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Effect of Different Steeping and Malting Regimen on the Amylolytic Activities of Some Improved Nigerian Sorghum Grain Varieties

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Authors' contributions

This work was carried out in collaboration among all authors. Authors CIN and UPA designed the study. Author TSE performed the laboratory procedures supervised by authors CIN and UPA who also performed the statistical analyses. Author OCA wrote the protocol. Author CIN wrote the first draft of the manuscript. All the authors contributed to the analyses and literature searches of the study. All authors read and approved the final manuscript.

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ABSTRACT

Three Nigerian improved sorghum varieties were evaluated to ascertain how different steeping and malting regimen affect their amylolytic enzyme development. Steeping incorporated air rest and continuous steep regime for 72 h. Samples were withdrawn every 12 h. Germination was then carried out for four days before kilning at 50°C for 24 h. Grain and malt parameters were examined. Results obtained showed variations in the response of sorghum root length to steep regimen and time. CSR-02 gave maximum root length (3.32 cm) after 72 h of air rested steeping. CSR-02, Samsorg 44 and Samsorg 14 had germinative energies of 92.00 ± 4.24, 94.00 ± 1.41 and 96.00 ± 1.41%; germinative capacities of 91.00 ± 1.41, 75.50 ± 2.12 and 88.00 ± 2.83; water sensitivities of 6.50 ± 2.12, 13.50 ± 1.44 and 1.00 ± 0.41 respectively. TKW results were 29.73 ± 0.32, 33.85 ± 1.54 and 33.51 ± 0.41 kg for CSR-02, Samsorg 44 and Samsorg 14 respectively. Variations in the response of the sorghum varieties to various conditions of steep regime and steep period were also observed. Steeping for 48 h seems to be the optimum time for the development of

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amylolytic activity in all the sorghum varieties at both steeping regimens. Samsorg 14 gave the highest total amylase activity (355.44 µg glucose equivalents), followed by Samsorg 44 (278.08 µg glucose equivalents). Samsorg 14 also showed the highest α-amylase development (276.93 µg glucose equivalents). Air rest was found to be show greater effect on β-amylase development in all the sorghum varieties.

Keywords: Amylase; cereals; malting; sorghum; steeping.

1. INTRODUCTION

Sorghum is a traditional African cereal that is well adapted to the semiarid drought-like Sudan-Sahelian conditions prevalent in many parts of Africa [1]. Just like rice and barley, sorghum belongs to the family of Gramineae and is ranked fifth both in terms of production and area planted compared to other common cereals [2]. Approximately 90% of global sorghum is cultivated in developing countries with Africa and Asia having the highest production rate [3]. Sorghum has a very high content of starch, protein, minerals and vitamins and therefore serves as a major food source consumed by both humans and animals for many people residing in many developing countries of Africa and Asia [4,5]. With that richness sorghum produces highly nourishing wort which explains increasing interests in making it one of the choice raw materials in the brewing process [6]. Despite the fact that sorghum has been used for centuries to brew traditional (opaque) beer in Africa, it was only in recent years that the brewing of sorghum beer was development and adapted to use in modern breweries. To a large extent that adaptation started in Nigeria following the ban on importation of barley due to scarcity of foreign exchange prevalent then. This gave rise to the efforts that today are responsible for the use of sorghum in brewing [7].

An essential part of beer production is malting during which cereal grains are made to germinate and quickly dried before further development occurs [8]. This malting process results in the synthesis of amylolytic enzymes that will degrade complex molecules into low molecular weight compounds. These enzymes contribute to the diastatic power (DP) or ability to convert malt starch or adjuncts into fermentable sugars is a major criterion for sorghum malt quality and its application in brewing processes [9].

A critical part of the malting process is steeping, which simply means the soaking of grains in water. Steeping is known to be a key factor, if not the most important stage in the malting process of cereals [10,11]. The significance of steeping has to do with the fact that it is the point of initiation of germination which thereupon induces the activity of endosperm modification enzymes especially amylases, proteases and similarly working enzymes. These enzymes and their activities then ultimately determine how the cereal endosperm modification will occur especially as it concerns the production of the desired malt [12]. Considering that it plays such a critical role in the malting process, we sought in this work to ascertain how different steeping and malting regimen affect the development of amylolytic enzymes in three improved Nigerian sorghum varieties.

