Invited article

125th Anniversary Review: The science of the tropical cereals sorghum, maize and rice in relation to lager beer brewing

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Mainstream lager beer brewing using the tropical cereals sorghum, maize and rice, either as malt or as raw grain plus commercial enzymes, is becoming widespread. This review examines the differences in composition between these tropical cereals and barley and their impact on brewing processes and beer quality. All of these cereals have a starch gelatinization temperature some 10 °C higher than barley. The sorghum prolamin proteins are particularly resistant to proteolysis owing to disulphide cross-linking involving γ-kafirin. Unlike barley, the major endosperm cell wall components in sorghum and maize are arabinoxylans, which persist during malting. The rice cell walls also seem to contain pectic substances. Notably, certain sorghum varieties, the tannin-type sorghums, contain considerable levels of condensed tannins (proanthocyanidins), which can substantially inhibit amylases, and probably also other brewing enzymes. Tropical cereal malts exhibit a similar complement of enzymic activities to barley malt, with the notable exception of β-amylase, which is much lower and essentially is absent in their raw grain. Concerning beer flavour, it is probable that condensed tannins, where present in sorghum, could contribute to bitterness and astringency. The compound 2-acetyl-1-pyrroline, responsible for the popcorn aroma of maize and also the major aroma compound in rice, presumably affects beer flavour. However, much more research is needed into tropical cereals and beer flavour. Other future directions should include improving hydrolysis of prolamins into free amino nitrogen, possibly using prolyl carboxypeptidases and investigating tropical cereal lines with useful novel traits such as high amylopectin, high protein digestibility and low phytate. Copyright © 2013 The Institute of Brewing & Distilling

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Introduction

Since the beginning of the twentieth century there has been increasing development of lager beer brewing using high proportions of cereals other than barley malt. Here, the term 'lager beer' is used to denote common traditionally barley malt-based beers. This process of barley malt replacement in brewing is accelerating. Today, there are several totally non-barley lager beers being brewed across the world, such as Eagle in Africa (1) and Redbridge (2) and Bard's Tale (3) in the USA.

Several drivers of barley malt replacement can be identified, in particular grain cultivation. Barley is a cool-season, temperate cereal (4), and in tropical and sub-tropical regions its cultivation is generally far less viable compared with the major tropical cereals of maize (5), rice (6) and sorghum (7). An early example of this driver was the demonstration of locally developed sorghum malt beverages in 1917 at the Madras Exhibition in India (8). Related to this are government policies of import replacement and support for local farmers. In Nigeria, a temporary ban on

barley and barley malt imports from the mid 1980s to 1999 has resulted in the continuing general use of sorghum and maize in lager beer brewing (9). Favourable taxation has also played a role. In Japan, in the early 2000s low barley malt lager beers called Happoshu, which were less taxed than beers produced with a high proportion of malt, captured 40% of the beer market (10). Similarly, lower taxation to favour locally grown cereals has helped the development of sorghum lager brewing in East African countries such as Uganda (1). Another driver is the enormous growth of grain bioethanol production based predominantly on maize, which is resulting in major developments in commercial enzyme technology (11). Lastly, the most recent driver is the gluten-free trend (12). While still a very small market in the USA, where in 2009 gluten-free beers accounted for less than 0.1% of beer sales, growth rates of up to 35% have been reported (13).

Although brewing a 'beer' with cereals other than barley malt is straightforward, achieving economic process efficiency and producing a consumer-acceptable product is an ongoing research challenge. This review examines four interrelated areas of non-barley malt brewing science: impacts of differences in chemical composition of the major constituents of tropical

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cereal grains; the enzyme activities of the tropical cereals; potential deficiencies in yeast nutrition; and beer sensory characteristics, in respect of refined raw grain (essentially starchy endosperm) and whole raw grain as well as malted cereal brewing. Throughout the review comparisons will be made between these cereals and barley. The emphasis in the review is on the science, since large-scale commercial brewing practice, especially with sorghum, has often rapidly overtaken or even pre-dated technology research developments reported in the public domain (14,15). The review concerns only sorghum, maize and rice, since these are the cereals used in mainstream low- or non-barley malt beers and they are all 'gluten-free' cereals (16).

Major grain chemical components of sorghum, maize and rice

In barley malt brewing, the process of solubilization (enzymatic hydrolysis and physical solubilization) of the chemical components of the grain – starch, non-starch polysaccharides (NSPs), proteins, lipids, minerals, vitamins and phytochemicals – is facilitated by enzymatic modification of the grain structure during malting. Notably, the endosperm cell walls are degraded and there is limited hydrolysis of the endosperm protein matrix and starch granules (17). The tropical cereal grains are similar in general proximate chemical composition to barley with only a few clear differences. However, there are important detailed differences in the composition, structure and properties of some of the chemical components between them and barley, and between themselves, which can restrict solubilization even if these grains are malted.

Starch

The starch gelatinization temperature range of the tropical cereal starches is some 10–20 °C higher than that of barley starch, 62–78 and 51–60 °C, respectively (18). The temperature optimum of, for example, sorghum malt α-amylase is around 70 °C and that of sorghum malt β-amylase is around 50 °C (19,20), with complete inactivation of β-amylase taking place at 68 °C within 15 min (21). Because of this, simultaneous starch gelatinization and hydrolysis as takes place in barley malt mashing (22) is not effective. Thus, in practice the tropical cereals, whether in the form of raw grain or malt, must be cooked first to gelatinize their starch, cooled, then saccharified using barley malt or commercial enzymes (22,23). Thus, in commercial lager brewing using sorghum malt, the malt is primarily an adjunct rather than the source of hydrolytic enzymes (23). This is despite much research on rising temperature and decoction-type mashing regimes to obviate the problem of sorghum starch's high gelatinization temperature (24–28).

