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ORIGINAL ARTICLE

The Efficacy of Alkaline Steeping in Extracting Proanthocyanidins and its effects on Malt quality in some Sorghum varieties

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ABSTRACT
Sorghum malt is an important raw material in opaque beer brewing. Use of high tannin sorghum varieties reduces malt quality due to binding of tannins to proteins. Tannins are usually leached from sorghum grain using formalin during the steeping stage of malting. However, formalin has been shown to have potentially harmful health effects thus driving the need to find alternative methods of tannin removal. In this study, the effectiveness of alkalis (calcium and sodium hydroxides) was assessed. The amount of tannins in red (NS5511) and brown (SMILE) malting sorghum varieties were determined by the butanol-HCl assay and compared to other food varieties. The NS5511 and SMILE were steeped in 0.02M and 0.04M concentrations of each of $Ca(OH)_2$, NaOH and formalin for 6hrs, subjected to germination conditions (26°C for 115hours) and kilned off at 60°C for 50hours. The germination rate, as measured by the chit count was determined and the alkali treated samples had the highest germination rates compared to formalin. Furthermore, the alkali treated samples had highest diastatic units. The amount of residual tannins was determined, and formalin treatment leached slightly more tannins than the alkalis. We conclude that alkalis can replace formalin since they are almost equally effective and have less serious health effects.
Key words; alkali, formalin, malting, proanthocyanidins, sorghum, tannin,

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INTRODUCTION

Sorghum (*Sorghum bicolor* (L.) Moench) is a cereal grass whose malted form is widely used in opaque beer brewing where it is the main source of hydrolytic enzymes, starch, yeast nutrients, beer flavor and color substances [1]. Some varieties contain polyphenolic compounds called condensed tannins or proanthocyanidins (PAs) located in the testa and these confer some agronomic advantages such as fungal resistance and reduction of bird predation [2]. A major drawback of these tannins is that they bind with the grain proteins and the enzymes of the digestive tract thereby reducing the nutritional value of the grain.

In sorghum malting, the main objectives are, to mobilize endogenous hydrolytic enzymes of the grain, which then modify the constituents of the grain during malting so that they are readily solubilized during brewing. Furthermore, these enzymes solubilize the unmalted starchy adjuncts during the mashing process of beer brewing [3]. Polyphenols, especially tannins, react with the enzymes to form insoluble compounds which are resistant to decomposition, making the enzymes unavailable for substrate hydrolysis [4]. In malt, this results in low sorghum diastatic units (SDUs), which is a measure of the joint α and β amylase activities. Traditionally, Formalin (a formaldehyde solution) has been used to relieve the negative effects of polyphenols by extracting them from the grain during steeping [5], through the formation of an inert resin like structure which is leached out in the first water of steeping.

Some studies have shown that formaldehyde is potentially carcinogenic amongst other health hazards such as causing eczema, cornea damage and respiratory problems [6], these side effects of formaldehyde have driven the need to research on the use of other chemicals. Alkaline steeping has been proved to be effective in improving malt quality [1] [7] but however, the response to the alkaline treatment has been shown to be variety dependent [8] and for this reason, in this study, alkaline steeping using calcium hydroxide and sodium hydroxide was assessed on local Zimbabwean sorghum varieties that are being used for commercial malting. Formaldehyde is still in use and once the effect of alkali on the grain has been assessed, the findings can be useful in acting as a platform for advocating discontinued use of the chemical.

MATERIALS AND METHODS

Sorghum grain

Two sorghum varieties namely NS5511 (red) and SMILE (brown) used for malting were obtained from the Sorghum Breeding and Procurement division of Delta Beverages. The other 10 varieties used for comparison of the tannin levels were obtained from the Department of Research and Specialist Services, Sorghum Improvement section at the Matopos Research Station. These were the white varieties SV4, Chibonda and Khakhi, the red varieties NL 9708, NL9602, Red Swazi and Town and the brown varieties Vincennes, Dwarf Wonder and NL 9605.

