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Changes in catalase activities during malting of some improved Nigerian sorghum grain varieties

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Abstract

The catalase activities of ten germinating improved sorghum varieties were monitored over a 4-day period to determine the effect of malting on them. This was done using standard methods that involved catalase assay at the appropriate experimental intervals. Results obtained showed that the different varieties of sorghum differed in their expression of catalase, a difference that was also reflected across the different stages of the malting process. The highest overall catalase activity (20.54 ± 0.74 U) was given by variety SK5912 after 72 hours of germination followed in second place by that from variety Nafelen (18.65 ± 0.99 U) obtained after steeping. The third and fourth highest value (17.88 ± 1.24 U and 17.08 ± 1.64 U) were given by KSV8 and Boboje after 72 and 48 hours of germination respectively. These values are probably indications that no single stage of malting was best for catalase expression among all the varieties. However, the fact that most of them (varieties ICSV 400, SK5912 and KSV 8, CSRO2 and ICSV III) all expressed their highest catalase activities after 72 hours of germination showed that 72 hours is probably the best germination stage for the elicitation of catalase among sorghum grains. The next best stage should be after 48 hours during which point three varieties (Boboje, NRL 3 and KAT 487) had their highest catalase activities. As a unity, all the sorghum varieties had their lowest catalase expression after 24 hours of germination, followed by those obtained after 96 hours germination.

Keywords: catalase, sorghum, malting, peroxidase, cereal enzymes

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INTRODUCTION

Catalases (EC. 1.11.1.6) are heme carrying enzymes usually classified as belonging to the second peroxidase superfamily (Hiraga et al., 2001; Sooch et al., 2016). They catalyse the reduction of hydrogen peroxide using two electron donors, which could either be a second molecule of hydrogen peroxide in the catalytic reaction, or organic compounds such as alcohols and aldehydes, in a peroxidation reaction (Dunford, 2010). Catalases occur in all aerobic organisms including plants (Yoruk et al.,

2005) and also in many anaerobic organisms (Switala and Loewen, 2002). Together with superoxide dismutase and peroxidase, catalase forms the most potent enzyme-based defence system of biological cells against reactive oxygen species and hydrogen peroxide (Scandalios, et al., 1984) effectively degrading them before they cause damage to important cellular components (Harb et al., 2015). These various reactive oxygen species are said to have many chemical and biological effects in the various processes associated with brewing (Clarkson *et a*l., 1992; Frederiksen et al., 2008). They are formed when partial reduction of oxygen causes it to acquire additional electrons in the p orbitals with the resultant successive formation of superoxide, $O2^-$, hydrogen peroxide, H_2O_2 and hydroxyl radical, OH, all of which are toxic because they can inactivate important brewery enzymes and cause lipid peroxidation (Halliwell and Gutteridge, 1989). The presence of these partially reduced forms of oxygen could lead to problems at various stages of the brewing process especially at the early part of malting such as during steeping and germination and also at the mashing stage (Clarkson et al., 1992). Not surprisingly therefore, biological systems protect themselves against these deleterious oxygen free radicals using the above-mentioned scavenging enzymes: superoxide dismutase, peroxidase and catalase. The occurrence of these critical enzymes has been indicated in important brewery and allied industrial grains as wheat, barley and sorghum (Kruger, 1977; Nwanguma and Eze, 1995; Bakalova et al., 2004; Dicko et al., 2006; Ishibashi et al., 2008). Additionally, these enzymes are known to influence important brewing properties such as lipid peroxidation and flavour instability among others (Nwanguma and Eze, 1995; Bamforth, 2009).

The cereal we are concerned with in the present work is sorghum, the the fifth most important cereal in total world production, after wheat, rice, corn and barley (Wong et al. 2009; USDA, 2011). Sorghum has become recognized as a veritable alternative in the brewing and malting industries, especially in the tropics where it is extensively cultivated as a result of the advantages it possesses such as being physiologically robust and able to withstand extreme environmental circumstances like extreme drought and heat as well as temporary water-logging among others (Purselove 1972; Taylor et al. 2006; Ejeta and Knoll, 2007; Nnamchi et al., 2013).