There is some evidence that the gelatinization temperature of sorghum and rice starches may be generally slightly higher than that of maize starch (29). In the case of sorghum, this is possibly because the amylose degree of polymerization (DP) and average number of side chains appear to be higher than those of maize starch (18,30). However, the proven, considerably higher gelatinization temperature of tropical starches seems to be related to the longer side chains of their amylopectin compared with those of the temperate cereals. For example, it has been found that approximate weight-average chain lengths of sorghum, wheat and barley amylopectin are 30.1, 26.8 and 27.9, respectively (31). Amylopectin chain length is related to starch synthase II

activity (32) and rice starch gelatinization temperature has been shown to be controlled by the starch synthase IIa (SSIIa) gene (33). It was found that SSIIa protein content and starch gelatinization temperature were positively correlated with amylopectin chain lengths of high DP and negatively correlated with chain lengths of low DP. Rice is unusual in that its starch is stored in compound starch granules consisting of at least 16 small granules of 3–5 μm diameter (32). However, the compound granule structure does not appear to affect end-use quality (32). For brewing, long-grain rice is generally avoided because of gelatinization and viscosity problems in mashing (34). Stickiness of long-grain rice is apparently correlated with the proportion of amylopectin A and short B chains (35).

There also exist waxy cereals, which are essentially 100% amylopectin (0% amylose) maize (36), rice (37) and sorghum (38) types. The high proportion of amylopectin exerts considerable effects on starch physical properties. Waxy barley and maize starches have been found to exhibit much greater granule swelling than their normal counterparts, which have 27.5 and 29.4% amylose, respectively, despite the waxy starches having 1–2 °C higher gelatinization temperature (39). Similarly, waxy sorghum starch exhibited a much higher and considerably earlier pasting peak viscosity than normal sorghum starch, despite its gelatinization temperature being 2 °C higher (40).

Presumably because of the better granule swelling properties of amylopectin starch, there has been substantial interest in using waxy sorghum and maize in lager beer brewing (23,41,42) and recently for bioethanol production (43,44). Significantly, it has been found that waxy maize gives a 93% starch to ethanol conversion, some 5% higher than normal maize, in a 'cold fermentation' process, that is, without starch cooking (44). This seemed to be a consequence of the waxy maize having shorter average amylopectin chain length than normal maize.

Proteins

Quantitatively, the major proteins of barley, maize and sorghum are the prolamin storage proteins, which are endosperm-specific (29). Uniquely, in rice, glutelin-type storage proteins with an 11S globulin type amino acid sequence (45) are the major proteins. Whole grain rice also has a much lower protein content (~7%) (46) than the other cereals of barley (8–15%) (47), maize (~10%) (36) and sorghum (~11%) (48). The maize zein and sorghum kafirin prolamins are very similar in composition, amino acid sequence and conformation, consisting predominantly of small, 19–25 kDa α-prolamins (49) and hence are less diverse than the barley hordein proteins, which more closely resemble the wheat gliadins and glutenins (50). Kafirin and zein are also notably more hydrophobic, or more strictly speaking less hydrophilic, than the storage proteins of the triticeae cereals such as wheat and barley (51).

Importantly with respect to the provision of free amino nitrogen (FAN), the wet cooked protein digestibility of sorghum is substantially lower (~30% lower) than that of the other cereals (52). Having said this, there is also some evidence that the endosperm storage proteins of cooked rice are very resistant to hydrolysis (53). Concerning the low protein digestibility of cooked sorghum, this is as a result of extensive polymerization of the kafirins (and possibly other endosperm proteins) through disulphide bonding involving the cysteine-rich γ-kafirin sub-class (51,52,54,55). The cross-linking of the kafirin containing endosperm matrix protein may limit starch gelatinization (more strictly speaking starch granule expansion)

during cooking (56,57) and in turn hydrolysis of the starch to fermentable sugars (58). The disulphide-bonded crosslinking involving γ-kafirin seems to exacerbate the problem of the high content of proline in cereal prolamin proteins, for example γ-kafirin with 23 mol% proline (59). The high content of proline makes the prolamin oligopeptides, released by endopeptidase activity, resistant to degradation to free amino acids by conventional carboxypeptidases (60).

Concerning protein types involved in beer foam, barley lipid transfer protein 1 (LTP 1) appears to play a key role in beer foam stabilization (61). Tropical cereal grains also contain LTPs (62), and like in barley LTP (62,63), they seem to be primarily expressed in the aleurone and germ (64). LTP 1's foaming properties are dependent on changes in the protein brought about during malting and brewing, in particular glycation by Maillard reactions during malting (61). Thus, grists comprising high proportions of raw tropical cereal refined starchy endosperm adjunct are undoubtedly deficient in foam-active LTP.

Non-starch polysaccharides

A fundamental difference in structure and chemical composition of rice compared with sorghum and maize is that paddy (rough) rice has a fibrous husk (hull) like barley, which is not threshed off the true grain. The rice hull comprises some 31–36% cellulose, 18% pentosans, 10–18% lignin, 3–12% hemicelluloses and 13–21% ash (mainly silicon) (65). Rice hulls could presumably function as a filter bed in lautering.

The cell walls of the starchy endosperm, like the starchy endosperm matrix protein, may limit starch granule expansion during cooking (66) and subsequent starch hydrolysis. Also, importantly the endosperm cell walls can retard or limit wort separation owing to their hydration (67). As is well known, this can be a major problem in barley brewing where the water-extractable (1,3) (1,4) β-glucans, are by far the major cell wall component (68). In contrast, in maize (69) and sorghum (70–72) the predominant components of starchy endosperm cell walls seem to be pentosans, specifically glucuronoarabinoxylans (GAX), which are water inextractable. In maize, the ratio of heteroxylans to β-glucans is at least 8:1 (73), whereas in barley, arabinoxylans account for only some 20% of cell wall NSP (69). In sorghum grain, the level of β-glucan is very low, ~0.2% of grain weight (74). The sorghum (75) and maize (69) GAX are also more complex and highly substituted than the arabinoxylans of barley. Further, their GAX are highly cross-linked. It now appears that this takes place by oxidation of feruloyl esters to form oligoferuloyl esters and ether-like bonds (76) rather than diferulates as has been widely proposed (77,78). Presumably, on account of their inert nature, the maize and sorghum endosperm cell walls do not seem to constitute a problem with regard to wort separation.

