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STEEP-LIQUOR TREATMENTS, GERMINATIVE ENERGY AND APPARENT DEGREE OF ATTENUATION OF THREE SORGHUM VARIETIES DURING MALTING AND BREWING

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ABSTRACT

The influence of steep-liquor treated with hydrogen peroxide, indole acetic acid and gibberellic acid on the germinative energy and apparent degree of attenuation of three sorghum varieties (SKV8, SKV4 and ICSV400) during malting and brewing was studied using standard methods. Samples steeped in the treated steep-liquors had 3.22 to 7.00 % improved germinative energy, which were significantly ($p<0.05$) different, more than the controls. Worts of the treated samples also showed 37.43 to 40.3 % improved apparent degree of attenuation, which were significantly ($p<0.05$) different, more than the controls during the five-day fermentation period. The results were also significantly ($p<0.05$) different, from the controls. Steep-liquor treatments however, influenced both the GE and ADA of the three sorghum varieties significantly ($P<0.05$) different along varietal lines. Thus, variety KSV8 steeped in H_2O_2 had the highest GE of 99.0 ± 0.10% while variety ICSV400 steeped in GA_3 had the lowest GE of 94.0 ± 0.16%. Similarly, wort samples from variety KSV8 steeped in H_2O_2 had the highest ADA of 70.0 ± 1.25% while those of KSV4 steeped in GA_3 had the lowest ADA of 31.7 ± 0.53%.

Keywords: Attenuation, brewing, fermentation, malting, modification, sorghum

INTRODUCTION

Malting which is the controlled germination of a cereal grain and involves steeping, germination and kilning has been found to be aimed at activating enzyme systems that catalyze the hydrolysis of polymerized food reserves, notably, proteins, starches, glucans and other cell wall materials into extractable fermentable materials (**Macleod, 1977; O'Rourke, 2004; Ogbonna, 2011**).
Steeping hydrates grains to a moisture level that will meet its water requirements for germination, enzyme production and migration through the multicellular endosperm complex (**Palmer and Bathgate, 1976)**. To produce high quality malt, the grain employed must have minimal post-harvest or storage dormancy and be able to germinate vigorously while during during germination the hydrolytic enzymes are developed to degrade the endosperm and cell wall materials to useful extract (**Palmer, 1983**). This degradation, which is technically referred to as modification, was reported by **Ogbonna (2011)** to be important and essential for the production of high quality malt.
Various methods which have been used to optimize the malting process include the steep liquor treatment. Steep liquor treatment involves the use of various substances and alkaline washes in steep-liquor to influence malt quality. These substances include Lime water [$Ca(OH)_2$] to check microbial growth and leach out some polyphenolic materials from the grain; Formaldehyde to reduce phenols and improve germination; Gibberellic acid (GA_3), a plant hormone, to accelerate all aspects of grain dormancy; Hydrogen peroxide (H_2O_2) to secure rapid and even germination and break dormancy; Indole acetic acid (IAA), another plant hormone, to promote cell wall enlargement and improved acrospire growth (**Palmer,1989; MacGregor, 1996; Dewar et al., 2001; Igyor, 2001; Berlin, 2002; Ogbonna, 2011**). The manipulation of steeping, germination and kilning conditions gives various types of malt such as pilsner malt, vienna malt, roasted malt, acid malt, roasted malt, brown malt, amber malt (**Kunze, 2004; Ogbonna, 2011**).
Furthermore, during fermentation the extract derived from malt is continually converted to metabolites (alcohol, esters, aldehydes, ketones, organic acids, etc.) by a pure yeast culture and the extent of the conversion is referred to as the *degree of attenuation* (V) which could be either apparent or real, depending on whether the measurement was done with or without alcohol in the medium (**Piesley and Lom, 1981; Kunze, 2004**). The degree of attenuation measured over a given period of time reflects the extent of fermentation. However, the proportion of *potential* fermentable extract present in the wort and the extent to which particular yeast can ferment it is known variously as the fermentation limit or attenuation limit or end of fermentation which determines the final attenuatin of beer (**Piesley and Lom, 1981**).
Traditionally, the cereal of choice for making malt is barley but other cereals like rye, wheat, rice, maize, and oat are also being used (**Osmanzi, 1992; Okrah, 2008**). However, the ban on the importation of barley malt in Nigeria in 1988 which was economically motivated triggered brewing-related researches on sorghum, centered mainly on its suitability and potentials as a possible replacement or alternative to barley malt. The objective of this study, therefore, was to investigate how different steep-liquor treatments influenced the GE (germinative energy) and ADA (apparent degree of attenuation) of three sorghum varieties during malting and brewing.

