International Journal of Food Science and Nutrition
www.foodsciencejournal.com
ISSN: 2455-4898
Received: 17-09-2024, Accepted: 18-10-2024, Published: 02-11-2024
Volume 9, Issue 4, 2024, Page No. 35-41

Impact analysis of Biostimulants on sorghum peroxidase activity during malting

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Abstract
Impact analysis of bio-stimulants on sorghum peroxidase activity during malting was studied in sorghum variety, KSV8. Preliminary experiments showed that alkaline steep (test) and the distilled water steep (control) had germination energy of 92± 2.87 % and 89± 0.57 % respectively. Malting properties showed appreciable results. Again, the results showed there was an appreciable increase in peroxidase activity from day 1 through to day 3 of germination for distilled water steep in regime I (control) when compared to the test with regression in peroxidase activity. There was a positive gradual increase in peroxidase activity influenced by air-rest cycle from day 1 through day 3 in regime II (distilled water steep for 24h). At the end of kilning at 60 °C for 7 h, peroxidase activity dropped sharply in both regimes. Consequently, the introduction of air-rest cycle as malting condition will be beneficiary to brewers. It reduces malting loss associated with sorghum beers. It will increase the germination energy and the defensive role of peroxidase against lipid peroxidation during malting. Conversely, the alkaline steep with final warm steep had an inhibitory effect on the development of peroxidase during malting. The PH and thermostability of partially purified and crude peroxidase are significantly within the reported range of 5.5-5.2 and 47.0- 50.0 °C. It was suggested that this work can offer a straightforward and practical method for enhancing catalytic activity and offer a crucially new viewpoint for researching and directing the development of peroxidase's malting potential.

Keywords: Alkaline steep, air-rest cycle, germination properties, malting, peroxidase and Sorghum

Introduction

Sorghum (*Sorghum bicolor* (L.) Moench) is the grain of choice to produce traditional cloudy and opaque beers throughout sub-saharan Africa. The key ingredient of these beers is sorghum malt, which provides hydrolytic enzymes (especially amylases) to ferment sugars into ethanol and carbon dioxide. Other enzymes produced in the process also include antioxidant enzymes like lipoxygenases, catalase and peroxidase. Sorghum is used for food, fodder, and the production of alcoholic beverages. It is both drought and heat tolerant, and is especially important in arid regions. Sorghum ranks fifth in the world cereal production, and as of 2008 the world annual sorghum production stood at 65.5 million tones Mundia, *et al*., 2019) [19]. It is an important food crop in Africa, Central America, and South Asia (Dabija *et al*., 2021; Gerik, Bean & Vanderlip, 2024) [4]. In some cases, Sorghum is used in the same way as barley to produce "malt" that can form the basis of a mash without gliadin or hordein and therefore suitable for coeliacs (Gumienna & Górna, 2020) [9].

Enhancing the brewing potential of Sorghum

The methods used to enhance the brewing potential of sorghum malt include manipulation of steeping sequence (alkaline steep treatment, air-rest cycle, cold and hot water extract and warm water final steep), appropriate cultivar selection, manipulation of germination time, germination temperature, kilning and mashing temperature and addition of exogenous enzymes (Onukogu, 2021; Ukwuru, 2010) [26, 22]. The germination temperature of about 25°C to 30°C seems to favour enzyme development, while Guzmán-Ortiz *et al*. (2019) [10] suggested that kilning grains in cycles of 45°C to 60°C tend to increase the number of enzymes than at a single temperature treatment. Mashing temperature of 65°C is generally used in mashing barley malt, but when sorghum malt was mashed at the same temperature the result was inadequate gelatinization of the starch and sub-optimal release of sugars even when commercial enzymes were added. However, at a mashing temperature of 85°C and above, sorghum starch was gelatinised effectively and sugars released into the wort were higher than at 65°C, and even higher when commercial enzymes were included at a very low rate. Although higher temperatures and added commercial enzyme preparations used in mashing sorghum malt dramatically increased the sugars released into the wort of sorghum mash, the ratio of glucose to maltose did not change. An industrial exogenous enzyme such as amyloglucosidase contributes more to the release of reducing sugars into the wort during mashing. For more sugar yield in the wort during yeast fermentation industrial amyloglucosidase was recommended as enzyme source (Serna-Saldivar & Rubio-Flores, 2017) [30]. Of course, there are other enzyme sources that can be used but not for wort sugar quality. For stabilization of lipid component of the wort because of the role of lipid in sorghum beer production peroxidase, catalase and lipoxygenase were recommended.