2. MATERIALS AND METHODS

2.1 Sorghum Sourcing, Sorting and Cleaning

The sorghum cultivars (CSR-02, Samsorg 44 and Samsorg 14) used in this study were obtained from the Institute of Agricultural Research, Ahmadu Bello University, Zaria. The grains were carefully and thoroughly sorted to remove foreign materials and broken kernels before being surface sterilized by immersion in 1% sodium hypochlorite and allowed to stand for a few minutes to ensure that only the good grains were used. Thereafter, the grains were thoroughly rinsed with tap and distilled water and placed evenly on a clean surface layered with soft absorbent paper and allowed to dry at room temperature overnight.

2.2 Sorghum Steeping, Germination and Kilning

Steeping was done by measuring out 300 g of each sorghum variety and immersing each of them in 600 ml of distilled water (to give a grain-water ratio of 1:2). Two types of steeping, with air-rest and without air rest (continuous steeping) were carried out with the steep water changed after every 12 h. Steeping was done for 72 h at 30°C with consistent withdrawal of 50 g of the sorghum samples after every 12 h.

The withdrawn samples were germinated for four days in the dark. This was achieved by embedding the grains in a thick jute bag placed inside a wooden chamber that has been previously cleaned and sterilized using a hypochlorite solution. The grains were sprinkled with water at regular intervals (morning and evening) to maintain a humid environment and mixed and carefully turned to prevent excessive root matting and maintain uniform temperature and moisture levels.

At the end of germination, the grains were dried (kilned) in an oven set at 50°C for 24 h. Shoots and rootlets were manually removed. The sorghum samples were milled to fine powder and kept in an air tight container at -20°C until needed for assay.

2.3 Grain and Malt Analyses

Thousand kernel weight (TKW) was determined by manually counting 1000 grains and weighing them afterwards [2]. Germinative capacity which measures the germinability (ability to germinate) of grains was measured by soaking one hundred sorghum kernels for 48 hours in 100 ml of 0.75% fresh hydrogen peroxide at room temperature (25-28°C) followed by additional re-soaking in fresh hydrogen peroxide for additional 24 hours at the same conditions. Germinative energy a measure of the number of grains that will germinate as an index of seed viability was done by placing 100 sorghum kernels of each variety on a filter paper in a Petri dish to which 4 ml of water had been added and counting the number of chitted grains. Water sensitivity was done by replacing the 4 ml above with 8 ml of water and noting the difference in terms of number of chitted grains between them (in %). All the analyses listed above were carried out using the Recommended Methods of the Institute of Brewing [13]. For the determination of the average root length of the grains, the Institute of Brewing Recommended methods of analysis [14] were used. Briefly, 20 germinated sorghum kernels were selected randomly and their root lengths measured using a ruler.

2.4 Enzyme Extractions and Analysis

Generally, the extraction of enzymes studied in this work was done with 0.16 g of milled sorghum in 10 ml of sodium acetate buffer (pH 5.7) containing 50 mM Na-acetate, 100 mM NaCl, and 10 mM $CaCl_2$. Extraction was with shaking for 2.5 h, followed by centrifugation at 3000 × g for 10 min. The supernatant which was used for enzyme assays were stored at -20°C.

2.5 Malt Total Amylase Activity

The total amylase action on soluble starch was determined according to Nelson and Somogyi procedure [15,16]. In this procedure, 0.25 ml of diluted extract was transferred into four tubes (A, B, C and D), each containing 0.25 ml of assay buffer (0.05 M sodium acetate buffer, pH 4.6). The contents were then incubated for 5 min at 30°C after which 0.5 ml of 1% soluble starch solution in assay buffer was added and further incubated for 15 min at 30°C. The reaction stopped by the addition of 1 ml of mixed Nelson reagent into tubes A, B and C. 0.5 ml of assay buffer was however added to the fourth tube, the enzyme control tube (tube D) in place of 1% starch solution with 1 ml of mixed Nelson reagent added almost immediately. A blank containing 1 ml of assay buffer and 1 ml of mixed Nelson reagent was also prepared. The reaction mixtures was boiled at 100°C for 25 min in a water bath, cooled to room temperature before 1 ml of arsenomolybdate reagent was added. It was then diluted with 7 ml of distilled water, vortexed and absorbance read at 565 nm using an S-20 spectrophotometer. The amount of reducing sugar formed was determined using a glucose standard curve.