MATERIAL AND METHODS

Source of Materials

Sorghum bicolor (L) variety KSV4 was obtained from the Michael Okpara University of Agriculture, Umudike while varieties KSV8 and ICSV400 were obtained from the Agriculture Research Institute of the Ahmadu Bello University, Zaria. Champion Breweries Ltd, Uyo provided a slurry of pure yeast culture: *Sacharomyces carlsbergensis* (with a history of 3.0×10^8 cells/mL, 62 % solid content, 98.5% viability; and number of cycles: 3, as supplied by the brewery). The treatment substances: H_2O_2 (hydrogen peroxide) made by Unipharm Chemicals Ltd, England; GA_3 (gibberellic acid) and IAA (indole acetic acid) made by Aldrich Chemical Co. Ltd, England; were purchased from a chemical shop at Aba, Abia State, in Nigeria. The grains were cleaned, screened and graded before use. All other reagents used were of analytical grade. This study was carried out in the Quality Assurance Laboratory and the Fermentation Cellars of Champion Breweries Ltd, Uyo.

Sterilization of Materials and Pre-malting Test

All equipment and other tools used in this study were first washed thoroughly with soap, brush and water, rinsed with hot sodium metabisulphite solution (100 grams $Na_2S_2O_5$ in 1 Litre distilled water) before being soaked in sodium

hypochlorite solution (NaOCl: 1 % (v/v) available chlorine). Pre-malting GE test was conducted on the three sorghum varieties which served as controls. One hundred grains each was steeped in 4 mL distilled water on two rings of filter paper in a petri dish incubated in a cabinet for 72 hours (**IOB, 1997**).

Malting

Each sorghum varietal malt sample was produced according to a modified malting method of **Ogbonna** (**2011**). Three hundred grams each of each variety was first thoroughly washed with tap water three times before being completely immersed in three different steep liquors each treated with 0.5% of H_2O_2, IAA and GA_3 respectively at a grain/liquor ratio of 1:2 for 45 hours in three cycles, each of which comprised a 6-hour wet steeping period followed by a 3-hour air-rest and another 6-hour re-steeping period at ambient temperature which varied between 25-30 ºC on different days. Grain samples of the different varieties steeped in untreated liquor served as control. The chitted grains were germinated at room temperature and seedling growth lasted for five days on a card board in a humid atmosphere. The germinating grains were turned daily and sprinkled with water to keep moist. On the third day of germination, they were covered with a transparent nylon in order for a cushion of CO_2 to build up and reduce respiratory loss (**Kunze, 2004**). At the end of the germinating period, the grains that did not germinate were peeled over the germ area and incubated on moist filter papers contained in petri dishes for an extra day. Thereafter, five random samplings of hundred grains from each varietal sample were evaluated for GE as follows:

$$GE = \frac{W_1 - W_2}{W_1} \times 100\ \%$$

Where:
W_1 = Total number of grains sampled.
W_2= Number of grains that did not germinate.

This determination was done in triplicate and mean values calculated. Thereafter, the germinated grains were spread on white tiles and dried in a blast air oven (model KX 350A made by Kenxin Int., Ltd). Drying was carried out at 60 ºC to the recommended moisture content of 5% for malts (**Palmer, 1983; Hough, 1985**). Twelve malt samples were produced as follows:

- A: KSV8 variety treated with GA_3.
- B: KSV8 variety treated with IAA.
- C: KSV8 variety treated with H_2O_2.
- D: KSV8 variety control.
- E: KSV4 variety treated with IAA.
- F: KSV4 variety treated with GA_3.
- G: KSV4 variety treated with H_2O_2.
- H: KSV4 variety control.
- I: ICSV400 variety treated with IAA.
- J: ICSV400 variety treated with GA_3.
- K: ICSV400 variety treated with H_2O_2.
- L: ICSV400 control.