Rice endosperm cell walls seem to be different from either barley or sorghum and maize, although arabinoxylans and β-glucans account for the major proportion (~47–49%) of endosperm cell wall NSP (73,79), there are also substantial proportions of cellulose (~23–28%) and pectic substances containing polygalacturonides (73,80) (~27%) and variable amounts of glucomannans (73). It does not, however, seem that these pectic substances have an adverse effect on wort filtration when rice is used as an adjunct.

A fundamental difference between sorghum and barley malt is that the starchy endosperm cell walls of sorghum are not degraded during malting to any appreciable extent (77,81) and remain visibly essentially intact even when the cell contents have been completely degraded (82). The persistence of the sorghum endosperm cell is probably due to the high levels of cross-linked GAX. Additionally, it has been suggested that the presence of fuco-xyloglucan may contribute to the cells walls being resistant to cellulolytic type enzymes (83). In view of the similarity in sorghum and maize endosperm cell wall composition (69), it is probable that the maize cell walls also persist during malting, but firm evidence seems to be lacking. In rice, it has been reported that malting brings about dynamic changes in the feruloylarabinoxylans, greatly reducing the molecular size and increasing the ferulic acid content of the soluble arabinoxylans 10-fold (84).

Notwithstanding the persistence of the endosperm cell walls in sorghum during germination, as sorghum malt is invariably treated as an adjunct and cooked prior to mashing, they do not seem to constitute a barrier to filtration in this type of brewing. However, there seems to be clear evidence that wort filtration is much retarded if sorghum malt is used conventionally as both a source of enzymes and as starch (85,86). It has been suggested that slow filtration of sorghum malt worts is as a result of low endo-β-(1–3) (1–4) glucanase activity, a suggested cause of persistence of sorghum endosperm cell walls (15,87). However, this seems unlikely in view of the relatively low β-glucan content, even if there is good evidence that the sorghum β-glucans do persist during malting (74). To resolve the cause of slow filtration of sorghum malt worts, the relative contributions of the persistent endosperm cell walls and incomplete saccharification need to be quantified.

Lipids

In brewing, lipid content is important with regard to foam and beer oxidative stability, both of which are adversely affected by high levels of lipids (22). However, caution has to be exercised when looking at data on cereal grain gross proximate composition. Nevertheless, whole grain maize has a considerably higher fat content (~4.4%) (36), compared with barley (2.3–3.7%) (88), barley malt (~1.8%) (48), rice (1.6–2.8%) (48) and sorghum (~3.2%) (89). The lipid content of the endosperm tissue is much lower. Maize endosperm grits and rice endosperm grits or broken polished rice are used as adjunct, with typical lipid specifications of 0.7% (90) and 0.2% (34), respectively. In contrast, sorghum can and is used as a whole grain adjunct (1), presumably because of its lower lipid content than maize and the fact that it is only milled directly before brewing and thus lipid oxidation is minimized.

Phenolics and tannins

All cereal grains contain phenolic acids, which are concentrated in endosperm and bran cell walls, and most cereals contain some flavonoid-type polyphenols, which are concentrated in the pericarp (91). However, sorghum is unique among the major cereals in that certain sorghum varieties contain significant levels of condensed tannin type polyphenols (≥1%) and that most types contain significant levels of flavonoids (92). However, despite valiant efforts by sorghum scientists, such as Professor Lloyd W. Rooney, to communicate the science and practical implications of polyphenols and tannins in sorghum (93), there remains a serious misconception that

the terms polyphenols and tannins are synonymous (15,94). Unfortunately, this compounds the problem that our knowledge of tannins and non-tannin polyphenols in sorghum is still far too incomplete.

Basically, three different types of phenolic compounds may be present in sorghum grain: phenolic acids, flavonoids and condensed tannins (95). All sorghum types contain phenolic acids such as hydroxycinnamic acids. For example ferulic acid is present in all cereal grains and coumaric acid is also present in barley, maize and rice (96), and the hydroxybenzoic acids such as vanillic and syringic acid are present in all four cereals (91). Sorghums visually appearing as red, brown and black, and even some types of white-appearing sorghums, additionally contain many different anthocyanin flavonoid-type polyphenols (95,96). The aglycones of some of these anthocyanins are pigmented, such as apigenidin and luteolinidin, and are responsible for the grain colour. Anthocyanins and anthocyanidins are also present in substantial quantities in pigmented barley, maize and rice varieties (97). However, sorghum is unique among cereal grains in that one of the anthocyanins that it contains is of the 3-deoxyanthocyanin type (98), which seems to have anticancer activity (99). Only the white-tan plant type of sorghum (referred to in the USA as food-grade sorghum) (96) contains minimal levels of polyphenols. This type of sorghum is used for sorghum lager brewing in East and Southern Africa (1).

Certain sorghum varieties additionally contain condensed tannins, more properly identified as proanthocyanidins or procyanidins, which are polymers of flavonoids (92). Tannin-containing sorghum varieties are properly classed as tannin sorghums (100) and are of two types – type II and type III – the latter containing a dominant B_1–B_2 spreader gene (38). We know that the type II tannin sorghums generally contain less tannins than the type III (92). However, comprehensive data on their tannin contents is lacking. The type II tannin sorghums are invariably white in appearance (38) and white type II tannin sorghum is widely used in sorghum malting and lager brewing in Nigeria (101,102). Type III tannin sorghums are invariably red or brown in colour (38). Thus, the situation is that there are many sorghum varieties that are white in colour, but contain tannins, and there are very many that are red and brown, or even black, that do not.

The major property of tannins of significance in brewing is that they bind irreversibly to proteins, probably through hydrogen bonding (103,104) and hydrophobic interactions (104,105). Tannins have an affinity for proline residues (103,105) and because of this sorghum-condensed tannins also complex with the kafirin prolamins (104,106) and other prolamins (104) and soluble proteins. The latter are rendered insoluble (107). It has been known for several decades that the tannins in sorghum malt can substantially inhibit malt amylase activity (108) and reduce sugar production during mashing (109). They also inhibit sorghum protein hydrolysis (107) and presumably other enzyme activities of importance in brewing. However, an important gap in our knowledge is what level of tannins in sorghum significantly inhibits malt and commercial enzyme activities in brewing.