2.6 Malt α- and β-amylase Activities

The method described by Sun and Henson [17] was used in the determination of α-and β-amylase activities. In this procedure, β-amylase was selectively inhibited by placing 3 ml of the extracts into a water bath set at 70°C for 15 min. The extracts were immediately transferred into chilled water to stop further heat action. Both the heated and unheated extracts were assayed using the Nelson and Somogyi procedure [15,16]. β-amylase was then calculated as the difference between total amylase and α-amylase activities. One unit of enzyme activity was defined as any amount of enzyme which is capable of releasing 1 *μ*g glucose equivalent/min/ml under the assay condition.

3. RESULTS

3.1 Preliminary Examination of the Sorghum Varieties

Table 1 shows the results of some preliminary tests conducted on the sorghum varieties.

Samsorg 14 had the highest germinative energy (96.00 ± 1.41%), followed by Samsorg 44 (94.00 ± 1.41%) while the least was CSR-02 (92.00 ± 1.24%). With respect to germinative capacity, CSR-02 which had the least germinative energy exhibited maximum germinative capacity (91.00 ± 1.41%) when compared with the other sorghum varieties. Samsorg 44 was the most water sensitive (13.50 ± 1.44%) of the grains studied, followed by CSR-02 (6.50 ± 0.72%) while the lowest value was given by Samsorg 14 (1.00 ± 0.41%). Furthermore, Samsorg 44 and Samsorg 14 had similar thousand kernel weights (TKW) of 33.85 ± 1.54 and 33.51 ± 0.41% respectively. The least TKW was given by CSR-02 (29.73 ± 0.32 g).

3.2 Effects of Steeping Time and Regime on Root Length Development

The effects of steeping time and steep regime on the development of average root length for the three sorghum varieties is as shown in Table 2. The sorghum varieties exhibited differences in the response of their average root length to steep regime and steeping time.CSR-02 recorded the least (1.53 cm) and maximum (3.36 cm) root length after 36 and 72 h of air rested steeping respectively.

3.3 Effects of Steep Regime and Time on Amylolytic Enzyme Development

The effect of steep regime and steep period on the amylolytic activity of the three sorghum cultivars is as shown in Fig. 1. The results showed that the total reducing power of two sorghum varieties (Samsorg 44 and Samsorg 14) generally increased with increasing steeping time, reaching its peak after which differences in responses was observed. However, variations were observed with respect to CSR-02 sorghum cultivar. Samsorg 44 and 14 varieties had 48 h as their optimum steeping time in both continuously steeped and air rested grains. For instance, maximum values of 250.19 and 303.15 µg glucose equivalents were observed for Samsorg 44 and Samsorg 14 varieties in which air resting was incorporated into the steep regime. Higher values were however observed for continuously steeped grains, without the incorporation of air rest, with the peak values of 278.08 and 355.44 µg glucose equivalents being observed for Samsorg 44 and 14 respectively. For CSR-02 however, peak activity of 143.52 and 128.46 µg glucose equivalents was observed after 36 and 60 h of steeping continuously (without air-rest) and with air rested conditions respectively.