Mashing and Fermentation

Each of the twelve sorghum varietal malt samples was milled with a knife-edge manual hand-mill (Corona, made by Landers in Medellin, Colombia) and mashed to produce twelve sweet wort samples using a three-stage decoction mashing system (**Ogbonna and Egunwu, 1994; Ogbonna, 2011**).The mashes were lautered hot through a sterilized sieve cloth and boiled at 100ºC for a minimum of one hour to 10 % (g. 100 mL^{-1}) original gravity (OG) content at 20ºC, which was measured with a saccharometer. At the end of boiling, the hot wort samples were respectively swirled in a beaker using a glass rod and allowed to stand for 30 minutes for trubs to settle. The sweet worts were decanted, cooled to 12 ºC in stoppered one-and-half Litre fermenting flasks equipped with thermometers and sampling points and aerated with filtered sterile air for 10 minutes by bubbling method. The essence of stoppering the flasks was to prevent any escape of the sterile air which ensured that its optimum physical dissolution in the cooled wort was achieved. Thereafter, sweet wort (1000 mL) from each malt variety was pitched with 15 mL slurry of pure yeast culture (*Saccharomyces cerevisiae*) respectively at the rate of 0.015ml of yeast slurry per ml of sweet wort. A minimum of 25% of the flask volume was given for headspace (foam) development, and the pitched wort fermented for five days at normal atmospheric pressure in a regulated temperature environment (the Fermentation Cellars of Champion Breweries Ltd, Uyo) which operated at a daily mean temperature of 11.0 ± 1.0 ºC, using the cold fermentation style of **Kunze (2004)**. The daily decreases in gravity, temperature and pH of each fermenting wort sample were monitored with a digital precision instrument: Akolyzer Plus beer analysis machine (Aton Paar/Physika Meβtechnik, Graz, A) and a pH meter (Orion Star A211, Thermo Fisher Inc., IL, USA). The ADA (V_s) for each fermenting wort sample was calculated as follows:

$$V_s = \frac{OG - PG}{OG} \times 100\ \%$$

Where:
OG = OG (Original gravity: extract content of sweet wort before pitching) in %.
PG = Present gravity (extract content of wort measured at any time, t, after pitching) in %.

The AL of the wort samples was determined with the Akolyzer Plus beer analysis machine. At the end of the fifth day, the fermented wort samples were cooled to 2 ºC and separated from the yeast for further analysis.

Experimental Design and Statistical Analysis

The three experimental units (sorghum varieties) were subjected to a total of eight treatments each. Each treatment was replicated in triplicate to give a total of seventy-two treatment units in a randomized complete block design (RCBD). Mean values and standard deviation of data generated were calculated. The influence of steep-liquor treatments on the GE, ADA and other fermentation factors of the three sorghum varieties was resolved by subjecting data obtained to statistical Analysis of Variance (ANOVA) using Statistical Package for Social Sciences (SPSS, 2006). Significant difference was accepted at $p<0.05$.

RESULTS AND DISCUSSION

GE and Steep-liquor Treatments

The sorghum varieties showed 3.22 to 7.0 % improved GE after being steeped in the treated steep-liquors. From Table 1, KSV8 variety (H_2O_2-treated) had the highest GE of 99.0 ± 0.10 % and ICSV400 variety (GA_3-treated) showed the lowest GE of 94.0 ± 0.16 %. The GE of both the treated samples and controls were significantly ($p<0.05$) different.

Table 1 Effect of treatment substances on the GE of sorghum varieties*

Treatment chemicals	Sorghum varieties		
	KSV8	KSV4	ICSV400
(%)	(%)	(%)	(%)
IAA	97.0 ± 0.19^{d}	95.0 ± 0.16^{b}	95.0 ± 0.20^{f}
GA_3	96.0 ± 0.08^{a}	93.0 ± 0.25^{f}	94.0 ± 0.16^{d}
HPO	99.0 ± 0.10^{f}	96.0 ± 0.19^{g}	97.0 ± 0.18^{a}
Control	92.0 ± 0.31^{c}	90.0 ± 0.28^{j}	92.0 ± 0.28^{i}

*Values are means of triplicate determinations ± S.D.
abcMeans with different superscripts on the same column are significantly different at $p<0.05$.

With increased moisture level resulting from steeping, the different steep-liquor treatments may have further minimised the dormancy of the samples and possibly enhanced the physiology of the treated varieties to increase their levels of metabolic activities. GA_3 and IAA are plant hormones known to accelerate enzyme development and cell wall development/improvement in acrospire growth respectively. H_2O_2 is an acceleratr and highly oxidative alkaline and an active donor of oxygen (a gas which is absolutely essential for germination, metabolism and enzyme formation in plants). High GE and minimal dormancy is essential for vigorous and even germination, high enzymatic activities and good modification during malting (**Woonton et al., 2005**).