Technologies such as steeping the sorghum grain in very dilute formalin or sodium hydroxide solution have been developed and are routinely applied in sorghum malting practice, whereby these chemicals react with the tannins and prevent amylase inhibition (23). In the formalin treatment, formaldehyde probably polymerizes the tannins to form phenol–formaldehyde resin (110). The mechanism of inactivation by sodium hydroxide is not known, but it has been suggested that it involves oxidative polymerization (108). These treatments undoubtedly also prevent inhibition of other enzymes in brewing, but concrete evidence is lacking.

Enzymes of sorghum, maize and rice malt and whole raw grain

This section will primarily address the question as to whether malted maize, rice and sorghum express the full spectrum of enzyme types and levels of activities to completely replace the enzymes of barley malt. With regard to brewing with raw grain, it will address the question whether raw whole maize, rice and sorghum contain useful levels of any enzymes for brewing.

Concerning α-amylase activity, using a somewhat non-specific assay (111) it was found that some 80% of a sample of 30 sorghum cultivars had similar or slightly higher malt α-amylase activity than a sample of 47 barley cultivars, although 20% were rather lower. These findings, together with other research that found lower α-amylase activity in sorghum malt compared with barley malt (85,112), are consistent with observations that there are fewer α-amylase isozyme forms in sorghum than barley (113,114). Maize malt also seems to have more α-amylase isozymes than sorghum (114). Using a specific dye-labelled dextrin assay for α-amylase (115), it was found that that α-amylase activity in maize malt was several times higher than that of sorghum malt, but lower than rice malt (116) (Table 1). However, the sorghum used had a germination percentage of only 32%, and despite a correction factor being applied, the data may not be accurate. The same study showed that rice malt had higher α-amylase activity than maize malt. In support of the caution, another study, using a relatively specific assay, showed rice malts to have only slightly higher α-amylase activity than sorghum malt (117). Further, using a specific assay of α-amylase (115), researchers have found medium to high activity in rice malt relative to barley malt (118,119). It can thus be concluded that maize, rice and sorghum malts all have adequate α-amylase activities. However, the raw grains of sorghum, maize and rice do not exhibit any appreciable α-amylase activity (118).

The situation is different with regard to β-amylase. There is clear evidence from both relatively non-specific and specific assays that the β-amylase activity of sorghum malt is very much lower than that of barley malt (111,21) and limiting in brewing (124). Maize malt also seems to have relatively low β-amylase activity (116). The low β-amylase activities of sorghum and maize malts can be attributed to the fact that, as tropical cereals, unlike the triticeae cereals such as barley and wheat, they only exhibit the tissue 'ubiquitous' form of the enzyme (125). Concerning rice malt, several workers all using a specific assay for β-amylase (126) have found that it has relatively good β-amylase activity (116,118,119), despite the fact that rice appears only to exhibit the ubiquitous form of the enzyme (125). It is probable that β-amylase is essentially absent in the raw grain of sorghum (21,127) and the other tropical cereals (119,125), notwithstanding the fact that significant levels were reported in black waxy rice raw grain (119).

Table 1. Comparative studies on the activity of enzymes important in lager brewing in barley, sorghum, maize and rice malts

Enzyme	Cereal	Malt enzyme activity
α-Amylase	Barley	206 IU/mg protein (112), 44 DU[a] (117), 365 IU/g (85)
	Sorghum	142–148 IU/mg protein (112), 20 IU/g (116), 31 DU (117),95 IU/g (85)
	Maize	98–106 IU/mg protein (112), 49 IU/g malt (116)
	Rice	120 IU/g (116), 28–42 DU[a] (117)
β-Amylase	Barley	234 IU/mg protein (112), 1017 IU/g (85)
	Sorghum	156–158 IU/mg protein (112), 23–80 IU/g (116), 48 IU/g (85)
	Maize	123–125 IU/mg protein (112), ~15–55 IU/g (116)
	Rice	23–175 IU/g (116)
α-Glucosidase	Barley	1.8 IU[a] (120)
	Sorghum	1.6 IU[a] (120), 0.06–0.30 IU/g (116)
	Maize	0.07–0.11 IU/g (116)
	Rice	0.22–0.30 IU/g (116)
Limit dextrinase	Barley	0.2–0.4 EU/g (121)
	Sorghum	1.0–1.4 EU/g (116)
	Maize	0.3–0.5 EU/g (116)
	Rice	3.3–6.0 EU/g (116), 2.2–3.2 EU/g (121)
Endo-β-(1,3) (1,4)-glucanase	Barley	100–135 U/g (87)
	Sorghum	15–20 EU/g (87), 0.4–2.4 U/g (116)
	Maize	0.0–0.1 U/g (116)
	Rice	0.0–0.1 U/g (116)
Pentosanase/xylanase	Barley	220–550 U/g (87)
	Sorghum	220–550 U/g (87)
	Maize	ND
	Rice	ND
Phytase	Barley	1.1 PU/g (122)
	Sorghum	0.4 PU/g (122), 0.4 PU/g (123)
	Maize	0.8 PU/g (122), 1.0 PU/g (123)
	Rice	0.1 PU/g (122), 3.0 PU/g (123)
Estimated proteolytic activity	Barley	36% KI (117)
	Sorghum	36% KI (117)
	Maize	ND
	Rice	37% KI (117)

[a]Reference does not give the amount of flour that contains stated activity
ND, No relevant data could be found. U, Unit definition could not be found. IU (international unit), Amount of enzyme which releases 1 µmol of *p*-nitrophenol from the substrate per minute at the defined pH and temperature. DU (dextrinizing unit), Quantity of α-amylase that will dextrinize soluble starch in the presence of an excess of β-amylase at the rate of 1 g/h at 30 °C. EU (enzyme activity unit), Amount of enzyme that releases 1 µmol of glucose reducing sugar equivalent per minute at 40 °C and pH 5.0 or 5.5. PU (phytase unit), Enzyme activity that liberates 1 µmol inorganic phosphate (122) or phosphorus (123) per minute. KI (Kolbach Index), Total soluble nitrogen in the wort as a percentage of the total nitrogen in the malt.