Malting

The Malting was done at Delta Beverages Aspindale Maltings Plant. One kilogram samples of the NS5511 and SMILE sorghum varieties were rinsed in tap water and steeped at 26°C for 6 h in 2L still solutions of $Ca(OH)_2$ (0.02 or 0.04M), NaOH (0.02 or 0.04M) or formalin (0.04M) in plastic containers. This was followed by a 10 h dry stand after which they were rinsed and subjected to similar germination conditions at an average temperature of 26°Cfor 115hours. Three water sprays (100ml each) were administered to all grain samples in early germination and the grain was turned to avoid matting. Chit counts and moisture content checks were done every 24 hours to check progression of germination. Kilning was carried out at 60°C for 50 h. The malt analysis was then carried out on the dried malt.

Malt analyses

Diastatic power determination

The diastatic power was determined according to the South African Bureau of Standards methods [10], at the Delta Beverages Kwekwe Maltings Plant Lab.

Flavonoid extraction from sorghum seeds

For the unmalted grain, seeds were surface cleaned by washing twice in distilled water. The samples were dried and ground in a mortar and pestle. A 0.25g of the finely ground powder was weighed into a centrifuge tube and to it 5ml of absolute methanol added and the tubes covered with aluminum foil. The tubes were placed on a bench shaker for 20 minutes then centrifuged at 3000rpm for 10minutes. The supernatant was transferred to a 12.5ml tube and stored at 4°C. A second extraction was carried out the same way on the pellet. This extract was added to the first extract and the volume made up to 12.5ml by adding methanol. The extract was stored at 4°C in the dark until analysis.

Butanol-HCl assay for proanthocyanidins

The amount of PAs (tannins) in the NS5511 and SMILE varieties was determined in the raw and malted samples using the butanol-HCl assay. Other varieties were also used for comparison, tannin free white varieties acted as the standards (SV4, Chibonda and Khakhi), the tannins in the red NS5511 were compared to the red NL 9708, NL9602, Red Swazi and Town whereas the brown SMILE was compared to Vincennes, Dwarf Wonder and NL 9605

For the assay, 6ml of freshly prepared butanol-HCl reagent (5ml of concentrated HCl to 95ml of butan-1-ol) was added to 1ml of the methanol extract form the seeds in a boiling tube closed with a cotton wool plug and aluminum foil. The blank contained no extract. The sample was shaken and the tubes placed in a boiling water bath for an hour than cooled under running tap water. The absorbance readings of the samples were taken at 550nm. These were reported as absorbance units per gram of sample.

The Butanol HCl assay was done in the Biochemistry Lab at the National University of Science and Technology, Zimbabwe.

Statistical analysis

A Completely Randomized Design (CRD)/ One Way Analysis of Variance (ANOVA) was used to assess the differences in the germination rate, diastatic power and residual tannin levels of the malt from the $Ca(OH)_2$, NaOH and formalin treatments. The differences were tested at the 5% level of significance. The Ho hypothesis represented the state of no difference amongst the malt produced by the three treatments whereas the Hi hypothesis stated that there are significant differences amongst the three treatments with respect to the malt produced.

RESULTS AND DISCUSSION

During steeping, the tannins were leached from the grain as was indicated by the change in colour of the steep water from an initial colourless to a brown-coke taint. In those samples treated in alkali, the water changed colour within the first 30 minutes of steeping. The intensity of the steep water color was in the order shown below with formalin treated samples still colorless after 30 minutes of steeping.

0.04M $Ca(OH)_2$ → 0.02M $Ca(OH)_2$ → 0.04M NaOH → 0.02M NaOH → 0.04M formalin

This observation was a first indication that alkali is surely better in leaching as compared to formalin. However, on the final malt, the samples treated in 0.04M calcium hydroxide had darker coloured malt, which had a burnt appearance (shown in fig 1). This is undesirable in malting and this may be an indication of too high a concentration of alkali used. High concentrations of alkali are known to have harmful effects on the grain [3]

Germination rate during malting

The chit count was done as a measure of the germination rate of the sorghum samples. On day 4 of germination (at 77 hours), the chit counts were as shown in Fig 2.

The overall observation was that alkali treated samples had higher germination counts than the formalin treated samples, specifically; $Ca(OH)_2$ had the highest count followed by NaOH. This observation suggests that the alkali steeping promotes a more rapid growth of the grain as compared to formalin. At the 5% significance level, one way ANOVA proved however, that there are no significant differences in the response to the different treatments.

