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Effects of gibberellic acid on the synthesis of α- and β-amylase during sorghum malting (*Sorghum bicolor* L. (Moench)

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ABSTRACT

Description of the subject. The use of red sorghum in brewing offers several benefits including the absence of gluten. But it is problematic because it contains huge amounts of polyphenols and has low α and β-amylase activations compared to barley, hence the need to improve them.
Objectives. The overall goal is to contribute to the utilization of red sorghum in modern brewing. Specifically, the study aims to evaluate the effect of added gibberellic acid on the synthesis of α- and β-amylase during red sorghum and corn malting.
Methods. To improve α- and β-amylase activities, the effects of a treatment factor with 3 modalities (CORN: corn; NDRS: non-discolored sorghum and DRS: discolored sorghum) in 4 soaking solutions (distilled water, distilled water +500 ppm gibberellic acid (GA), distilled water + 500 ppm acetone/water extract (70/30: V/V), distilled water + 500 ppm, GA + 500 ppm acetone/ water extract (70/30: V/V), a gibberellic acid content factor and an acetone / water extract factor (70/30: V/V) were studied.
Results. The results showed that GA has a significant effect on α-amylase synthesis during DRS malnutrition whereas no evidence is given in this study neither on the reasons for this lack of response during α-amylase malnutrition NDRS, nor on the lack of effect of GA addition on β-amylase synthesis.
Conclusion. This study showed that increasing the acetone/water extract content (70/30: V/V) decreases the synthesis of both α-amylase and β-amylase activity during of discolored red sorghum malting (DRS).

Keywords: Red sorghum, α- and β-amylase, phenolic compounds, gibberellic acid.

RESUME

Effets de l'acide gibbérellique sur la synthèse de l'α- et de la β-amylase lors du maltage du sorgho (*Sorghum bicolor* L. (Moench)
Description du sujet. L'utilisation du sorgho rouge en brasserie offre plusieurs avantages, notamment l'absence de gluten. Mais il pose problème car il contient d'énormes quantités de polyphénols et a des activés α et β-amylase basses par rapport à l'orge, d'où la nécessité de les améliorer.
Objectifs. L'objectif global est de contribuer à la processabilité du sorgho rouge en brasserie moderne. Spécifiquement, l'étude vise à déterminer l'effet de l'ajout de l'acide gibbérellique sur la synthèse de l'α- et de la β-amylase au cours du maltage du sorgho rouge et du maïs.
Méthodes. Pour améliorer les activités α- et β-amylase, les effets d'un facteur traitement avec 3 modalités (CORN : maïs ; NDRS : sorgho non décoloré et DRS: sorgho décoloré) dans 4 solutions de trempage (eau distillé, eau distillée+500 ppm d'acide gibbérellique (GA), eau distillée+500 ppm d'extrait acétone/eau (70/30: V/V), eau distillée+500 ppm, GA+500 ppm d'extrait acétone/eau (70/30: V/V); d'un facteur teneur en acide gibbérellique et d'un facteur teneur en extrait acétone/eau (70/30: V/V) ont été étudiés.
Résultats. Les résultats ont montré que GA a un effet significatif sur la synthèse de l' α- amylase lors du maltage du DRS alors qu'aucune évidence n'est donnée dans cette étude ni sur les raisons de cette absence de réponse lors du maltage du NDRS, ni sur le manque d'effet de l'addition de GA sur la synthèse de la β-amylase.

Conclusion. Cette étude a montré que l'augmentation de la teneur en extrait acétone/eau (70/30 : V/V) diminue la synthèse aussi bien de l'activité α-amylase que celle de l'activité β-amylase au cours du maltage du sorgho rouge décoloré (DRS).

Mots clés : Sorgho rouge, α- et β-amylase, composés phénoliques, acide gibbérellique.

1. INTRODUCTION

The dependence of the brewing industries in African countries on western brewing raw materials (barley malt, hops) is a ballast for the revival of the brewing industry, particularly regarding development in general. In the tropics, the difficulty of growing barley profitably is increasingly prompting brewers working in these regions to think of other cereals as substitutes for and / or supplements to barley malt (Bwanganga *et al.*, 2013a).

In developing countries, the technological interest in sorghum (*Sorghum bicolor* (L.) Moench) is due to its ability to generate a complex system of enzymes associated with starch hydrolysis. This in turn enables the preparation of low viscosity (boiled) weaning foods (Dillon, 1989, Larreta-Garde, 1997; Traore *et al.*, 2004), even though these enzymes do not allow, in terms of type and level of activity, the opportunity to optimize the use of sorghum in modern breweries (Bwanganga *et al.*, 2013a).