Data on the other amylase enzymes in malts of these tropical cereals is scanty. Limit dextrinase (debranching enzyme) of high activity was purified from sorghum malt as long ago as 1976 (128). Much more recently, comparative work, referred to above (116) has shown that rice malt has high limit dextrinase activity, followed by sorghum malt (notwithstanding the sample's low germination), with maize malt having the lowest activity. Interesting, whole grain rice and white (debranned) rice have been found to exhibit high levels of limit dextrinase activity (119,121), several times that of barley malt (121).

α-Glucosidase activity has been found in malt of black non-waxy and black waxy rice (119) and small amounts in the raw grain of these rice types (119). In the case of sorghum malt, α-glucosidase is present and active but in a water-insoluble form (129). As a consequence of this and probably the high gelatinization temperature of sorghum starch (120), sorghum

malt worts can contain a very high ratio of glucose to maltose, 1:1 (129,120). It has also been reported that maize, rice and sorghum malts exhibit amyloglucosidase (glucoamylase) activity (116). However, this may be a misinterpretation, as the assay used employed *p*-nitrophenyl β-ᴅ-maltoside as substrate (130), as this also acts as a substrate for α-glucosidase (131).

There is also very scanty information concerning the cell wall degrading of malts of the tropical cereals. According to a 2009 review on cereal xylanases, it is not even confirmed on the basis of specific assays whether xylanase activity is even present in germinating maize (132), despite the fact that, as explained, the arabinoxylans are the major endosperm cell wall components in maize and sorghum (69,71,72). Concerning xylanase activity, a comparative study using their cereal pentosans as substrates indicated that sorghum malt had higher pentosanase activity than barley malt (87) and that there was essentially no

pentosanase activity in the raw barley or sorghum grain. In apparent contrast, another study by the same first author stated that extracts of sorghum malt enzymes failed to hydrolyse sorghum endosperm cell walls (70). However, the data suggests that in fact the enzymes removed the arabinose side chains from the xylose backbone. This would be consistent with the persistence of the endosperm walls in sorghum malt (77,81).

With regard to β-glucanase activity, several studies have shown that the endo-β-glucanase activity in sorghum malt is considerably lower than in barley malt (70,87,111) (Table 1). A single study showed that the level of end-β-glucanase activity in maize and rice malts was very low, even in comparison to sorghum malt (116). There seems to be negligible endo-β-glucanase activity in raw sorghum (87,116), maize (116) and rice (116) grains. Although limited, the literature clearly indicates that maize, rice and sorghum malts contain low levels of endosperm cell wall-degrading enzymes. Because of this and the fact that the endosperm cell walls of all the cereals probably persist during malting, supplementation with exogenous cell wall-degrading enzymes in brewing is beneficial.

The problem of hydrolysing the proline-rich cereal prolamin storage proteins into free amino acids has been referred to earlier. Barley malt contains up to 42 different endoproteases alone, which are of four different classes: metallo, serine, cysteine and aspartic (133,134). Zymography on two-dimensional electrophorograms indicated that sorghum malt had a similar pattern of endoproteases to barley malt, but the enzymes had low pI (134). High levels of metalloproteases were found in sorghum and apparently also cysteine- and serine-type proteases (133). Both the metallo- (135) and cysteine-type proteases (136) have been purified from sorghum malt. The sorghum malt proteases, like those of barley malt, are insoluble in simple aqueous solvents (137,138). This presumably means that enzymatic sorghum malt extracts, as have been proposed in lager brewing (23), would be very deficient in protease activity. Interestingly, it has been observed that the level of endoprotease activity in sorghum did not increase substantially during malting (139).

With regard to malted maize, four endoproteases, apparently of the cysteine type, which could degrade zein have been isolated from the endosperm of germinating grain (140). Rice malt has been found to exhibit much lower endoprotease activity than barley or sorghum, with possibly the cysteine class being most important (134).

It has been proposed that the unusual prolyl type carboxypeptidase is of major importance with regard to hydrolysis of the proline-rich peptide products of endoprotease cleavage of prolamins into free amino acids (60). Such prolyl carboxypeptidases have been found in germinated barley (141) and there are indications that that they are present in maize, rice and sorghum (60). It has also been shown that there is carboxypeptidase activity in sorghum malt at brewing type acidic pH (138,139) and importantly that the activity releases FAN from endopeptidase hydrolysed kafirin prolamin (139). Unlike the situation with endoprotease activity, carboxypeptidase activity in sorghum was found to increase substantially during malting (139).

In germinating rice, m-RNAs for several types of serine-type carboxypeptidases have been found to be abundant in the germ and to increase during germination (142) and high neutral metallo carboxypeptidase activity has also been found (143). This enzyme was not detected in germinating barley or maize. However, importantly with respect to brewing, the levels of acid carboxypeptidase activity in both germinated rice and maize were low in the scutellum and very low in the endosperm, when compared with germinating barley (143). Whether these carboxypeptidases in germinated rice and maize can release free amino acids from the peptide products of endosperm storage protein hydrolysis does not seem to have been investigated.

Information on the differences between the phytase (*myo*-inositol-hexakisphosphate 6-phosphohydrolase) activity in barley, rice, sorghum and maize whole raw grains and malt is lacking. This may be due to the fact that it is very difficult to purify plant phytases from contaminating non-specific phosphatases (145). Contradictory increases and decreases in phytase activity owing to germination have been observed in barley (145,146,122) and rice (146,123), while the phytase activity of sorghum (122,123) and maize (122,123) has only been found to increase substantially during germination. The phytase activities of sorghum, maize and rice malts are similar to that of barley malt (Table 1). It has also been found that malting reduced the phytate content of sorghum by 29% (122) to 81% (123), that of maize by 34% (122) to 88% (123) and that of rice by 54% (147) to 65% (122) and 83% (123). It appears that, with malted sorghum, maize and rice, phytate degradation is similar to that of barley malt (122,145,146,148). Therefore addition of exogenous phytase when brewing with malts of these cereals would not seem to be necessary.