Role of antioxidant enzymes in beer stability

As part of the physiological protection against various forms of toxicity caused by reductive forms of oxygen, living cells synthesize and use a number of enzymes referred to collectively, as oxygen radical scavenging enzymes or simply, antioxidant enzymes. These enzymes include superoxide dismutase (E.C. 1.15.1.1), catalases (1.11.1.16) and peroxidase (E.C.1.11.1.7). Superoxide dismutase (SOD) catalyses the dismutation of superoxide radicals to ground state oxygen and hydrogen peroxide (Case, 2017) [3]. The potential stages in brewing where oxygen radicals might be formed include malting, kilning and mashing (Koren *et al*., 2019) [16]. However, the products of their oxidative reactions with malt lipids survive in the finished beer and contribute to loss of flavour stability during storage. All the three antioxidant enzymes have been demonstrated in barley (Baldus *et al*., 2021) [1]. Their activity in barley increases during malting and they help limit the rate of oxidation reactions occurring at this and later stage of brewing. Although the role of all these enzymes is important for long shelf- life of beer, only peroxidase has been shown to survive the heat treatments of kilning because of its high thermostability.

Rational for the work

Lipid peroxidation is undesirable in malting and brewing because the products from the reaction, namely the hydroperoxides and their decomposition products, the aldehydes affect the availability of wort nutrients and affect the beer flavour and colloidal stability. Among the three antioxidant enzymes (superoxide dismutase, catalase and peroxidase) peroxidase is reported to have survived the kilning process due to its high temperature stability (Nwanguma and Eze, 1995) [22]. The survival of peroxidase during the kilning stage demonstrates that its antioxidant function or role is guaranteed in the wort, thereby, contributing to the enhancement of the shelf life of beer. Again, scientists continue to research cheap and alternative source of enzyme for wild scale industrial applications. The study of sorghum peroxidase serves the two broad functions: as a possible cheap source of peroxidase and to promote the longevity of commercial beer.

Aim

Steeping sorghum in 0.1N NaOH (alkaline steep) and the introduction of air-rest cycles have been recommended for enhancing or improving the malting parameters of different sorghum varieties.

Before these modifications, standard steeping methods received widespread application. The need to determine their effect on a number of other processes that take place during malting becomes necessary.

Thus, the aim of this study was to determine the impact of bio-stimulants such as alkaline steep and air-rest cycle on the development (activity) of peroxidase during malting of sorghum. The interest in peroxidase is based on an earlier report that its activity is important in controlling lipid peroxidation during malting and mashing. The adopted air-rest cycle and alkaline steep regimes were those already recommended for commercial scale malting because of their desirable effect on some malting properties of sorghum.

Methods

Steeping methods

The alkaline steep and air-rest cycle methods used were those suggested by Okolo and Ezeogu (1996) [25] in their study.

The steep regimes are: Steep regime I (alkaline steep): The test grains (500) were steeped in alkaline water (0.1% sodium hydroxide solution) at pH 13.25 for 8 h at 30°C. It was followed by air-rest cycle of 4 h dry; 6 h wet for 40 h with final steep temperature maintained at 40°C for 6 h. The control experiment for regime 1 had distilled water at pH 5.97 instead of alkaline water.

Steep regime II: The test grains (500) were steeped in 40ml of distilled water at 30°C for 24 h; it was then subjected to a steep cycle of 10 h wet and 1 h air-rest. In the control experiment a continuous steep was applied.

In the germinative measurement tests: Evaluation of water sensitivity and germination energy were done according to the method described by Okolo and Ezeogu (1996) [25]. Average root length and malting loss were performed in line with Ogbonna, Obi and Okolo (2003) [24] and Okolo and Ezeogu (1996) [25] respectively.

Enzyme assay

Sorghum Peroxidase was extracted, partially purified and assayed according to the instructions provided by Nwanguma and Eze, (1995) [22] and as modified in Nnamchi *et al*. (2022) [20]. Protein determination and reagent preparation were consistent with Lowry *et al*., (1951) [17].

Statistical analysis

$$\bar{x} = \frac{\sum x_n}{n}$$

Where:

- $\bar{x}$ is the sample average of variable x.
- $\sum xn$= sum of n values.
- n = number of values in the sample.