Relationship of ADA with Steep-liquor Treatments

Figure 1 shows the influence of steep liquor treatments on the ADA of worts from malt varieties differently treated. The ADA over the fermentation period was a measure of the fermentation level. The ADA of the fermented wort samples were significantly ($p<0.05$) different. The treated samples showed improved degrees of attenuation. The controls recorded low apparent ADA during the five-day fermentation period.

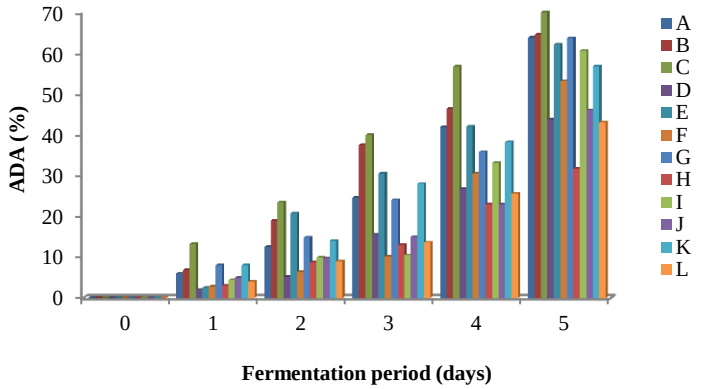

Figure 1 Influence of steep liquor treatments on the ADA of wort samples from different malt varieties

Legend: A=KSV8 variety treated with gibberellic acid, **B**=KSV8 variety treated with indole acetic acid, **C**=KSV8 variety treated with hydrogen peroxide, **D**=KSV8 variety control, **E**=KSV4 variety treated with indole acetic acid, **F**= KSV4 variety treated with gibberellic acid, **G**=KSV4 variety treated with hydrogen peroxide, **H**=KSV4 variety control, **I**=ICSV400 variety treated with indole acetic acid, **J**=ICSV400 variety treated with gibberellic acid, **K**= ICSV400 variety treated with hydrogen peroxide, **L**=ICSV400 control.

The AL of the different wort samples at the end of fermentation was significantly ($p<0.05$) different as follows: A = 88.2 % (1.18 % residual extract), B = 88.0 % (1.20 % residual extract), C = 88.8 % (1.12 % residual extract), D = 78.2 % (2.18 % residual extract), E = 88.0 % (1.20 % residual extract), F = 88.8 % (1.12 % residual extract), G = 87.6 % (1.24 % residual extract), H = 78.0 % (2.20 % residual extract), I = 84.5 % (1.55 % residual extract), J = 86.9 % (1.31 % residual extract), K = 85.8 % (1.42 % residual extract), L = 77.6 % (2.24 % residual extract).

The variations in fermentation rates may be attributed to the quality of fermentable extract of the wort samples. This may have influenced yeast metabolism which eventually determined the rate of daily decrease in the gravity (extract content) of the respective wort samples as reflected in Figure 2. The fermentable extract content of the wort samples was a function of the development of enzyme systems which hydrolyzed polymerized food and cell wall materials during the germination (seedling growth) phase of the malting process. The implication was that germination improved the extent of modification which suggested that the nexus of these effects (modification vis-à-vis quality of extract) may have been the different steep-liquor treatments. It is well established that extract values are influenced by the extent of endosperm modification during malting (**Stuart et al, 1988**).