The effect of calcium hydroxide and sodium hydroxide on high tannin sorghum is attributable to the ability of alkalis to increase water uptake. It is thought to be as a result of the alkali disrupting the sorghum pericarp cell wall structure. Alkali is known to saponify acetyl groups and other ester linkages [9], cause cellulose to swell and disrupt the hydrogen bonds between hemicelluloses resulting in the solubilization of hemicelluloses [10]. This makes it easy for the alkalis to react with the tannins. Due to the tannin removal, there is an increase in metabolic activity attributed to the rapid hydration [10]. In this study, the alkali treated samples had higher chit counts, which means they had a faster germination rate due to rapid hydration. However the varieties had different rates. These differences can then be attributed to varietal differences but the common observation being that alkali promotes a faster germination rate as compared to formalin.

The presence of moulds and mycotoxin infection is a factor known to reduce the germination rate of most sorghum grains [11]. If mycotoxin contamination had occurred in the batch sample, then formalin treated samples could have been affected since there is no clear evidence of its ability to inhibit fungal or microbial growth. Alkalis have been used as antimicrobials since ancient times and their antimicrobial ability is a function of the degree of dissociation of the OH^- and H^+ in the cytoplasmic membrane of the microbial cells, resulting in their death [12]. Also, addition of dilute $Ca(OH)_2$ during barley steeping has been shown to inhibit microbial and fungal growth [3], which could be an advantage in later fermentation as the alkalis may present a "cleaner" malt, in terms of microbial load. It has been shown that steeping in 2000ppm prevented bacteria and mould growth without affecting the malting loss, diastatic power, cold and hot water extracts of sorghum malt [13]. Steeping in dilute NaOH has also given malts with improved diastatic power and free amino nitrogen, reduced malting loss, enhanced carbohydrate and protein mobilization without any adverse affects to the grain [7] [10] [14].

Diastatic power

The diastatic power (amount of α and β amylases) of the treated malts were measured and plotted in a graph (figure 3). Considering the varietal responses to the treatments, SMILE had the highest SDU in the $Ca(OH)_2$ treated samples followed by the NaOH treated samples. Formalin had the lowest SDU compared to the alkaline treatments. In the NS5511 variety, the sample treated in 0.04M alkalis had the highest SDUs followed by the 0.02M alkali concentrations and here, again, formalin treated samples had the lowest SDUs.

The diastatic power was determined as a measure of malt quality. If high amounts of tannins have been removed from the grain, more enzymes should be available in the malt produced hence a higher SDU. The overall result was that $Ca(OH)_2$ treatment produces malt with higher diastatic power followed by NaOH and lastly, formalin. However, at the 5% significance level, the differences were insignificant. The high DP of the $Ca(OH)_2$ treated malts has been attributed to the presence of Ca^{2+} ions. Several studies on enzyme development in germinating cereal grains have addressed the effects of Ca^{2+} on hydrolytic enzyme secretion [9]. Ca^{2+} is associated with enhancements in the production and secretion of α amylase, the rate determining enzyme in endosperm starch modification of cereal grains and a key component of the malt diastase enzymes. However, the ability of grains to respond to Ca^{2+} has been said to be genetically dependent [15], this might explain the slight differences observed in the response to $Ca(OH)_2$ steep treatment. This has been attributed to the inability of some cultivars to synthesize particular enzyme isoforms when subjected to specific treatment or the inability of the grains to activate their amylases by degrading α amylase inhibitor substances [8] [16]. NaOH is also a good leachate for tannins, it reacts readily with carboxylic acids to form their salts and is a strong enough base to form salts with phenols [14].

The SMILE variety had higher SDUs with $Ca(OH)_2$ treatment as compared to the NaOH treatments whereas the NS5511 variety had higher SDUs with NaOH treatments, supporting the observations on varietal response differences to treatments by other researchers [8] [14].

Tannin levels

Tannin levels of the NS5511 and SMILE varieties were determined and compared to other established varieties. The levels of the tannins in the raw grain as estimated by the butanol-HCl assay are shown in figure 4. From the graph, it is evident that the SMILE and NS5511 varieties are high tannin varieties; NS5511 had the highest tannin levels amongst the red varieties whilst SMILE had the second highest tannin levels after the brown NL 9605.