Different workers had tried to determine the course of these enzymes at the different stages of brewing with sorghum (Nwanguma and Eze, 1995; Dicko et al., 2002; Nnamchi et al., 2013). Unfortunately, the quest for the role of these antioxidant enzymes in malting and brewery-related works have been mostly restricted to works with peroxidase. Few if any work had been dedicated to studying the incidence and course of catalase during malting and brewing with sorghum. We have therefore tried in the present work to bridge some of that gap by following the course of catalase expression and change during a 96-hour malting period of ten different sorghum varieties.

MATERIALS AND METHODS

A total of ten different varieties of sorghum grains (*Sorghum bicolor* L. Moench var. SK 5912, KSV 8, ICSV 400, ICSV III, White Kaura, Boboje, CSRO2, NRL-3, KAT 487 and Nafelen 6) all grown in 2009 and purchased from the Institute for Agricultural Research of the Ahmadu Bello University Zaria, Nigeria were used for this work. The method of Ogbonna et al. (2003) was used to clean and sort the grains.

Grain steeping

Grain steeping of the sorghum varieties was done by measuring out 200 grams of each sorghum variety in triplicates and immersing them in 400 ml of distilled water such that the grain/water mixture was in a ratio of 1:2. Steeping was carried out for 24 hours at room temperature. The steep water was changed at 6 hourly intervals to remove any untoward microbial contaminant.

Germination

Germination of the sorghum grains was carried out for 4 days using the methods described by Ogbonna et al. (2003). After germination the grains were immediately freeze-stored at -80^{o}C (Thermo Scientific) for at least 24 hours or until required for assay. Before assay, the very cold and now brittle radicles and plumules were carefully removed manually.

Assay for Catalase Activity

Sorghum catalase was extracted using the procedures outlined by Clarkson *et al*. (1992). Appropriately weighed quantities of each

sorghum grain variety were ground with mortar and pestle and catalase extracted by incubating the ground grains in a 1:2 (w/v) ratio with 50mM phosphate buffer, pH 7.0 for 30 minutes at 4°C. Thereafter, the resulting homogenate was centrifuged at 5,000g for 30 minutes in an SS 34 Sorvall Evolution centrifuge. The supernatant produced was used as the crude extract. Catalase activity was assayed using the method of Haywood and Large (1981). Exactly 0.1ml of diluted enzyme preparation was added to 2.9ml of a freshly prepared solution containing 0.1ml of 30% (v/v) H_2O_2 in 50ml of 50mM phosphate buffer, pH 7.0. The decrease in absorbance at 240nm was followed and the time taken for the A240 to fall by 0.05 absorbance units was determined at room temperature (28 ± 2°C). This corresponds to the decomposition of 3.45 micromole of H_2O_2 in the 3ml solution. Catalase activity (expressed in sigma units) is equal to 3.45/ {time (min) required}.

Statistical Analysis

Values of catalase activity were calculated as mean ± SE (standard error) of triplicate measurements/assay obtained for each improved sorghum variety at the different malting stages.