Results

Table 1: Germinative properties of KSV8

Regimes	Germination Energy (%) Germinative Energy (%)	Germination Capacity (%) Germinative Capacity (%)	Water Sensitivity (%) Water sensitivity (%)	Malting loss (%)	Malt yiel (%)	Root lengt (cm) (%)
Regime (test 0.1N NaOH	92.0 ± 2.87	98.0 ± 0.57	6.0	20.1 ±0.93	79.9	1.3 ± 0.47
Regime I Control	89.0 ± 0.71	96.0 ± 1.14	4.0	20.0 ±1.28	80.0	1.4 ±0.28
Regime II Test	95.0 ± 1.41	99.0 ± 0.0	6.0	5.6 ± 1.28	94.37	1.5 ±0.07
Regime II Control	95.0 ± 0.57	97.0 ± 1.15	5.0	15.84± 0.19	84.16	2.44± 0.54

a. Results are means of triplicate trials
b. Calculated as 100 % - malting loss (%)

The germination energy (GE) and germination capacity (GC) of the seeds steeped in 0.1N NaOH were considerably high with values of 92.0 ± 2.87 and 98.0 ± 0.57 respectively. The water sensitive test for the sorghum seed was found to be low as well as malt yield and root length.

The germination properties of sorghum variety KSV8 steeped in distilled water in regime I (control) showed that the germination energy and germination capacity of the sorghum seeds were considerably high and are 89.0 ± 0.71 and 96.0 ± 1.14. Yield malt was low (80.0) and most of the malts were lost in form of root length or used in root growth.

The germination properties of sorghum variety KSV8 steeped in distilled water for 10 h and exposed to 1 h air-rest cycle is shown in regime II (test). Results obtained showed that the germination energy (95.0 ± 1.41) and germination capacity (99.0 ± 0.00) of the sorghum seeds were considerably high as well as malt yield. However, malting loss (5.6 ± 1.28) and root length (1.5 ± 0.07) of the sorghum seed were low.

The sorghum seed in the control experiment (regime II) had high germination energy (95.0 ± 0.57) and high germination capacity (97.0 ± 1.15). Malting loss of 15.84 ± 0.19 was recorded in the control. Similarly, the root length had a value of 2.44 ± 0.54. The result also showed there was a decrease in the yield malt.

Results of Enzyme activity over time

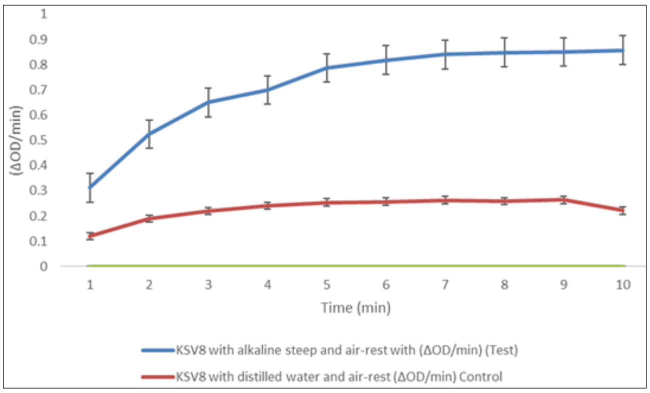

Fig 1: Peroxidase activity for alkaline and warm steep at 40°c for regimes I. Results are means of triplicate trials

Figure 1 represents the plot of the effect of alkaline steep on the development and levels of peroxidase activity at the end of steeping. The level of peroxidase activity in the seeds steeped in 0.1N NaOH (alkaline steep) demonstrated a much higher level of activity than the seeds steeped in distilled water (control).