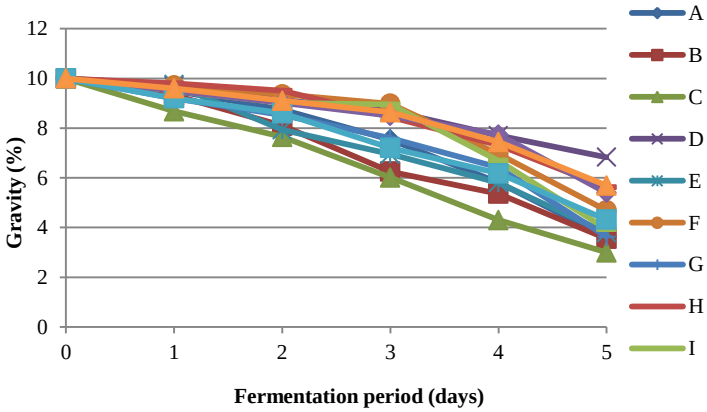

Figure 2 Pattern of gravity drop of wort samples during fermentation

Legend: A=KSV8 variety treated with gibberellic acid, **B**=KSV8 variety treated with indole acetic acid, **C**=KSV8 variety treated with hydrogen peroxide, **D**=KSV8 variety control, **E**=KSV4 variety treated with indole acetic acid, **F**= KSV4 variety treated with gibberellic acid, **G**=KSV4 variety treated with hydrogen peroxide, **H**=KSV4 variety control, **I**=ICSV400 variety treated with indole acetic acid, **J**=ICSV400 variety treated with gibberellic acid, **K**= ICSV400 variety treated with hydrogen peroxide, **L**=ICSV400 control.

The extract content and pH of the wort samples as factors of fermentation were not under control during the process. However, while the extract content was significantly ($p<0.05$) different and varied from 6.83 ± 1.34 - 10 ± 1.26 % for sample D and 3.01 ± 1.28 10 ± 1.00% for sample C, the fermentation temperature, in contrast, was not. The fermentation temperature of the wort samples was under an external influence: the temperature of the cellars. It, therefore, varied according to the level of cooling in the cellars at any point in time during the process (Figure 3). Thus, the temperature readings were not the true reflections of the quantities of heat produced by yeast metabolism during the exothermic fermentation process.

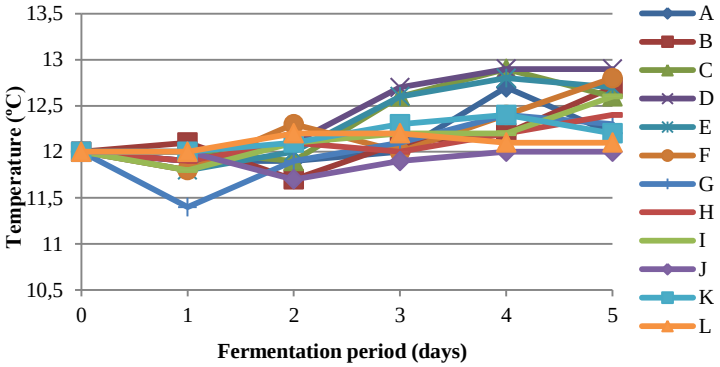

Figure 3 Temperature variations of wort samples during fermentation

Legend: A=KSV8 variety treated with gibberellic acid, **B**=KSV8 variety treated with indole acetic acid, **C**=KSV8 variety treated with hydrogen peroxide, **D**=KSV8 variety control, **E**=KSV4 variety treated with indole acetic acid, **F**= KSV4 variety treated with gibberellic acid, **G**=KSV4 variety treated with hydrogen peroxide, **H**=KSV4 variety control, **I**=ICSV400 variety treated with indole acetic acid, **J**=ICSV400 variety treated with gibberellic acid, **K**= ICSV400 variety treated with hydrogen peroxide, **L**=ICSV400 control.

The pH was not significantly ($p<0.05$) different but decreased during fermentation which indicated acidification and decreased reduction potential of the wort samples (Figure 4). This acidic nature confers some level of microbiological stability to the resultant beer. The maximum pH range was 5.20 ± 0.02 (sample B) - 5.71 ± 0.01% (sample K) while its minimum range was 3.78 ± 0.02 (sample I) – 4.01 ± 0.01% (sample C). The pH is a function of the ions of metabolites formed as a result of the actions of yeast enzymes. These enzymes perform maximally at different optimum pH ranges.