Tannin levels were again measured in the malted samples and they had been greatly reduced (figure 5). Formalin treated samples had the lowest residual tannins suggesting that formalin is better than the alkali treatments in tannin removal, however the alkalis also leached high amounts of the tannins. Considering the varietal response, both alkaline treatments removed more tannin in SMILE than in NS5511. This shows that the response in alkaline treatment depends on the sorghum variety. At the 5% significance level, one way ANOVA proved however, that there are no significant differences in the residual tannin levels left by the different treatments.

The tannin assays carried out before and after alkaline malting proves the efficacy of alkaline treatment. The raw grain had very high amounts of tannins as compared to the malted samples. Both SMILE and NS5511 varieties are high tannin sorghums. The alkalis removed more than 90% of the tannins in the grains but here formalin had the best results leaching up to 98% of the tannins. Since these differences were insignificant according to the statistical model, alkalis are just as efficient in tannin removal.

Safety

Safety concerns associated with residual alkali in the product also have to be considered, as well as downstream processing (brewing) effects. For $Ca(OH)_2$, no carcinogenic, teratogenic, developmental toxicity or mutagenic effects in humans have been reported as yet, but with a dose of 1200mg/kg ascities tumors may develop in rats. Health issues that have been reported include skin irritations, gastrointestinal tract irritations, vomiting and nausea in cases of over exposure. For NaOH, only solutions of 0.5M concentration have been labeled as corrosive, chemical burns and blindness can result from such high concentrations. This makes the use of these alkalis safer than formalin.

Basing on the findings of this study, after considering the germination rate, diastatic power, tannin removal and safety, it can be concluded that alkali treatment, specifically calcium hydroxide, is a better way of leaching tannins prior to germination in the malting of high tannin sorghums. Since there is no difference in the quality of the malt produced then safety concerns become the driving force to replace formalin with the safer alkalis. However, to determine the most effective concentration for use, it becomes necessary to expose the variety in question to different concentrations and assessing the response.

Figure 1. The NS5511 variety malt colour profiles showing, from left to right, the normal formalin malted sorghum, the 0.02M $Ca(OH)_2$ malted sorghum and the 0.04M $Ca(OH)_2$ malted sorghum.

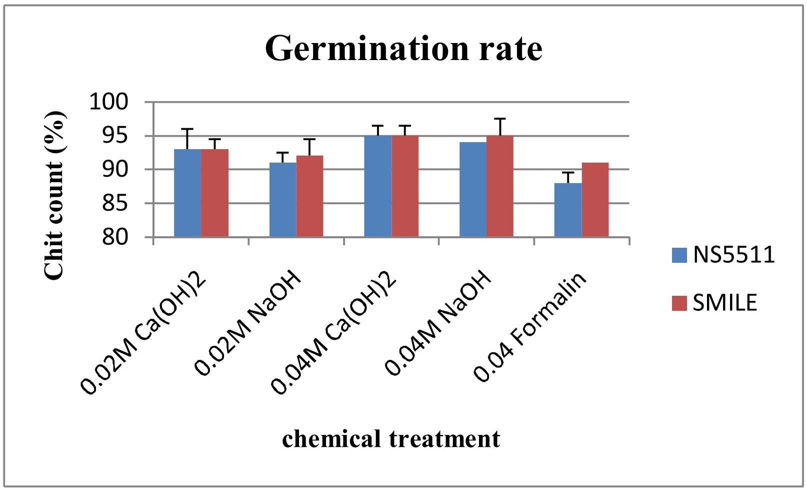

Figure 2. Chit counts for germination day 4 (at 77hours) with +SD values plotted as error bars