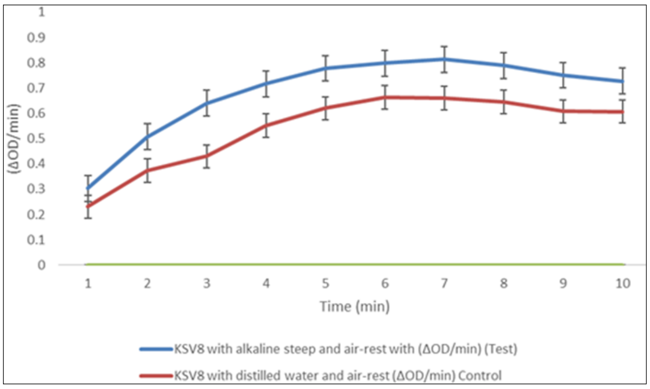

Fig 2: Peroxidase activity at the end of 24h germination for regime I. Results are means of triplicate trials

Figure 2 shows the level of peroxidase activity at the end of 24 h germination (Regime 1). From the result, at the end of germination for 24 h, the difference between the levels of peroxidase activity in the test (0.728) and the control seeds (0.607) had narrowed down

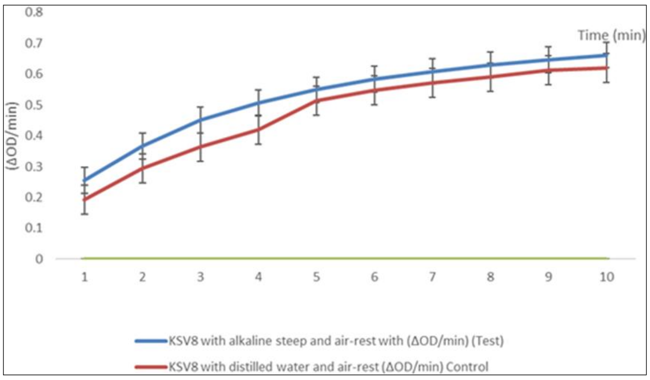

Fig 3: Peroxidase activity at the end of 48h germination for regime I

Figure 3 shows the effect of alkaline steep on the levels of peroxidase development at the end of 48 h germination. The results showed that the levels of peroxidase activity in the test (0.662) and the control seeds (0.620) had also narrowed down. Peroxidase activity in the control seed was high compared to peroxidase activity in the test seeds.

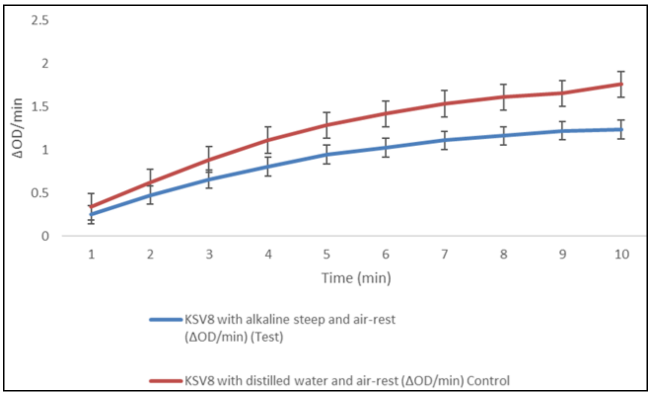

Fig 4: Peroxidase activity at the end of 72h germination for regime I. Results are means of triplicate trials

Figure 4 represents plot on the effect of alkaline steep on the development of peroxidase at the end of 72 h germination. From the result, the control seeds demonstrated a higher level of peroxidase activity (1.764) than the test seeds (1.239).

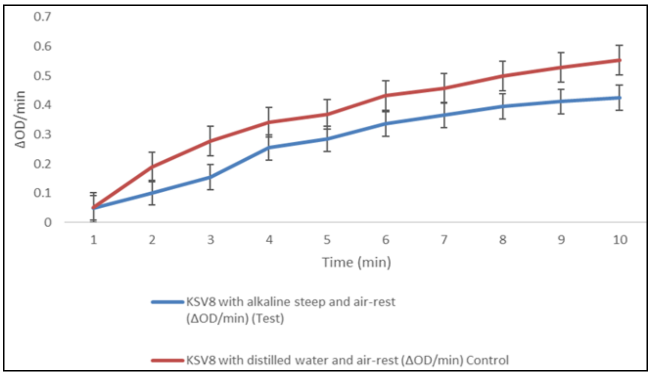

Fig 5: Peroxidase activity at the end of kilning for regime I. Results are means of triplicate trials

Figure 5 shows peroxidase activity at the end of kilning at 60 °C for 7 h. From the results, there was a variation in the levels of decrease in peroxidase activity in the test seeds and the control seeds. At the end of kilning, the level of peroxidase activity in the seeds (0.425) was much lower than the level in the control (0.553).