RESULTS

Figure 1 shows the changes in the catalase activity of variety ICSV 400 at the different malting stages. The highest catalase activity (11.31 ± 1.05 U) was obtained after 72 hours of germination followed by that obtained after 96 hours of germination (7.55 ± 0.32 U) while the lowest catalase activity (1.15 ± 0.38 U) was obtained after 24 hours germination. The raw (ungerminated) ICSV 400 grain has a catalase activity value of 2.99 U ± 0.36. In figure 2 is shown the same changes in catalase activity for sorghum variety SK5912. The highest catalase activity here (20.54 ± 0.74 U) was again obtained after 72 hours of germination, followed in second place by that obtained after 48 hours of germination (16.59 ± 0.44 U). Again, the least catalase activity value (2.77 ± 0.19 U) was obtained after 24 hours of germination. The catalase activity changes in variety KSV 8 is shown in figure 3 with germination after 72 hours giving the highest catalase activity value of 17.88 ± 1.24 U, followed in second place by that obtained after steeping (16.27 ± 0.39 U). The least catalase activity value (3.37 ± 0.88 U) was obtained again 24 hours of germination. Shown in figure 4 is the catalase activity of White kaura variety after different malting stages. Interestingly, the highest activity was observed after steeping (8.78 ± 0.30 U) followed in second and third places by those obtained after 96 hours of germination and the raw ungerminated grain with values of 6.9 ± 0.96 U and 6.63 ± 1.05 U respectively indicating here that germination really may not have affected the catalase activity of grain variety except reduce it somewhat as indicated by the least value obtained which as in the previous cases was after 24 hours of germination (3.57 ± 0.98 U). Figure 5 shows the catalase activity obtained for sorghum variety Boboje after different malting stages. The highest value here (17.08 ± 1.64 U) was obtained after malting the grains for 48 hours, while in second place was that obtained after malting for 72 hours (13.42 ± 0.34 U). The least value of 2.1 ± 0.58 U was obtained after 24 hours of germination. Presented in figure 6 is the changing catalase activity values after different malting regimes for sorghum variety ICSV III. The highest value (10.55 ± 1.34 U) was obtained after 72 hours of germination, followed with only little change in second place by that obtained after 48 hours of germination (10.39 ± 0.65 U). In third place is that obtained from the raw ungerminated grain (7.84 ± 0.36 U) which is many times greater than that obtained as the least value after 24 hours of germination (1.43 ± 0.68 U). Figure 7 shows the catalase activity values obtained with variety CSRO2 after different stages of malting. As has mostly been the case thus far, the highest catalase activity (12.55 ± 1.04 U) was obtained after 72 hours of germination. In second place is the value of 10.39 ± 0.99 U obtained just after steeping while the least value of 4.41 ± 1.08 U was obtained as usual after 24 hours of germination. The catalase activity values as they occurred in respect of variety NRL-3 after their malting is presented in figure 8. The only change

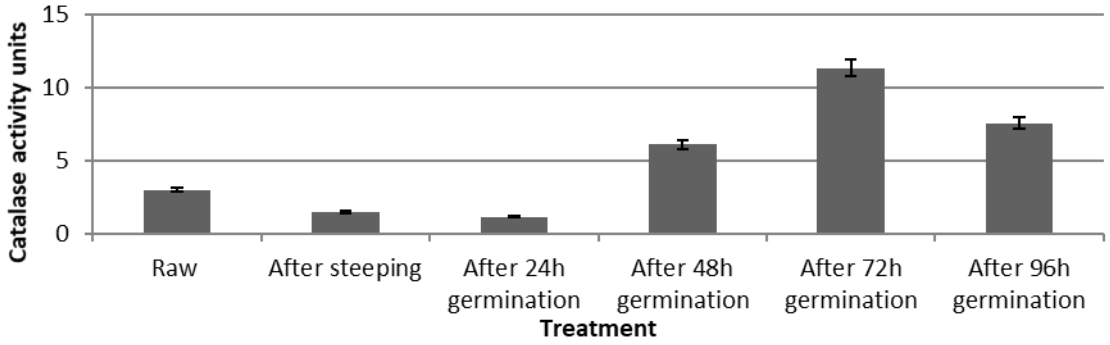

Figure 1: Changes in catalase activities of ICSV 400 after different malting stages