Sorghum however, would be the ideal alternative cereal of barley in the modern brewery. This is primarily because it meets the climatic and agronomic needs of the brewing industry, as well as most of the other demands they require. Moreover, it is cultivated in the semi-arid and tropical areas of the world because of its good yield and adaptation to hostile environments (Dicko, 2005). As such, its high content of polyphenols (Dicko, 2005) makes it resistant to fungal attack and pests (which is an agronomic advantage), albeit reducing its processability.

Polyphenols present in large amounts of red sorghum grains may be responsible for the low grain response to amylase synthesis induction, when using gibberellic acid in soaking solutions (Bwanganga, 2013b). It has thus been proposed that the application of gibberellic acid takes place sometime after the start of soaking and after the quenching solution has been renewed, to reduce the possible interactions between gibberellic acid and the phenolic compounds of sorghum. Which in turn would be at the origin of the weak response to the induction of sorghum amylase synthesis (Bwanganga, 2013b).

In this study, the elimination of phenolic compounds was carried out before the soaking. Gibberellic acid and acetone-water extracts (70/30: V/V) were added at the same time to the dipping solutions. The effects of the elimination of phenolic compounds and acetone/water extracts (70/30: V/V) on the synthesis of these same enzymes were thus evaluated in terms of reducing the rate of hydrolysis of the specific substrates.

2. MATERIAL AND METHODS

A red sorghum cultivar (*Sorghum bicolor* Moench) and a corn cultivar (*Zea mays*) sold locally were used in this study. The homogeneity of the grains was obtained after a visual inspection and the gnawed grains (which attacked by insects), have been eliminated from the sample. Acetone and gibberellic acid have been provided by Sigma-Aldrich, China. The AMYLAZYME and BETAMYL kits were fortified by Megazyme, Irland for the measurement of α- and β-amylase activities. A Jinnuo scale balance, JT3003B, a UV-VIS spectrophotometer (Hach DR 600), a water bath (Suart, 24L), a centrifuge (Sigma 2-16), an oven (Memmert, Modell 100-300), and other laboratory equipment and glassware were also used.

Phenolic compounds were extracted as described by Amisi *et al.* (2019) and Bwanganga (2012) with some modifications. To do this, in 50 ml of the acetone-water solvent system (70/30: V/V), 500 mg of red sorghum grains were added and mixed for 30 minutes, before the mixture was distributed in tubes and then centrifuged (5000 rpm for 5 minutes). The collected supernatant was placed in the oven set at 50°C for 24 hours to remove the solvent. The resulting extract was then kept in the freezer for later use.

Four (4) quenching solutions were used, namely: distilled water, distilled water +500 ppm gibberellic acid, distilled water + 500 ppm acetone/water extract (70/30: V/V) and distilled water +500 ppm gibberellic acid + 500 ppm acetone/water extract (70/30; V/V). In a first trial, a device comprising of a treatment factor (with 3 modalities: CORN (maize), NDRS (unbleached sorghum) and DRS (discolored sorghum)), and Gibberellic acid content factor (with two modalities: 0 ppm and 500 ppm) was used to test the effect of gibberellic acid on the induction of synthesis of α- and β-amylases during malting. In a second trial, a factor of acetone/water extract (with 2 modalities: 0 ppm and 500 ppm) was used to evaluate the effect of adding the

acetone/water extract whilst adding gibberellic acid during the synthesis of α- and β-amylases during malting (CORN and DRS). The discolored sorghum grains were obtained after extractions of the phenolic compounds using acetone/water mixture (70/30: V/V).

Soaking was then performed for 16 hours, as proposed by Bwanganga *et al.* (2012, 2013b, 2013c). Finally, after soaking, seeds were placed in the dark for 72 hours to germinate. The green malts obtained were kilned at 40°C for 48 hours in the oven and then ground and sieved (5 mm). Additionally, the flours obtained were kept in the refrigerator at 4°C. α- and β-amylase activities were measured as detailed by Bwanganga *et al.* (2013a and 2015).
The data obtained was processed using the Minitab 17 software.

3. RESULTS

The effect of gibberellic acid content was evaluated during the malting of sorghum (discolored and undyed grains). The effect of the Treatment factor (3 modalities: CORN (maize), NDRS (unbleached sorghum) and DRS (bleached sorghum)), the Gibberellic acid content factor ("Added GA" with two modalities: 0 ppm and 500 ppm) as well as their interaction have been studied.