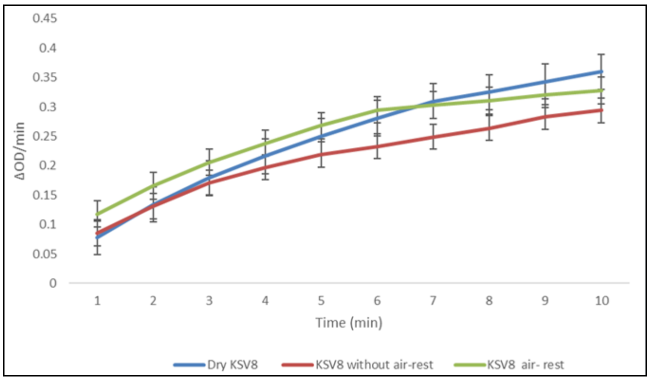

Fig 6: Peroxidase activity at the end of 24 h of steep for regime II. Results are means of triplicate trials

Figure 6 shows the effect of air-rest cycle on the level of peroxidase activity in the sorghum malt at the end of 24h steep. From the result, peroxidase activity in the test seeds demonstrated a slightly higher activity when compared to that in the control seeds.

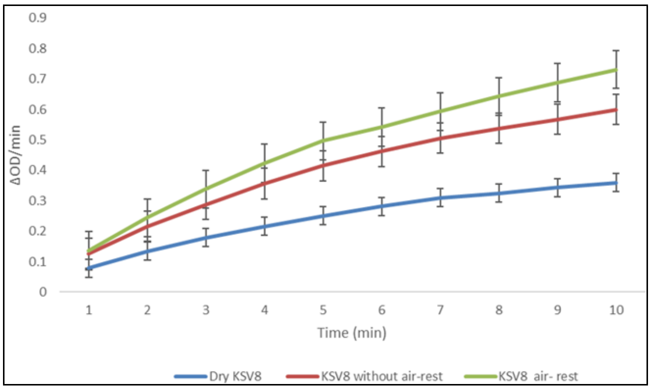

Fig 7: Peroxidase activity at the end of 24 h of germination for regime II Results are means of triplicate trials

Figure 7 shows the effect of air-rest cycle on the level of peroxidase activity at the end of 24h germination. From the results, peroxidase activity in the test seeds and the control seeds had increased considerably. However, peroxidase activity in the test seeds were slightly higher than peroxidase activity in the control seeds.

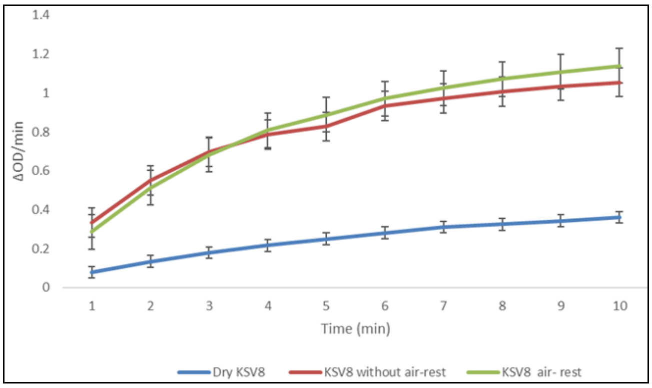

Fig 8: Peroxidase activity at the end of 48h germination for regime II. Results are means of triplicate trials

Figure 8 represents the plot of the effect of air-rest cycle on the level of peroxidase activity at the end of 48 h germination. The test seeds and the control seeds demonstrated appreciable level of peroxidase activity overtime. There was a slight difference in the level of peroxidase activity between the test seeds (1.055) and the control seeds (1.139)

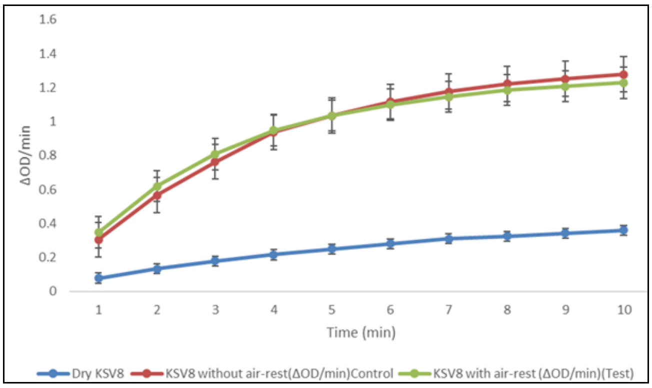

Fig 9: Peroxidase activity at the end of 72 h germination for regime II. Results are means of triplicate trials

From the results the level of peroxidase activity in the control seeds had increased appreciable (1.279) with that in the test seeds (1.229).