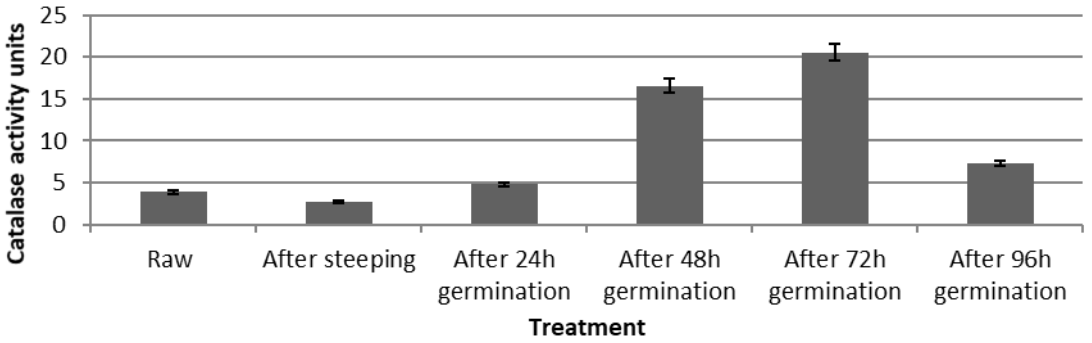

Figure 2: Changes in catalase activity of SK5912 after different malting stages

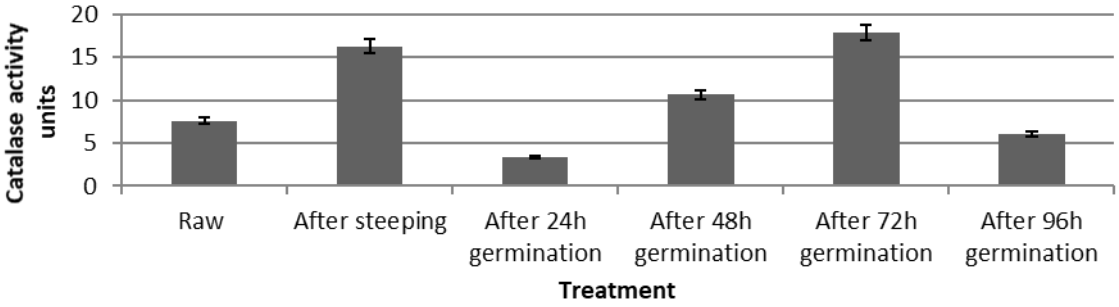

Figure 3: Changes in catalase activity of KSV8 after different malting stages

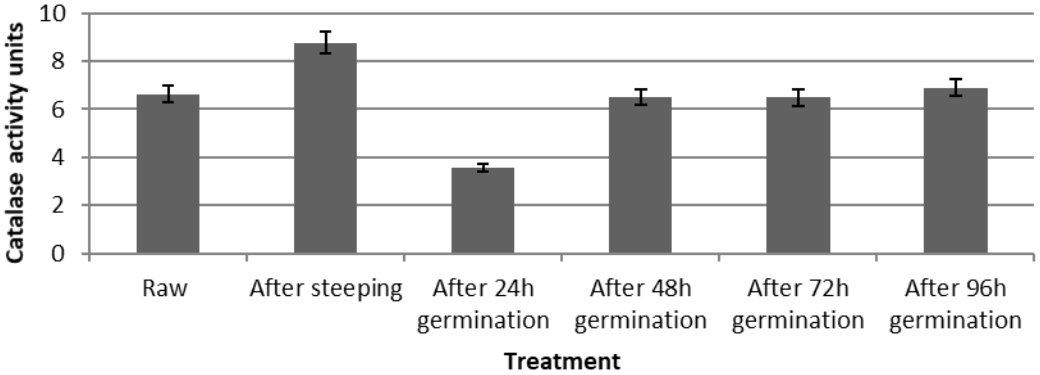

Figure 4: Changes in catalase activity of White kaura after different malting stages

