sorghum grain composition, properties, morphology and anatomy have been reviewed (Ogbonna, 1992; Owuama, 1999). In grain sorghum, there are both soluble and insoluble amylase fractions (Owuama & Okafor, 1990). The insoluble amylases which adhere tenaciously to insoluble substances still remain active in certain varieties of sorghum and are solubilised by breaking the link through a prolonged grain protease action during aqueous extraction. However, the activity of grain amylases varies with sorghum variety and are apparently involved in hydrolysis during mashing. Optimal temperatures for β-amylase (60-65°C) and α-amylase (72-75°C) in grain sorghum differ slightly from one variety to another while optimal pH of the enzymes fall between 5 and 6 (Owuama, 1999; Owuama & Okafor, 1990).

Contradictory reports on the necessity to gelatinise starch adjuncts for amylase to act (Agu & Palmer, 2013; Elkonin et al., 2013; Ezeogu & Okolo, 1996) has been attributed to differences in fineness of grinding, thickness of mash or quantity of enzymes (Briggs et al., 1981). However, mashing gelatinised sorghum grits, at different proportions with barley malts produce worts of varying contents (Owuama, 1999) while adding an industrial enzyme, "thermamyl", used by Nigerian breweries for mashing unmalted sorghum, increases yield of extract in wort when combined with malt (Agu et al., 1995). The introduction of external enzyme to 100% gelatinised sorghum malt during mashing produces lager beer comparable to commercial brands obtained from barley malt (Olatunji et al, 1993). Nevertheless, a 40-70% substitution level of sorghum for barley malt is considered adequate for brewing lager beer with virtually the same organoleptic properties as beer produced with only barley malt (Dhamija & Singh, 1978; Ogbonna & Obi, 1992; Owuama, 1999). Utilization of sorghum adjunct, at 5 to 20% level, showed a progressive decrease in extract recovery, solubilisation of nitrogen, and production of free amino nitrogen and peptide nitrogen in the wort. Sorghum adjunct has been shown to release higher levels of FAN and peptide nitrogen in extracts than barley adjuncts, a difference that may influence fermentation potential of the wort (Agu, 2002).

Brewing grits from four different decorticated sorghum genotypes, brown normal (BNO), white normal (WNO), white waxy (WWX) and white hetero-waxy (WHWX) show that decorticated kernels have lower protein, crude fibre, ash, and colour values and higher starch contents than their respective whole kernels. The extract yield of brewing adjuncts from decorticated BNO, WNO, WWX and WHWX were 81%, 87.4, 89.9, and 90.0 respectively. Worts from WWX brewing adjuncts filter faster than the hetero-waxy, white normal and brown normal. Worts from all the sorghum genotypes standardized to 14°P, show similar viscosity, α-amino nitrogen, pH and colour values. White sorghums with hard and waxy endosperms are most suited for use as brewing adjuncts (Osorio-Morales, et al., 2000). Sieving analysis of some sorghum grains as well as their hot water extractable (HWE), hot water extractable protein (HWEP) and free amino nitrogen (FAN) show that cultivars with high starch and amylose contents plus low protein and fat percent will make better adjuncts based on their HWE and HWEP yields. However, the suitability of sorghum variety as brewing adjunct for lager beers is apparently not determined by the grain size (Ratnavathi et al., 2000).

Fermentation of wort from all barley malt (ABM) mash and commercial enzyme/barley malt/sorghum adjunct (CEBMSA) mash of similar wort gravity reveals similar glucose to maltose ratios and similar amino acid spectra. ABM yields 27% more glucose and 7% more maltose than CEBMSA. After yeast fermentation, ABM mash produce 9.45% alcohol by volume (ABV) while the commercial enzyme/barley malt/sorghum adjunct mash produced 9.06% ABV (Okolo et al., 2020).

XI. Conclusion

Variations in physical and biochemical characteristics of sorghum cultivars, steeping solution without or with amendments such as ions and koji, *Aspergillus oryzae*, as well as temperature and period of germination influence optimal malting conditions and eventually malt quality. Consideration of a reasonable number of malting variables are necessary for selecting proper sorghum malt for brewing beer. Equally essential are optimising conditions for mashing and fermentation of worts to achieve the expected goal of producing sorghum beer comparable to barley beer. The wort filtration problem encountered from brewing with sorghum may be resolved by using the filter press instead of lauter tun and artificial husks from nylon materials of plant tissue (Owuama, 1999).

However, the distinct differences that exist between the structure and physiology of the aleurone, embryo and starchy endosperm cells of sorghum and barley grains (Aisen & Palmer, 1983; Palmer, 1989; Palmer et al., 1989), questions the expectation of producing similar character of lager beer from the two different grains. Also, disparities in their malt characteristics, such as β-glucan and pentosan levels, as well as amino acid profiles of malt worts add to the unlikelihood of obtaining beers of exactly the same physical and organoleptic properties from barley and sorghum malts (Owuama, 1999). Thus, it is expected that sorghum beer of a slightly different character eg. in colour, flavour and taste will be produced. Producing beer with 100% sorghum immensely benefits coeliac disease sufferers who are allergic to gluten, which is present in barley beer (Tanner et al., 2013). Currently, wholly sorghum beer is commercially available and does have great appeal to coeliac disease patients. Hopefully, sorghum beer will attract a wider range of consumers in the near future, particularly among the younger generation.

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I designed, searched literature and prepared the manuscript for submission.

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