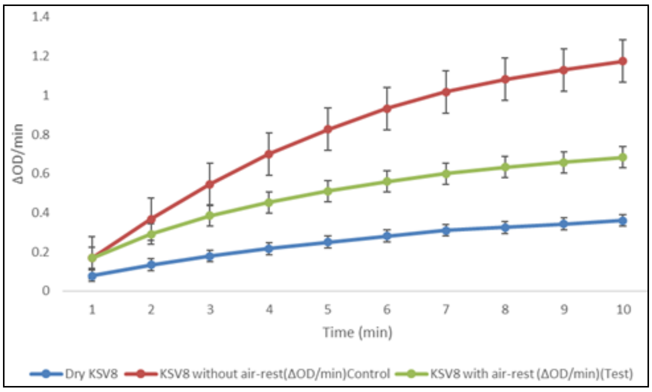

Fig 10: Peroxidase activity at the end of kilning for 7 h for regime II. Results are means of triplicate trials

Figure 10 shows the effect of air-rest cycle on the level of peroxidase activity at the end of kilning. The results showed that the peroxidase isolated from both the control and the test seeds survived kilning. However, much lower level of peroxidase activity was observed in the test seeds

Discussion of results

This present study showed that the cultivar KSV8 had good germination energy as shown in Table 1-4. The results are within the acceptable range of (> 95%) prescribed for malting grains. In the control part of regime I, germination energy was recorded as (89% ±0.71) and it was below the recommended value which may be attributed to grain size and storage methods. Critically, planting and harvesting time are also factors that can influence malting quality, especially when it has to do with improved malt quality. The depth and complexity of these remains insufficiently understood notwithstanding, the observed differences in germinative properties from crops grown in different environments are complex to explain, and their interpretation might represent an opportunity to improve other varieties of sorghum. For instance, the high value recorded in the alkaline steep (regime I) such as high germination capacity of (98± 0.57 %) for test sample and a value of (96±1.14 %) for the control. Germination capacity (GC) was also high in regime II (distilled water steep with air rest) while the test recorded a value of (99 ± 0.00 %). On the other hand, the control had a value of (97 ±1.15 %). Based on these results and works by different researchers, it can be stated that the variation in germination properties recorded were functions of conditions surrounding the malting of the sorghum variety especially the protein content and the storage method used for KVS8. The complexity of their growth is generally influenced by internal and/or agroclimatic nature of KVS8 growth. There is a debatable possibility here, it is possible that the composition of the cotyledon and endosperm played a role. If more of the nutrient is sourced for the development of is embryonic leaf and root of the seeds, then high malting loss will occur. Conversely, in the seeds that had high germination capacity the nutritive tissue, which stores nutrients required by the development of embryo during seedling were properly used. Malting loss in regime 1 and Alkaline steep can also be attributed to what is happening within the seed coat of sorghum malt. If there is a leak within the seed coat of the sorghum malt it is then likely that protection of the inner embryo from physical or microbial damage is impossible or limited hence the lower yield in form of malting loss. This is because some seedling physical or microbial damage are minor and may not be noticed with physical eyes.

Furthermore, physiologically, sorghum malt contains significantly lower levels of salt soluble proteins which influence some of the biochemical changes that take place during malting. Therefore, the possibility of enhancing the enzyme activity through improvement in malting methods such as alkaline steep and air rest cycle is necessary. Irrespective of the steep treatment applied, the chiting ability of the grains was suppressed by alkaline steeping. This trend was more pronounced in regime 1 control (alkaline steep) which showed more sensitivity to regime change than the other steep treatment. Moreover, grain germinability was high in alkaline steep and in the distilled water steep, since the grains were of the same cultivar it is possible that steeping pre-history and air-rest cycle had influenced seed germinability. 95% germination energy could be due to possible seed contamination during malting and perhaps the methods used to store the seeds after harvest.