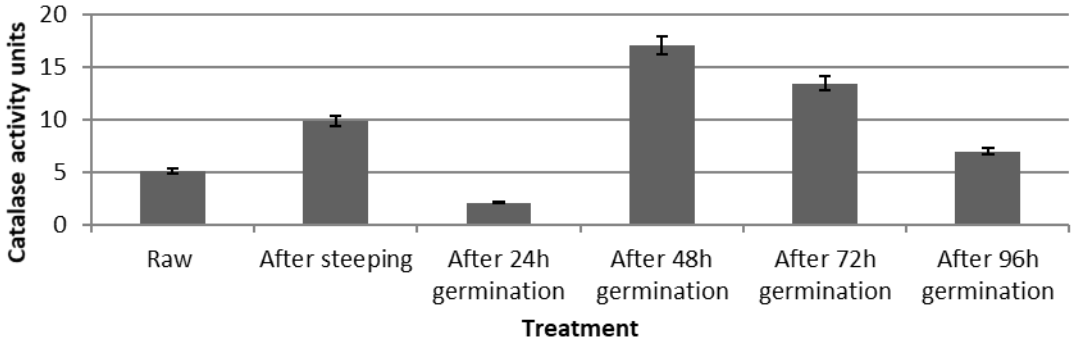

Figure 5: Changes in catalase activity of Boboje after different malting stages

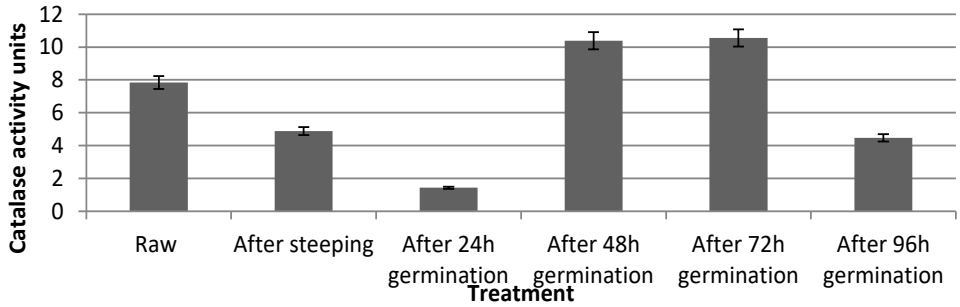

Figure 6: Changes in catalase activity of ICSV III after different malting stages