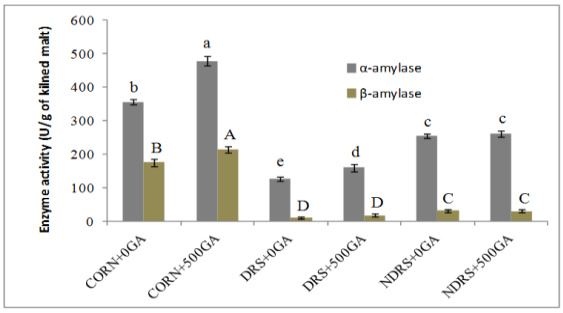

Figure 1. Effect of addition of gibberellic acid on synthesis of α- and β-amylase during malting of red sorghum and maize

Treatment factor (with 3 modalities: CORN (maize), NDRS (unbleached sorghum) and DRS (bleached sorghum)); Gibberellic acid content factor (with two modalities: 0 ppm and 500 ppm). On the ordinate: enzymatic activity in units per gram of malt. Means sharing no letter (lower or upper case respectively for α- or β-amylase) are significantly different at the 5% threshold.

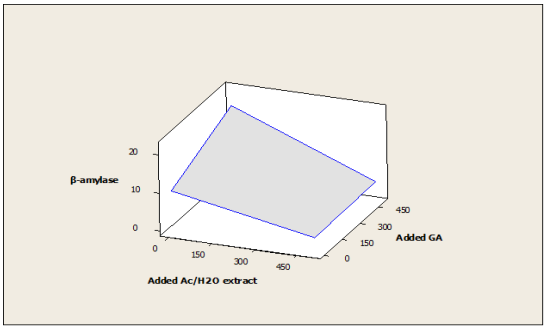

Figure 2. Surface diagram describing the effect of addition of gibberellic acid (Added GA) and acetone / water extract (70/30: V / V) (Added Ac / H_2O extract) on the synthesis of β-amylase during malting of red-colored sorghum

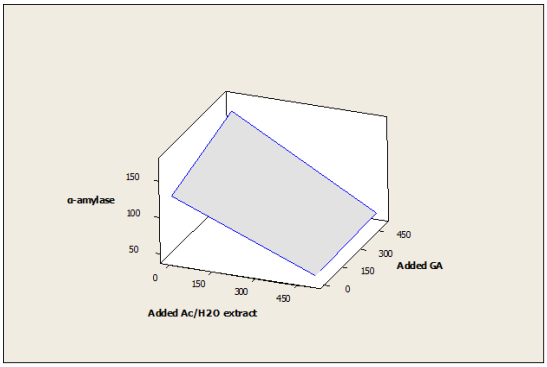

Figure 3. Surface diagram describing the effect of addition of gibberellic acid (Added GA) and acetone/water extract (70/30: V / V) (Added Ac / H_2O extract) on synthesis of α-amylase during malting of discolored red sorghum

4. DISCUSSION

The results of Tables 1 and 2 show that the two factors, as well as their interaction, have significant effects both on the synthesis of the α-amylase and that of the β-amylase. The comparison of the averages performed according to the Tukey test (FIG. 1) shows that the various treatments can be classified in the following descending order, according to their α-amylase activities:

CORN + 500GA> CORN + 0GA> NDRS + 500GA = NDRS + 0GA> DRS + 500GA> DRS + 0G and in descending order according to their β-amylase activities:

+ 500GA CORN> CORN + 0GA> NDRS 500GA + = + NDRS 0GA> DRS DRS + = + 500GA 0GA

With: CORN + 0GA (corn without gibberellic acid added); CORN + 500GA (corn with 500 ppm added gibberellic acid); DRS + 0GA (discolored red sorghum without added gibberellic acid); DRS + 500GA (discolored red sorghum with 500 ppm added gibberellic acid); NDRS + 0GA (undyed red sorghum without added gibberellic acid) and NDRS

+ 500GA (undyed red sorghum with 500 ppm added gibberellic acid).

NOTE HERE that if the gibberellic acid content significantly affects both α- and β-amylase synthesis during the malting of the maize cultivar used (significant difference between CORN + 0GA and CORN + 500GA treatments), then the effect of gibberellic acid on α-amylase synthesis is significant only when the sorghum grains are discolored. Contrastingly, this effect was not significant during the malting of un-decolored sorghum (no significant differences observed between DRS + 500GA and DRS +0 GA treatments).
The effect of gibberellic acid is indeed recognized in germination, the induction of enzyme synthesis, the release of dormancy, and so on (Davies, 2004; Rodrigues *et al.,* 2011), although this has never been proven for sorghum.