Whole raw grain sorghum (24 –111 U/kg) (122,148), maize (12–130 U/kg) (122,148,149) and rice (120–190 U/kg) (122,150) seem to have negligible to low phytase activity compared with barley whole raw grain (582–1830 U/kg) (122,148). Therefore when whole raw sorghum, maize and rice are used in brewing, phytase addition appears to be useful. For example, it has been found that adding phytase in rice sake brewing increased yeast growth and fermentation performance, without the osmotic stress produced by the addition of large amounts of inorganic phosphate salts (151). Also, adding phytase to raw sorghum and maize mashing was shown to decrease the phytate content of sorghum spent grain, while the phytate content of the maize spent grain was negligible before the phytase addition (152).

With the notable exception of β-amylase, it appears that tropical cereal malts have all the enzymes required to produce a well fermentable wort. However, the raw grains of these cereals are almost completely devoid of all the enzymes required in mashing, including β-amylase. Thus, when brewing with solely raw grain, the added exogenous enzymes need to provide all the activities.

Potentially limiting micronutrients for yeast fermentation

The magnesium, zinc, lipid and FAN contents of sorghum, maize and rice refined grain, whole raw grain and malt and will be discussed with respect to yeast nutrient requirements. In general, wort from barley malt provides all the nutritional requirements of yeast during fermentation with the exception of zinc (22,153). It has been found that, to achieve optimal fermentation during lager brewing, the zinc and magnesium concentration of wort should be 0.01–0.15 mg/L (153) and 50–100 mg/L (154), respectively. The magnesium contents of barley, sorghum and maize whole raw grains are not substantially different from each other (Table 2), with the exception of a very high and possibly anomalous value

Table 2. Magnesium and zinc contents of barley, sorghum, maize and rice refined grain, whole raw grain and malt

	Refined grain	Whole raw grain	Malt
	Magnesium (mg/kg)		
Barley	780 *(159)*, 78[a] *(160)*, 670 *(161)*, 468 *(160)*	1330 *(159)*, 890 *(161)*, 1410 *(160)*, 1971 *(162)*	ND
Sorghum	1200 *(159)*, 1590 *(163)*,	1840 *(163)*, 188 *(162)*, 4480 *(155)*, 1452 *(152)*	3510 *(155)*
Maize	470 *(161)*	1270 *(159)*, 511–571 *(164)*, 806 *(152)*, 1800 *(165)*	440–560 *(164)*
Rice	350 *(159)*, 310–406 *(161)*,	230–1120 *(159)*, 326–415 *(161)*	ND
	Zinc (mg/kg)		
Barley	21 *(159)*, 5[a] *(160)*, 28 *(161)*, 16 *(160)*	28 *(159)*, 24 *(160)*, 74 *(162)*	ND
Sorghum	3–8 *(161)*	3 *(162)*, 64 *(155)*, 22 *(152)*, 31 *(161)*,	53 *(155)*
Maize	14 *(159)*, 5 *(161)*	22 *(159)*, 26 *(165)*, 18 *(152)*, 13 *(164)*	18–20 *(164)*
Rice	11–21 *(161)*, 8 *(159)*	12–25 *(159)*, 18 *(159)*, 20 *(147)*	6–13 *(147)*

[a]Hand dissected endosperm.
ND, No relevant data could be found.

reported for sorghum *(155)*. The sorghum was soaked in tap water, which may explain the high levels of magnesium. It seems, however, that in general whole raw grain rice has a lower magnesium content compared with the other grains. The zinc contents of all the whole raw grain cereals are similar, with some high values being reported for sorghum and barley. It is not clear if there are really barley and sorghum cultivars with such high zinc contents or if this is due to other reasons such as contamination *(156,157)* or high soil mineral content *(158)*.

The potential for magnesium and zinc deficiencies in wort from refined barley, sorghum, maize and rice grains is even higher as up to 50–90% of magnesium and zinc can be lost when cereal grains are refined, owing to the removal of the mineral-rich pericarp and sometimes the germ *(163)*.

According to an authoritative review, slightly higher amounts of certain vitamins and minerals have been found in cereals after germination, but it was cautioned that most of the increases in nutrients are not true increases and could simply be due to the loss of dry matter, mainly carbohydrates, owing to respiration *(150)*. Other authors have, however, reported substantial increases in magnesium and zinc during germination [maize *(164))*, decreases (sorghum *(155)*, maize *(164)*, rice *(147)*] and no significant effect (maize *(164))*. Despite the significant reduction in magnesium and zinc contents, which occur in all the grain species during refining, it appears that the variation in these mineral contents within a cereal type owing to environmental *(166,167)* and genetic *(167,168)* variation is as large as between cereal species.

The grain mineral contents alone do not, however, give the full picture. Components in the grain inhibiting mineral solubilization substantially affect the amount of minerals that is eventually available in the wort to the yeast. Phytate (*myo*-inositol hexaphosphate), which chelates divalent minerals such as magnesium and zinc, has been found to be present in barley, sorghum, maize and rice at 0.6 *(169)*, 0.8 *(169)*, 0.1 *(169)* to 1.1 *(164)* and 0.4 *(169)* to 1.3 *(147)* g/100 g, respectively. A study on the effect of the phytate content in sorghum and maize on the solubilization of minerals into the wort during mashing showed that, when the phytate content of the spent grain was lower, the percentage of minerals solubilized into the wort was higher *(152)*. If tannin containing sorghum (discussed previously) is used for brewing,

the tannins probably also bind these minerals *(170)*, further reducing their solubilization into the wort.