3.4 Effects of Steep Regime and Time on α-Amylolytic Enzyme Development

The effect of steep regime and steep period on the α-amylolytic activity of the three sorghum cultivars is as shown in Fig. 2. The results showed that the α-amylase activity of Samsorg 44 and Samsorg 14 sorghum cultivars generally increased with increasing steeping time. Like total reducing power, this was not the case with

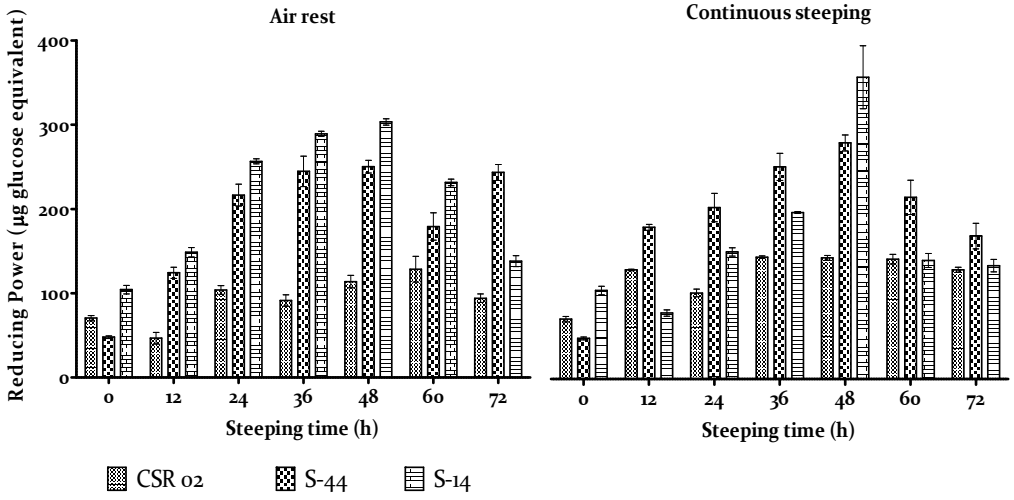

Fig. 1. Amylolytic enzyme development of CSR-02, Samsorg 44 and Samsorg 14 sorghum varieties as influenced by steep regime and Steeping time

Table 1. Grain quality parameters of the sorghum varieties studied

Parameters	CSR-02	Samsorg 44	Samsorg 14
Germinative energy (%)	92.00±1.24	94.00±1.41	96.00±1.41
Germinative capacity (%)	91.00±1.41	75.50±2.12	88.00±2.83
Water sensitivity (%)	6.50±0.72	13.50±1.44	1.00 ± 0.41
Thousand kernel weight (g)	29.73 ± 0.32	33.85±1.54	33.51±0.41

Table 2. Development of average root length (in cm) for the three sorghum varieties

Steeping time (h)	CSR-02		Samsorg 44		Samsorg 14	
	Air rest	Continuous	Air rest	Continuous	Air rest	Continuous
12	3.41	2.61	2.64	3.00	2.86	2.60
24	2.53	2.13	2.36	2.46	2.23	2.02
36	1.53	2.44	2.11	1.71	1.86	2.14
48	2.65	2.70	2.29	2.59	2.17	3.00
60	2.82	3.18	2.88	2.78	3.33	2.62
72	3.36	3.22	2.69	2.80	2.63	2.81

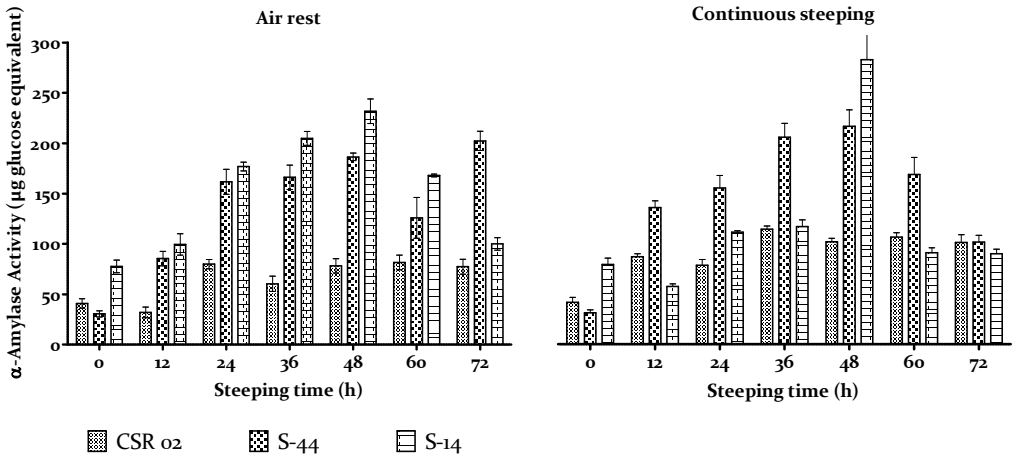

Fig. 2. α-Amylolytic enzyme development of CSR-02, Samsorg 44 and Samsorg 14 sorghum varieties as influenced by steep regime and steeping time