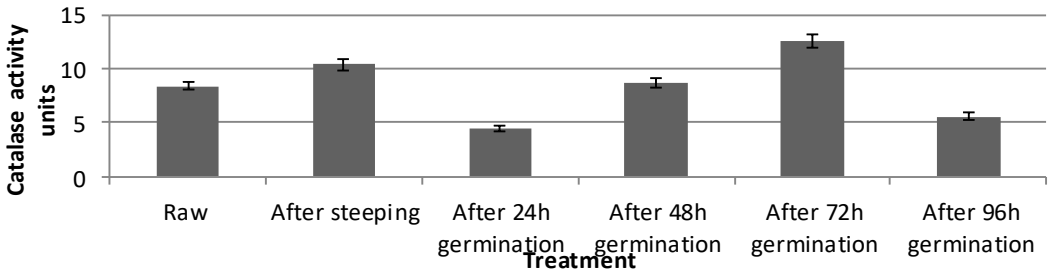

Figure 7: Changes in catalase activity of CSRO2 after different malting stages

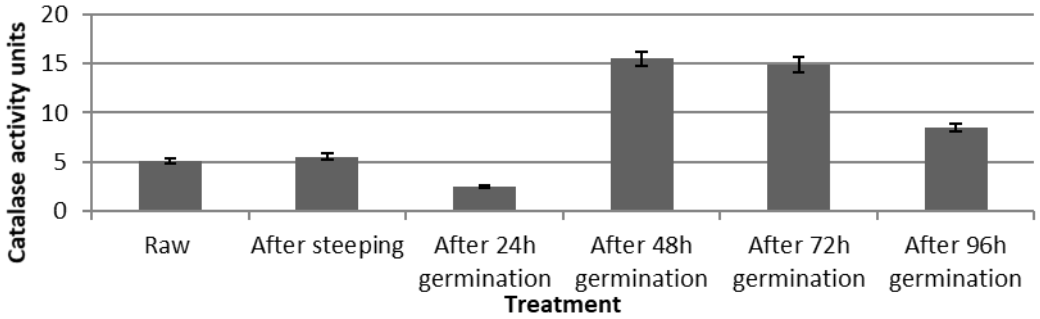

Figure 8: Changes in catalase activity of NRL 3 after different malting stages

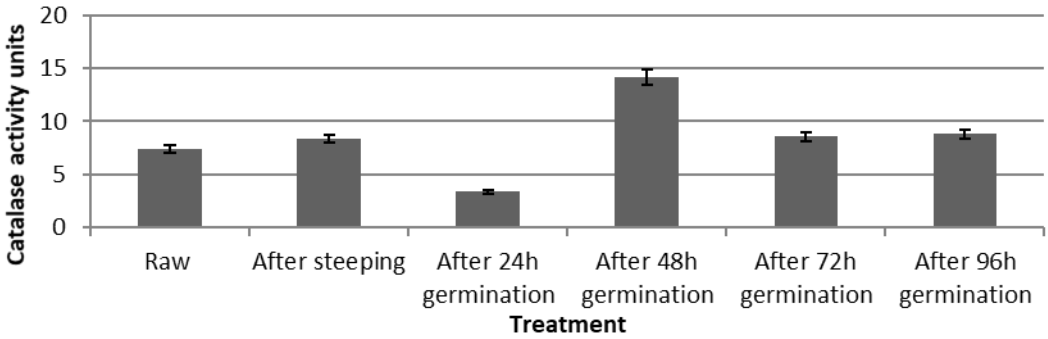

Figure 9: Changes in catalase activity of KAT 487 after different malting stage

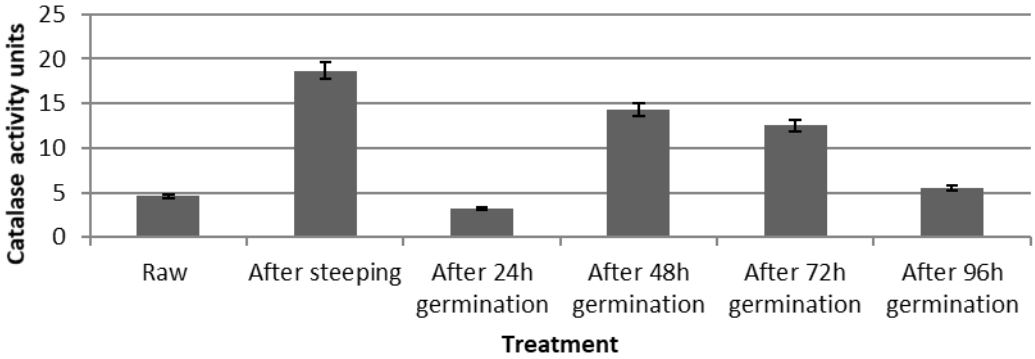

Figure 10: Changes in catalase activity of Nafelen 6 after different malting

observed here is that as was noticed once before, the highest activity value (15.47 ± 1.51 U) was obtained after 48 hours of germination, followed by that obtained after 72 hours (14.87 ± 0.94 U). The least value of 2.52 ± 0.38 U was obtained once more, after 24 hours of germination. Shown in figure 9 is the changing catalase activity values obtained with variety KAT 487. The highest value of 14.2 ± 1.15 U was attained again after 48 hours of germination while in second, third and fourth places were the values of 8.8 ± 0.56U, 8.56 ± 0.74 U and 8.35 ±

0.39 U respectively obtained after 96 and 72 hours of germination and after steeping. Those values were not too far off from that (7.37 ± 0.46 U) obtained from the raw ungerminated grain. The least value of 3.35 ±0.81 U was obtained after 24 hours of germination. Finally, the catalase activity values got for Nafelen 6 after the different malting regimen is shown in figure 10. The highest value of 18.65 ± 0.99 U was obtained this time after steeping, followed by that (14.26 ± 1.25 U) obtained after 48 hours of germination while the least value (3.19 ± 0.29 U) was obtained after 24 hours of germination.

DISCUSSION

In this work we demonstrated the presence and relative incidence of the antioxidant enzyme catalase in ten improved sorghum grains at the different malting and brewing stages of steeping and germination. Several workers have demonstrated the presence of catalase and many other antioxidant enzymes such as peroxidases and superoxide dismutase in many important industrial and brewery cereals (Kruger, 1977; Nwanguma and Eze, 1995; Bakalova et al., 2004; Dicko et al., 2006; Ishibashi et al., 2008). As a rule, these important antioxidant enzymes play such vital roles as combating the many excesses caused by free oxygen radical usually generated during the many metabolic activities of these plants or other organisms concerned, by reducing the energy of free radical groups. They do this by donating electrons to the free radicals which help to stabilize them by forming stable products. They may even preclude the formation of the free radicals in the first place (Elliot, 2006).

An important case of these free radical induced activities in brewing is flavour instability and lipid peroxidation (Nwanguma and Eze, 1995). These problems which are often critical during brewing are considered complex and therefore necessarily demands a multi-enzyme approach in the quest for its solution (Bamforth, 2009). Unfortunately, the numerous works done on sorghum in recent times has focused primarily on peroxidases to the exclusion of catalase (Nwanguma and Eze, 1995; Dicko et al., 2006; Nnamchi et al., 2013). The few works done on catalase-related activities had been on other cereals such as barley, wheat and rice (Palmiano and Juliano, 1973; Clarkson et al., 1992; Ishibashi et al., 2008) but not on sorghum. Considering the importance of sorghum, (Nunes et al., 2004; Ejeta and Knoll, 2007) we have tried in this work to provide some information on catalase presence and activities during sorghum malting.