The fact that this effect is not significant in the synthesis of β-amylase could possibly be due to the low percentage of germination observed during the malting of the discolored sorghum (DRS treatment). This is also noted in the inferiority of this treatment when compared to NDRS, for which, despite the absence of the effect of gibberellic acid, both α- and β-amylase activities are significantly different from those of DRS. It is known however, that gibberellic acid and abscisic acid antagonistically regulate the activity of α-amylase in the cells of the barley layer (Gilroy & Jones, 1992), despite the fact that this is not clearly demonstrated during the germination of sorghum.

If this study shows that gibberellic acid can have an effect on the synthesis of α-amylase during the malting process of discolored sorghum (DRS) (21% increase in α-amylase activity obtained after bleaching of the grains (Figure 1)), there is no evidence for this lack of response when malting unbleached sorghum (NDRS), nor for the lack of effect of the addition of gibberellic acid on the synthesis of β-amylase.

Induction of β-amylase synthesis thus remains a major concern for better use of red sorghum malt in breweries. Heat shock (Bwanganga, 2014), use of *Bacillus subtilis* starters (Bwanganga *et al.,* 2012), use of caustic soda etc. are treatments which, despite their effectiveness in improving certain properties of sorghum malt, have remained limited in the induction of β-amylase synthesis. The acetone/water extract (70/30: V/V) added to the soaking solutions significantly affected the synthesis of amylases during malting (FIGS. 2 and 3). For that reason, it would most likely contain molecules with the same binding sites as the Gibberellic acid found on the receptor cells of both the aleurone layer and the scutellum, which in turn would reduce the hormonal response.

In fact, FIGs. 2 and 3 show that the increase in the content of acetone/water extract (70/30: V/V) decreases the synthesis of both the α-amylase activity and the β-amylase activity. The results of this study also show that the fading treatment used is not effective in that it affects the synthesis of the enzymes (50.5 and 68.1% reduction respectively for α- and β-amylase activities). Thus, when comparing NDRS + 0GA and DRS + 0GA (Figure 1)), studies should be conducted to either optimize it or find a technique that would affect the underlying germination process as little as possible.

Conclusively, the results of Figures 2 and 3 can be shown to be in agreement with that of Bwanganga *et al.* (2015), and show that the addition of the total polyphenols extracted by the acetone/water mixture (70/30: V/V) significantly reduces the synthesis of α-amylase activity during the malting of red sorghum. It is known that polyphenols can bind to starch and other polysaccharides, thus affecting their digestibility (Le Bourvellec & Renard, 2012). When the digestibility of starch is low, the cellular need for enzymes can also be affected considerably. Furthermore, phenolic compounds, in this case condensed tannins, can directly bind to amylases thus affecting their digestibility (Davis & Hoseney, 1979, Asquith & Butler, 1986, Beta *et al.*, 2000).

The response surfaces of FIGs. 2 and 3 show that the synthesis of the two enzymes is strongly affected by the addition of the acetone/water extract (70/30: V/V) (62% and 70% and β-amylases respectively when malting is carried out without the addition of gibberellic acid and 68 and 80% reduction of α- and β-amylase activities respectively when the malting is carried out with addition of 500 ppm of gibberellic acid). The fact that the percentage reduction further decreases when gibberellic acid is added to the soaking solution would probably be due to the interactions between gibberellic acid and the phenolic compounds often extracted by the acetone/water mixture (70/30: V/V) (Bwanganga, 2013c).

5. CONCLUSION

The purpose of this work was to contribute to solving the problems that are blocking the use of red sorghum in breweries. Phenolic compounds which are inhibitors of α and β-amylase were removed by the acetone/water extract (70/30: V/V) before malting to promote the best synthesis of these amylases. The malting was carried out using 4 quenching solutions: distilled water, distilled water + 500 ppm gibberellic acid, distilled water + 500 ppm acetone/water extract (70/30: V/V), distilled

water + 500 ppm gibberellic acid + 500 ppm acetone/water extract (70/30: V/V).

Gibberellic acid has been added to quenching solutions to induce the most possible amylases (α and β). The effect of adding gibberellic acid in the synthesis of α- and β-amylase during the malting of red sorghum and maize was evaluated. The results show that while gibberellic acid has a positive effect on the synthesis of α-amylase during the malting of discolored sorghum, no evidence is given for this lack of response when malting discolored non-sorghum (NDRS). Nor any indication towards the lack of effect in the addition of gibberellic acid on the synthesis of β-amylase. Experience shows that an increase in the content of acetone/water extract (70/30: V/V) decreases the synthesis of both the α-amylase activity and that of the β-amylase activity, as shown in the effect of adding GA and acetone/water extract (70/30: V/V) to the synthesis of α and β-amylase during the malting of red-colored sorghum.

Screening the acetone/water extract (70/30: V/V) would make it possible to study more specifically the inhibitory effects of each of the constituents on the synthesis of the amylotytic enzymes.

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