CSR-02 where variations in α-amylase responses were recorded. Enzyme activity for air rested grains peaked after 48, 60 and 72 h for Samsorg 14 (231.84 µg glucose equivalent), CSR-02 (81.58 µg glucose equivalent) and Samsorg 44 (202.29 µg glucose equivalent) cultivars respectively. However, maximum α-amylolytic enzyme development was recorded after 36 h of steeping for CSR-02 and after 48 h for both Samsorg 44 and 14 sorghum varieties. Continuous steeping of the three sorghum cultivars generally showed better α-amylase development at their optimum steeping time.

3.5 Effects of Steep Regime and Time on β-Amylolytic Enzyme Development

Fig. 3 shows the time course development of β-amylase activity in the three sorghum cultivars. The data obtained depicts that air resting improved β-amylase activity at their peak values in all the three sorghum cultivars. For CSR-02, this was achieved after 60 h of steeping. However, varieties Samsorg 44 and 14 showed optimal β-amylase activity after 36 h of steeping incorporating air rest into the steep regime. At peak diastatic activity, β-amylase constituted

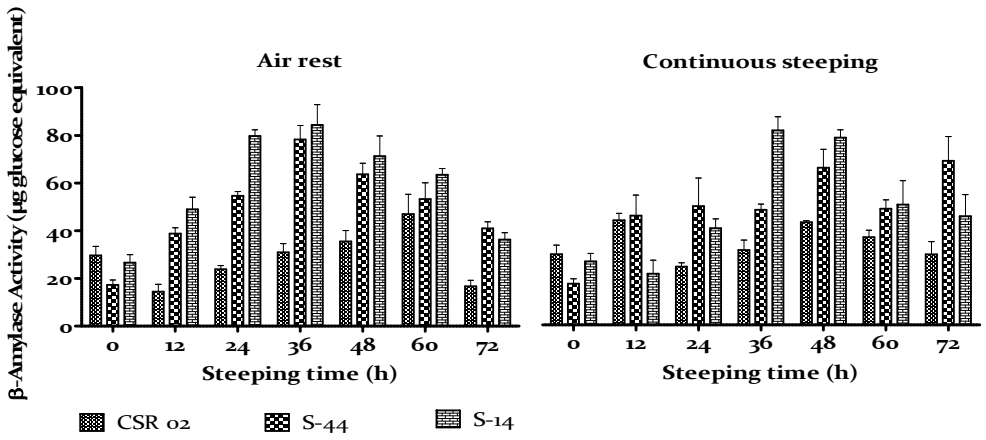

Fig. 3. β-Amylolytic enzyme development of CSR-02, Samsorg 44 and Samsorg 14 sorghum varieties as influenced by steep regime and steeping time

23.71, 22.09 and 21.89% of total reducing power for Samsorg 44, 14 and CSR-02 cultivars, respectively.

4. DISCUSSION

The three sorghum varieties investigated had good germinative energies as there was no tendency of dormancy (Table 1). CSR-02 cultivar had a germinative capacity of 91.00 ± 1.41%, followed by Samsorg 14 (88.00 ± 2.83%) while the least was Samsorg 14 (75.50 ± 2.12%). Samsorg 44 exhibited high water sensitivity values of 13.50 ± 1.44 whereas CSR-02 had values of 6.50 ± 0.72%. The least water sensitive of all the sorghum varieties studied was Samsorg 14 which had a value of 1.00 ± 1.41%. The thousand kernel weight of the sorghum grains, Samsorg 44 and 14 were 33.85 ± 1.54 and 33.51 ± 0.41 g respectively while the least value of 29.73 ± 0.32 g was obtained with variety CSR-02. The thousand kernel (or corn) weight is a measure of the size of cereal grains. Larger kernels are known to be more preferable to smaller ones because they tend to have higher extract yield and other positive attributes such as having less husk and therefore a higher starch content among others [2].