The grains were not water sensitive (Ws) (Table 1-4) possibly because of the cultivar type, season of grain harvest and method of preservation. But most importantly, the grains were designed to survive in drought conditions so are capable of withstanding exogenous stress. However, malting loss was higher in regime I (alkaline steep) in both the test and the control (20.0 ± 0.93 %; 20 ±1.28 %) respectively. A higher value of malting loss was also recorded in regime II (distilled water steep) control (15.84 ± 0.19 %), while the lower value for the test (5.63 ± 1.28 %) would be attributed to the impact of air-rest cycle on the malt. The 5.63% malting loss observed in the test was low because an average of 10 -15 % respiration / metabolic loss is expected in a well malted sorghum with good diastatic power according to Sawadogo-Lingani, Owusu-Kwarteng and Jespersen (2023) [29].

The average root lengths in regime I (alkaline steep) were short, and this could be due to the effect of alkaline steep. Alkaline steep liquor is known to suppress root development, causing short root lengths as observed in regime I (alkaline steep). This is consistent with Juan Li *et al*. (2015) study based on soil condition. The mechanism underlying alkaline stress-mediated inhibition of root elongation remains a complex but ongoing study. The short root length also means that the malt if planted is likely not to survive the drought condition of African soil. In regime II (distilled water steep) root length was observed to be more developed (longer) than that for alkaline steep. Data observed in this study (of Table 1-4) suggest that the introduction of air-rest cycle during sorghum steeps would be highly beneficial in the reduction of malting losses of sorghum malts.

Enzymatic activity over time

Dry KVS8 demonstrated significant peroxidase activity to necessitate the continued testing of the various steeping conditions. At the end of 40 h steep peroxidase activity for the alkaline steep (test) was higher compared to the control (figure 1). Peroxidase activity in regime 1 control was well below that of regime 1 (test) which is attributed to the degree of germination suppression by final warm steep even though, both the text and the control had the same pre-steeping history.

For regime II (distilled water steep), at the end of 24 h steep, peroxidase activity decreased (control: no exposure to air-rest cycle) and was lower than that of the test (test: exposed to air-rest cycle of 10 h wet and 1h dry). This is possibly because steeping causes physical and biochemical changes that hasten grain modification and the effect of air-rest cycle on water retention capacity of the grains. This possibly may have led to the decreased peroxidase activity observed in the control seed (figure 6).

Peroxidase activity decreased at the end of 24 h germination in regime I (test) but maintained high activity compared to the control (figure 2). This is suspected and may be as a result of the suppressive effect of alkaline steep, whereas regime 1 control showed increase in peroxidase activity. Peroxidase activity increased steadily for both regime II (control: not exposed to air-rest cycle) and for the test (exposed to air-rest cycle) (figure 7). This was also in line with an earlier report by Owuama (1997) who demonstrated that peroxidase activity increases by about 14-fold during germination of grains steeped at 30°C for 24 h without air-rest cycle. The stress factor associated with alkaline at this stage of sorghum development could point to the diminished scavenging role of peroxidase. It is also possible that the reactive oxygen species (ROS) constantly generated in various metabolic activities of plant (Van Breusegem & Mittler, 2023) [34]. In malted sorghum grain it could have led to more production of peroxidase, but the converse is true. The imbalance in peroxidase production could have led to more oxyradical generation, protein and other macromolecular oxidation that affect endospermic and angiosperm changes in malted grains.

At the end of 48 h; in the control part of regime 1(alkaline steep), it continued its progressive increase in peroxidase activity. This is surprising despite the alkaline suppression of root length, this increase could suggest that a huge deposit of the peroxidase did not come from the root length but in the endosperm. Another possibility to this can be the degree of imbibition. It is known that imbibition reactivates the dormant seed and initiates metabolic activities therefore could have facilitated protein and enzyme synthesis (figure 3). When this finding was compared to the test result of the same regime which showed decreasing peroxidase activity. This decline in activity was ascribed to a fast rate of respiration, moisture and ion uptake engineered presumably by air-rest cycle. The exposure of seed to air-rest cycle on its own could attract bioactive agents that shaped the enzymatic activities observed. Similarly, this effect was also observed in diastatic activity where the extent of repression for ICSV400 was 9 % (Okolo and Ezeogu 1996) [25]. This repression effect could also be attributed to the fact that any character that influences protein binding properties of a molecule also influences its activity in other biochemical processes. The possibility exists that the differences in the capacity of alkaline steep liquor to influence protein binding properties of polyphenol /tannins is cultivar dependent and might have contributed to decreased peroxidase activity. KSV8 has high polyphenol compared to other sorghum cultivars (Valera Sanchez, Lima & da Silva, 2023) [33]. However, distribution and concentration of protein binding complexes in the grain is also species dependent. Polyphenols are known to inactivate a number of grain enzymes (Kageruka *et al*., 2024) [15] within the pericarp and testa. The possibility exists that these polyphenols might have penetrated the endosperm with the imbibed water. According to study by Nwanguma and Eze, (1995) [22] peroxidase in endosperm were classified as 44 % for acrospires and the rootlet had 56 % of peroxidase activity. Based on this, polyphenol may have complexes with reserve seed and enzyme proteins to effect repression of enzyme synthesis and subsequent inactivation of already synthesized enzymes; but the effect and degree of this is not known (Nwanguma and Eze, 1995) [22].