As is easily glimpsed from the figures, the ten improved varieties of sorghum were affected by the different malting stages in their expression of catalase. The first key observation is that most of the grains already have substantial endogenous contents of the enzymes as is seen in the good catalase activity values obtained for the raw grains in all of the grain varieties. However, the amounts contained in each of them were different signifying the variety-dependent nature of that property. That attribute, although with other enzymes, have also been observed in several other works with sorghum (Ezeogu and Okolo, 1994; Nwanguma and Eze, 1995; Nnamchi et al., 2013). Another important observation is the fact that in all cases during the malting process, the least expression of catalase was observed after the first day of germination (24 hours). This consistency is probably due to the fact that the grains will necessarily require some adjustment period after steeping to commence full germination and therefore begin to elaborate catalase and other important enzymes. Germination triggers the enzyme system of sprouting seeds, leading to the breakdown of proteins, carbohydrates and lipids into simpler forms (Nout and Ngoddy, 1997; Correia et al., 2008). Therefore, germination activates the activities of intrinsic amylases, phytases, fibre-degrading enzymes that disrupt protein bodies, thereby increasing nutrient accessibility (Taylor et al., 1985) and of course those of important free radical scavenging enzymes such as peroxidases and catalase. In the process, it improves the overall nutritional profile of seed grains by causing the bioavailability of various minerals, vitamins etc. and reduces levels of antinutritional factors present in seeds (Maneemegalai and Nandakumar, 2018). It is not surprising therefore that the malting of cereals is a processing procedure that is traditionally used in many parts of Africa, Asia and other parts of the world for the processing of many food types and the manufacture of alcoholic drinks (Dewar et al., 1997; Traore et al., 2004; Choon et al., 2010) as it causes the positive release of many of these important enzymes among other improvements. In a similar experiment where peroxidase was assayed for during different stages of sorghum

malting, we had observed that the least enzyme expression occurred during steeping (Nnamchi et al., 2013). Such differences may well lie with the fact that they are two different enzymes.

A few authors had written on the changes associated with catalase expression and accumulation during malting of cereals. Palmiano and Juliano (1973) had observed that catalase activity increased during rice germination, with the highest activity occurring on the 6th out of a 7-day germination period, whereas for Ito and Hayashi (1961), the highest catalase activity was observed on the first day of rice germination. The expression of catalase in different plants had been described as erratic (Siminis et al., 1994). This erratic tendency is probably another way of restating varietal influence. Therefore, it is necessary to point out here that apart from the lowest values being consistently obtained after 24h germination in all the sorghum cultivars, no one point as such gave the highest catalase activity. However, in the majority of cases (five), that is, with cultivars ICSV400, SK5912, KSV 8, CRSO2 and ICSV III, the highest catalase activity was obtained after 72 hours of germination; cultivars Boboje, NRL 3 and KAT 487 (three) showed highest catalase activites after germination for 48 hours, while White kaura, and Nafelen 6 (two) showed their highest activities after steeping. Kar and Misra (1976) had observed such apparent inconsistent catalase activities in their works with leaf senescence where they had observed that whereas some authors had observed increase, some had seen decrease in catalase activities while working with leaves from different plant types (Farkas *et al*., 1963; Kisban *et al*., 1964; Parish, 1968). The explanation for all these variations may lie in the differential abilities of the different varieties to synthesize catalase or the presence of different levels of endogenous regulators or activators of catalase in different types and varieties of plants (Chapman, 1987; Nwanguma et al., 1995).

Conclusion

In the foregoing work we found that the catalase activity among ten improved sorghum varieties showed variations at the different malting stages and also among the different varieties. Whereas the highest catalase expression was observed after 72 hours of germination for five varieties, three varieties showed their highest values after 48 hours and then two after just steeping. Consistently, the lowest amounts of catalase were obtained after 24 hours of germination. None of the varieties showed their highest catalase expression values after 96 hours. In the majority of cases such as with variety SK5912, KSV8, Boboje and NRL-3, germination substantially increased catalase expression many times higher than those contained in the raw grains (control), whereas in a few others such as White kaura, ICSV III and CSRO2 the increase was not as high.

Conflict of Interest

Authors have no conflict of interest to declare

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