Sorghum has been reported by different authors to be used for various purposes, particularly in brewing where it is a key ingredient for the manufacture of alcoholic and non-alcoholic beverages [2,18,19,20]. Certain qualities are desirable if any cereal is to be used efficiently for brewing processes. A key desirable quality is whether the cereal has the ability to produce malts with high diastatic power with which it can breakdown starch molecules and produce reducing sugars [18]. As illustrated in the result, the diastatic activity of varieties Samsorg 44 and 14 generally increased with increasing steeping period, followed by a peak before a decline was recorded. This trend however was not observed with CSR-02 cultivar which was found to be the least amylolytic under the conditions employed in this study. Obviously, different sorghum varieties behave differently with respect to their optimum malting conditions. This fact may be responsible for the difference observed especially with this third variety, although further work may be needed to find out the conditions that will suit enzyme development in this cultivar. Possibly, alkaline steeping, which have been reported to improve the amylolytic potential of sorghum malts [21] may offer an opportunity to enhance the maltogenic powers of these sorghum varieties. Incorporation of air rest into the steep regime of Samsorg 44 and 14 varieties showed peak amylase activity of 250.19 and 303.14 µg glucose equivalents respectively after 48 h of steeping. However, when these same varieties (Samsorg 44 and 14) were steeped continuously for 48 h, they gave values of 278.08 and 355.44 µg glucose equivalents respectively, which showed that that steep condition was the most beneficial of all the steeping options, for total amylase development.

α-Amylases are of great interest in cereals because they are central to the degradation of carbohydrates located in the starchy endosperm.

Like total reducing power, the α-amylase activity of Samsorg 44 and Samsorg 14 varieties generally increased with increasing steeping time until the peak values were attained. As was the case with total diastatic power, variations in enzyme responses was also observed in the synthesis of α-amylases with variety CSR-02. In this cultivar, maximum α-amylase (112.10 µg glucose equivalents) was obtained after 36 h of continuous steeping. This value was however, far below what was obtained with the other sorghum varieties.

Air resting during steeping, was beneficial to the development of β-amylases although varietal differences were also observed. In Samsorg 14, the peak β-amylase activity (84.33 µg glucose equivalents) which occurred after 36 h of steeping with air-rest, was not significantly different when compared with their continuously steeped counterparts (81.61 µg glucose equivalents). Unlike continuous steeping of Samsorg 44 where maximum β-amylase activity occurred after 72 h, that of air rested steeped grains was noticed after 36 h of steeping. This probably implies that the use of air-resting during steeping could have the capacity of inducing enzyme development faster than continuous steeping. Cultivar CSR-02 was observed to show the least β-amylolytic expression under the conditions employed in this study. Therefore, possible means of enhancing its activity should be exploited by maltsters and brewers.

In general malting improved the expressions of the amylolytic enzymes studied in this work. Similar increases in the incidence of different enzymes due to malting of sorghum grains have also been observed previously, in respect of peroxidases [11,22], catalases [20] and also amylases [23].

5. CONCLUSION

Variations were observed in the response of three improved sorghum grains to steeping time and malting regime in this study. α-Amylase was found to be the main contributor to total diastatic power in all the varieties studied. From the results, continuous steeping, without air-rest, seemed a more suitable option for enzyme development. Steeping for 48 h was found to be the optimum time for the development of amylolytic activity in all the sorghum varieties at both steeping regimens. Of the three improved sorghum varieties, Samsorg 14 gave the highest total amylase activity. Another observation was that irrespective of the steeping time and regime employed, the synthesis of β-amylase remained low when compared to the other amylolytic enzymes assayed. It may therefore be necessary to seek further opportunities and options to improve the both the maltogenic powers and β-amylase activity of these sorghum varieties, such as by using alkaline steeping among others.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

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