At the end of 48 h, regime II (distilled water steep) test and control: peroxidase activity increased steadily (figure 8). This might be due to an increase in protein and enzyme synthesis that increases as germination time increases.

At the end of 72h germination, peroxidase activity in the control continued to increase and surpassed that of regime 1 (test) which demonstrated increased peroxidase activity at the end of 72 h (figure 4). This sudden increase at the end of 72 h for regime1 (test) is perhaps as a result of increased concentration of the imbibed ion, the rate of formation of protein complexes and time dependent effect of ion protein binding. It is also possible that peroxidase protein structural integrity has been repaired. Furthermore, a possible depletion in the suppression effect of final warm at 40°C for 6h could be attributed to this sudden increase in peroxidase activity. It is also possible that the effect of germination times on the development of proteins and enzymes synthesis may have led to the change in peroxidase activity. Increased peroxidase activity was also observed in regime I control because it was susceptible to all the factors that contributed to favoring peroxidase function and synthesis.

In regime II (distilled water steep) peroxidase activity increased in both the test and the control but was higher in the control relative to the test (figure 9). This might be due to the degree of imbibitions and sufficient moisture uptake. The level of oxygen uptake may have led to increased peroxidase development. These possibilities are also influenced by the degree of air-rest cycle the grains were exposed to.

At the end of kilning, peroxidase activity in regimes I and II decreased as expected (figure 5 and figure 10). This is because proteins generally denature when they are exposed to high temperature. It is therefore possible that some of the peroxidases were denatured at this stage. In regimes I and II, the peroxidase that survived the heat treatment were more in the control compared to the test. This perhaps could be due to the nature of the steeped liquor and its physical properties such as vapor density, evaporation temperature, etc. Heat effect on the ions of the steeped liquor and their likely interaction with peroxidase proteins could also be a contributing factor. It was also possible that some of the denatured proteins regained their conformational integrity afterwards. The survival of peroxidase at this stage (kilning) suggests that peroxidase activity may be guaranteed even at the mashing stage of beer production.

Peroxidase properties

The pH and thermostability of partially purified and crude peroxidase are significantly within the reported range of 5.5-5.2 and 47.0- 50.0 °C. Protein content for the crude and partially purified enzyme followed the lowly curve.

Conclusion

From the present study, introduction of air-rest cycle as malting condition would be beneficial to brewers during malting development of peroxidase. This is because the study suggested, as were earlier studies, that alkaline steep and air-rest cycle reduces malting loss associated with sorghum malts used for beers. It increased the germination energy and guarantees the defensive role of peroxidase against lipid peroxidation during malting. Conversely, the alkaline steep had an inhibitory effect on the activity of peroxidase during malting. Heat treatment of dry sorghum and dry sorghum peroxidase test showed divergent activity levels marking it as one of the know thermostable enzymes. So, its use for immobilization and industrial scale experiments were reinforced. The value of peroxidase cannot be overemphasized in brewing industry notwithstanding, the mechanism of most of these are yet to be fully understood. The genetic modification of sorghum variety continues in Nigeria for a reduced dependent in imported barley. Researchers will continue to experiment on this conditions for better quality beer.

Recommendation/ Suggestion for further studies

Since the results had shown that air rest cycle increases peroxidase activity during malting. It is also important to look at its effect in other antioxidant enzymes such as catalase and superoxide dismutase; so as to employ it effectively in reducing lipid peroxidation. The inhibitory effect of alkaline steep should also be investigated in other antioxidant enzymes since the nature and mechanism of these enzymes vary.

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