Concerning lipids, sterols and unsaturated fatty acids, these can be present in sub-optimal quantities in wort *(171)*, despite the high concentration of lipids in whole cereal grains. As lipids are concentrated in the germ, they may be lost in substantial amounts when the grains are refined *(172)*. All the unsaturated fatty acids present in the grain may also not be solubilized into the wort, as it has been found that the concentration of oleic acid in barley malt wort was approximately 52% less than that of its malt *(22)*. As with the minerals, the fatty acid contents of these grains seem to vary substantially within each cereal species. However, there do not seem to be substantial differences between the palmitic, oleic and linoleic acid contents of barley, sorghum, maize and rice whole raw grains (Table 3). While data on the effect of malting on the fatty acid contents of barley, sorghum, maize and rice are limited, it is clear that malting does not substantially affect the fatty acid contents.

Concerning FAN, whole raw grain sorghum and maize, but possibly not rice, seem to have a lower FAN content than that of whole raw grain barley (Table 4). The FAN contents of cereal malts are affected by a number of factors, such as germination time, temperature and watering level *(179)*, explaining the substantial variation in the data between different studies. However, the wort FAN levels from whole raw grain and malted barley, sorghum and rice (maize no data) seem to be similar (Table 4). It has been found that, when whole raw grain maize and decorticated sorghum were mashed with the same commercial enzymes, the maize FAN (110–169 mg/L) was higher than that of the sorghum (84–142 mg/L) *(180)*. This suggests that refining of sorghum decreases the FAN that can be produced by proteolysis during mashing, presumably as result of the removal of a part of the protein-rich corneous endosperm and germ.

The effects of yeast nutrient limitations are exacerbated during high-gravity brewing, which subjects yeast cells to high osmotic pressure *(192,193)* and high ethanol levels *(193)*. High-gravity brewing may necessitate supplementation of the wort with minerals *(153)*, vitamins *(194)* and lipids *(195)* to avoid stuck fermentations. Yeast requires oxygen during the first few hours of fermentation for lipid synthesis by the yeast *(196)*. However, oxygen solubility is poor in high-gravity worts, possibly resulting in even less optimal concentrations of sterols and lipids *(196)*.

Table 3. Palmitic, oleic and linoleic acid contents (percentage of total fatty acids) of barley, sorghum, maize and rice whole raw grain and malt

	Whole raw grain	Malt
16:0 (Palmitic acid)		
Barley	24 *(161)*, 18–19 *(173)*	16–19 *(173)*
Sorghum	12–13 *(174)*	12–15 *(174)*
Maize	13–16 *(175)*	ND
Rice	18 *(176)*	19 *(176)*
18:1 (Oleic acid)		
Barley	12 *(161)*, 35 *(159)*, 13–14 *(173)*	10–12 *(173)*
Sorghum	35 *(159)*, 31–49 *(177)*, 34–40 *(174)*	37–41 *(174)*
Maize	39 *(159)*, 21–36 *(175)*	ND
Rice	12–43 *(176)*	44 *(176)*
18:2 (Linoleic acid)		
Barley	57 *(161)*, 56–58 *(173)*	61 *(173)*
Sorghum	28–51 *(177)*, 42–50 *(174)*	42–43 *(174)*
Maize	48–59 *(175)*	ND
Rice	19 *(176)*, 39 *(178)*	18 *(176)*

ND, No relevant data could be found.

Table 4. Free amino nitrogen (FAN) contents of barley, sorghum, maize and rice whole raw grains, their malts and worts

	Whole raw grain (mg/100 g)	Malt (mg/100 g)	Wort –whole raw grain (mg/L)	Wort – malt (mg/L)
Barley	47–48 *(181)*,[b] 73 *(182)*[a]	106 *(182)*, 125 *(183)*, 140–196 *(181)*	46 *(183)*, 65 *(182)*	118–120 *(184)*, 126 *(185)*, 136 *(186)*, 157 *(183)*, 158 *(184)*, 177 *(182)*
Sorghum	12 *(187)*, 13–19 *(188)*, 18 *(54)*, 22 *(152)*	83 *(187)*, 167–213 *(179)*	29 *(152)*, 29 *(189)*	152 *(186)*
Maize	22 *(152)*, 25 *(188)*,	ND	22 *(152)*	110–169 *(179)*
Rice	45 *(188)*	30–50 *(150)*, 99–104 *(119)*, 170 *(190)*	ND	67–188 *(191)*, 95–138 *(184)*

[a]Results obtained using the EBC Congress mashing procedure on a grist of 50% malted barley and 50% unmalted barley.
[b]One-day germinated grain.
ND, No relevant data could be found.

A review on the effect of increasing the magnesium and zinc contents of worts by enrichment and supplementation concluded that increasing these minerals significantly improves fermentation performance in high-gravity brewing *(197)*.

Impact of brewing with sorghum, maize and rice on beer flavour

It has been stated that most of the flavour of barley malt beer originates from the hops or is developed through yeast metabolism and that the majority of malt flavours originate during kilning *(198)*. However, the same authors further stated the barley may contribute astringency and perhaps body owing to the polyphenols and tannins *(198)*. In fact, barley malt (and presumably the grain) contains the flavonoid catechin, plus procyanidin dimers and trimers based on catechin and gallocatechin units *(199)*. However, the level of all these flavonoids is probably only 20% of that in red non-tannin sorghums and only some 3% of that in

tannin sorghums *(91)*. In addition, polyphenols and tannins, aldehydes, which are lipid oxidation products, are key aroma compounds in barley *(200)*.