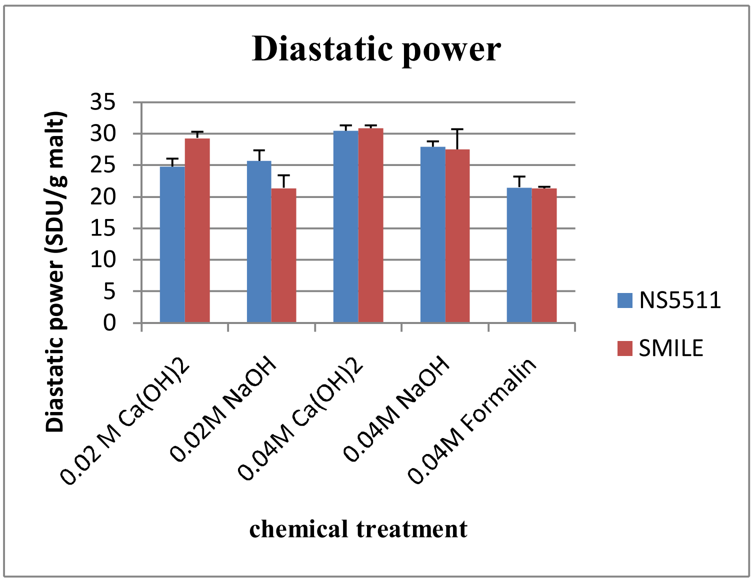

Figure 3. Diastatic power represented in Sorghum Diastatic Units (SDUs), with +SD values plotted as error bars

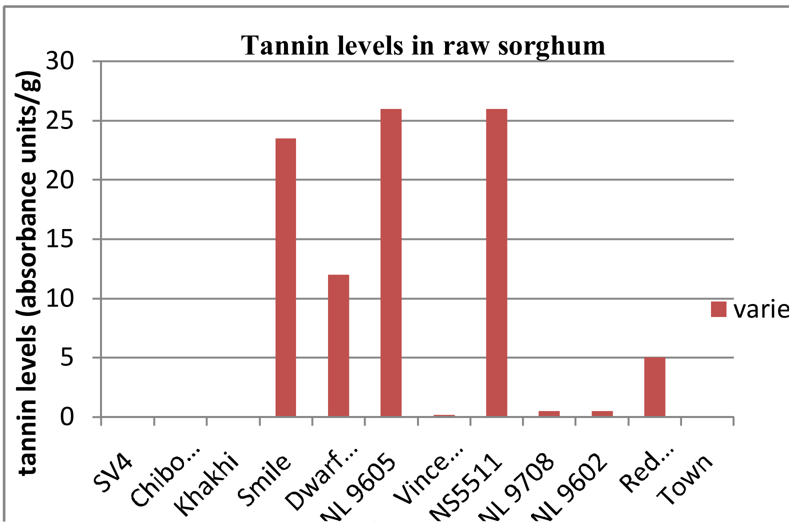

Figure 4.Tannin levels in the different sorghum varieties (in absorbance units per gram of sample)

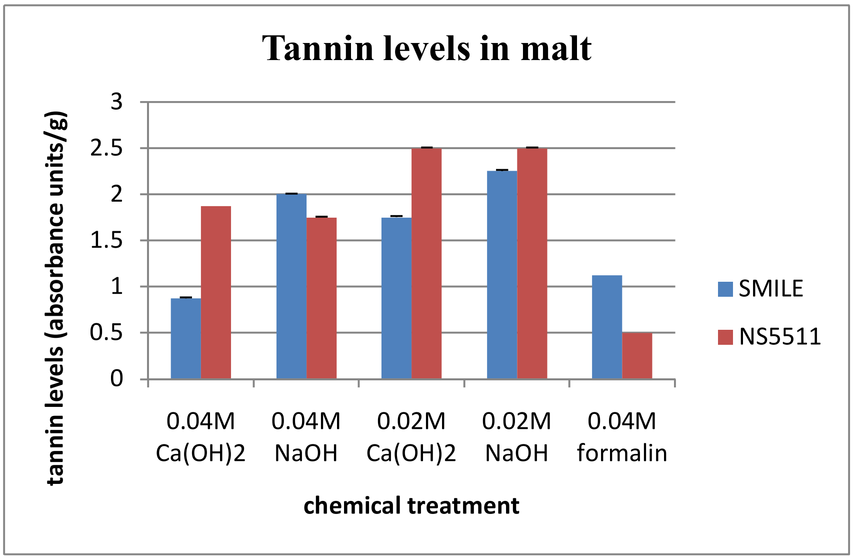

Figure 5. tannin levels in malted samples of NS5511 and SMILE (in absorbance units per gram of sample), showing the +SD values

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