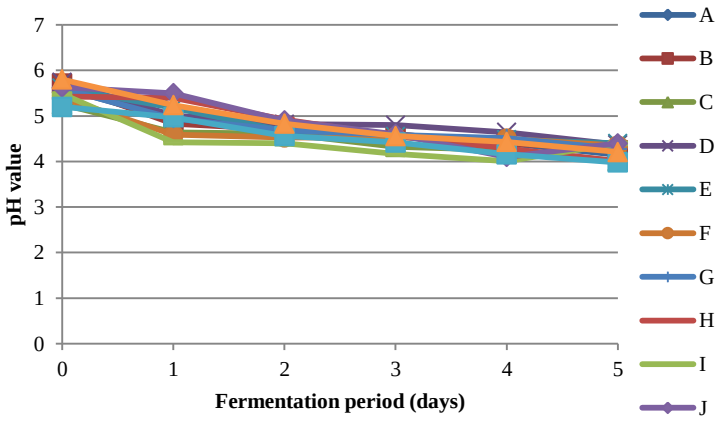

Figure 4 Changes in pH of wort samples during fermentation

Legend: A=KSV8 variety treated with gibberellic acid, **B**=KSV8 variety treated with indole acetic acid, **C**=KSV8 variety treated with hydrogen peroxide, **D**=KSV8 variety control, **E**=KSV4 variety treated with indole acetic acid, **F**= KSV4 variety treated with gibberellic acid, **G**=KSV4 variety treated with hydrogen peroxide, **H**=KSV4 variety control, **I**=ICSV400 variety treated with indole acetic acid, **J**=ICSV400 variety treated with gibberellic acid, **K**= ICSV400 variety treated with hydrogen peroxide, **L**=ICSV400 control.

Relating Figures 2 and 3 with Table 1, it was observed that with the exception of sample K, other varietal samples with high GEs had the lowest OG and pH values, hence high ADA amongst their kinds at the end of day 5 of fermentation. High GE is associated with vigorous and high level germination within a sample batch which results to better modification and high quality fermentable extract for yeast nutrition during fermentation.

Some other factors of fermentation including the quality and quantity of yeast added, yeast type and variety, temperature and pressure of fermentation were under control and were not significantly ($p<0.05$) different. In addition, comparing the results of treated samples with those of controls, it was observed that the ADA of the wort samples displayed a trend of consistent relationship with the GEs of the malt samples from which they were produced (Figure 5).

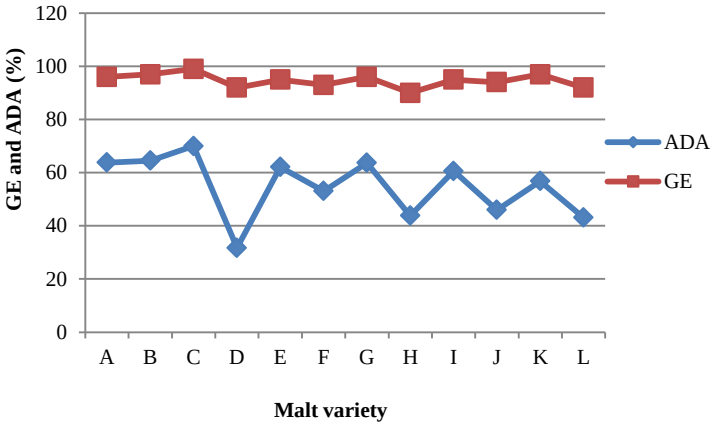

Figure 5 Trend of relationship between GE and ADA of malt samples

Legend: A=KSV8 variety treated with gibberellic acid, **B**=KSV8 variety treated with indole acetic acid, **C**=KSV8 variety treated with hydrogen peroxide, **D**=KSV8 variety control, **E**=KSV4 variety treated with indole acetic acid, **F**= KSV4 variety treated with gibberellic acid, **G**=KSV4 variety treated with hydrogen peroxide, **H**=KSV4 variety control, **I**=ICSV400 variety treated with indole acetic acid, **J**=ICSV400 variety treated with gibberellic acid, **K**= ICSV400 variety treated with hydrogen peroxide, **L**=ICSV400 control.

CONCLUSION

The objective of this study was to investigate how different steep-liquor treatments influenced the GE and ADA of the three sorghum varieties (SKV8, SKV4 and ICSV400) during malting and brewing. The results revealed that samples of different treated sorghum varieties had more significantly improved GE than the controls. Worts of treated samples showed significantly improved ADAs during the five-day fermentation period while the controls had low ADA respectively. However, the GE and ADA of the three sorghum varieties were observed to have differed ($p<0.05$) significantly along varietal lines with KSV8 variety steeped in H_2O_2-treated-liquor having the highest GE of 99.0 ± 0.10 % while ICSV400 variety steeped in GA_3-treated-liquor had the lowest GE of 94.0 ± 0.16 %. Similarly, wort of KSV8 variety treated with H_2O_2 had the highest ADA of 70.0 ± 1.25 % while that of KSV4 variety treated with GA_3 had the lowest ADA of 31.7 ± 0.53 %.

Comparing the results of treated samples with those of controls, the ADA of the wort samples displayed a trend of consistent relationship with the GEs of the samples of malt varieties from which they were produced which suggested that some of the underlying influence on the improvements observed in both the GE, extract content and ADA may have resulted from the different steep-liquor treatments.

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