Concerning sorghum, apart from its polyphenols, little is known about the compounds responsible for sorghum flavour. As described, some types of sorghum contain substantial quantities of condensed tannins. Tannins are known to impart the dry and puckering sensation of astringency in the oral cavity, which seems to involve binding of the tannins to the salivary proline-rich proteins *(201)*. However, it has been shown that bran extracts of all the major types of sorghum, including the white tan-plant type, which contains very low levels of polyphenols *(91)*, were perceived by a trained descriptive sensory panel to be both bitter and astringent *(202)*. In general, however, the tannin sorghums were more bitter and astringent, although one tannin sorghum variety was found to be similarly bitter and astringent to a red non-tannin type, notwithstanding the fact that it contained condensed tannins. Notably, the white tan-plant sorghums also had a sweet, maize-like flavour, unlike

the tannin and red non-tannin types. Unpublished descriptive sensory work from our laboratory on the flavour of boiled sweet wort from raw whole grain sorghum revealed that wort from red tannin sorghum was substantially more sour, bitter and astringent than worts from white tan-plant, red non-tannin and even white type II tannin sorghums. In apparent contradiction, a commercial brewing syrup made from white sorghum is stated not to have the unpleasant aftertaste associated red sorghum syrup (203).

Regarding the impact of maize and rice on beer flavour, a predominant flavour of cooked maize is the 'popcorn aroma', which is caused by 6-acetyltetrahydopyridine, 2-acetyl-1-pyrroline (2-AP) (roasty popcorn-like aroma) and its analogue 2-propionyl-1-pyrroline (204). In corn (maize) tortillas, a potent flavour compound is 2-aminoacetophenone (205), apparently formed from tryptophan under the alkaline cooking condition. It is not known whether this compound would be formed under the slightly acidic conditions of brewing. The aroma of sweetcorn is predominantly due to dimethyl sulphide (206). Concerning polyphenols, beer brewed from a grist containing 86% maize adjunct contained somewhat less total phenols than all barley malt beer, 280 and 337 mg/L, respectively, with both types of beers containing very low levels of flavonoids and tannins (207).

With regard to rice flavour, it is important to distinguish between the fragrant and non-fragrant types (208), the latter being used as adjunct as they are far less expensive. More than 200 volatile compounds have been identified in rice, but only 2-AP (the popcorn aroma compound) has been confirmed to contribute to rice aroma (208). The levels of 2-AP in non-aromatic rice are up to 0.008 ppm, some 10 times less than in aromatic rice (209). Apart from 2-AP, as with barley aldehydes, lipid oxidation products, such as 2-nonenal and 2–4 decadienal clearly also contribute to rice aroma or flavour (210). In a study of beer and its ingredients, one aldehyde, glyoxal (ethanedial) (which has an off-flavour reminiscent of sour milk) was found in highest concentration in rice (211).

An important point made concerning rice, but also applicable to all cereals, is that aroma and flavour are affected by many factors, including genetics, pre-harvest issues, time of harvest, harvest moisture, drying and storage conditions, degree of milling (which impacts lipid oxidation) and cooking method (208), and in particular malting and kilning (67). It has been observed that, with pale beers (presumably including regular type lager beers), few of the characteristic flavours of malt survive the brewing process, but the situation is very different when roasted malts and roasted barley are ingredients (212).

An analysis of a non-alcoholic malt beverage made from sorghum malt roasted at 200 °C revealed 28 volatile compounds, comprising pyrazines, furans, aldehydes, ketones, esters and alcohols (213), all of which with the exception of alcohols are typical of the Maillard reaction (214). Aldehydes, ketones and esters were present in the beverage in the highest concentration and it was described as having a nutty, sweet chocolate aroma (214).

Beer produced from sorghum malt germinated at 25 °C was found to contain somewhat lower levels of higher alcohols than that from sorghum germinated at 20 °C (28), possibly related to the better modification of sorghum malt at the higher temperature (215).

There is some indication that the differences in the free amino acid profile of sorghum malt worts compared with barley malt worts could influence beer flavour by affecting yeast metabolism. Sorghum malt worts were found to contain low levels of valine (111). This led to high levels of vicinal diketones (diacetyl), during fermentation owing to effects on the regulation of valine synthesis by the yeast. A more general problem is that brewing with a low proportion of malt (and undoubtedly also with just raw grain) can also impact beer flavour as a result of low levels of FAN. It has been proposed that, resulting from the fact that, when FAN is depleted during fermentation, yeast cell proliferation ceases, brewers raise fermentation temperatures to enable the small number of yeast cells to consume the same quantity of carbon sources (fermentable sugars) (216) and that this affects yeast metabolism, resulting in higher levels of volatile flavour compounds.

Future directions

As consumers of beers brewed from tropical cereals become discerning, more attention needs to be given to beer flavour. An interesting concept is to promote or repress higher alcohol and ester production by yeast by controlling fermentation parameters and the addition of limiting yeast nutrients such as free amino acids, zinc and linoleic acid (216).

Far more knowledge on the proteolytic enzymes of these cereals, especially regarding the activity of prolyl carboxypeptidases, is required in order to improve their wort FAN levels. It would also seem potentially attractive to genetically modify these cereals so that they have this particular protease activity and a similar level of β-amylase activity to barley malt. A candidate β-amylase is the lysine-rich protein from Hiproly barley (217). However, whether the economic benefit of these genetic modifications would justify the cost of development, especially including obtaining regulatory approval, is debatable.

A more viable alternative is to investigate lines of these cereals that have modified traits in respect of improved solubilization to produce a well fermentable wort. The waxy (high amylopectin) trait has been discussed. The bioethanol industry is beginning to employ a novel commercial amylase that can effectively hydrolyse raw (ungelatinized) starch (11), thus saving energy. Recent research indicates that waxy maize starch is better hydrolysed by this novel amylase than normal maize starch (44).

In many developing countries, a major research activity is to develop tropical cereals with improved nutritional traits for human nutrition, such as increased provitamin A and essential minerals, improved mineral bioavailability and improved protein quality and digestibility (218). These co-called biofortified cereals are being specifically developed for cultivation by small-holder farmers, and they could be a valuable crop for these farmers, as some of the traits are beneficial in brewing (152). For example, sorghum lines with high protein digestibility have been developed where synthesis of certain kafirin sub-classes, including specifically γ-kafirin, have been inhibited by chemical mutation (219) or genetic modification (220). Research has shown that FAN in sorghum malt (187) and FAN and extract in raw sorghum grain brewing (152) can be substantially improved with the use of such sorghums.

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