RESEARCH ARTICLE

Effects of malting conditions on enzyme activities, chemical, and bioactive compounds of sorghum starchy products as raw material for brewery

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Two cultivars of sorghum (red, RS and white, WS) were germinated at 25 or 30°C for 1, 2, or 3 days and were evaluated regarding enzyme activities (diastatic power: DP, β-amylase), chemical compounds (total starch, damaged starch, glucose, free amino acid), and bioactive compounds (free phenolic compounds: FPC and γ-aminobutyric acid:GABA). For WS and RS (on third germination day at 30°C), DP was 11.3 ± 1.4 and 10.8 ± 1.3 °DP, β-amylase activity: 33.0 ± 0.9 and 71.1 ± 0.7 UBetamyl/g d.b., respectively, while at 25°C, DP was 6.1 ± 0.7 and 18.0 ± 1.1 °DP, β-amylase activity: 9.3 ± 0.5 and 57.8 ± 0.3 UBetamyl/g d.b., respectively. Starch degradation, free amino acids, and free glucose content increased during germination, and depended on cultivar, germination time and temperature. The hydrolysis of the protein matrix influenced the release of starch granules, which were more accessible to amylolytic action. FPC and GABA levels were higher after grain germination and depended on sorghum cultivar and germination temperature. Germination at 30°C for 3 days represented the most suitable conditions for obtaining good bio-functional sorghum malt.

Keywords:
Bio-functional malt / Brewery / GABA / Phenolics / Sorghum

1 Introduction

Sorghum (*Sorghum bicolor* (L.) Moench) is a potential material for use in food, energy, and industry. It is an important source of proteins and minerals and a potential source of functional health promoting constituents, such as B group vitamins, fibers, antioxidant phenolic compounds (PC), and cholesterol-lowering waxes [1]. Sorghum grains are generally spherical, weighing about 20–30 mg, and can be white, red, yellow, bronze, or brown. It belongs to the grass family and is one of the cereals having internationally proven cost-effective, based on its low production cost and resistance to drought and thus, an important food source in semiarid regions of the world [2]. It has a lower cost than barley and sits in the fifth place in term of world grain production, after maize, rice, wheat, and barley. Besides, in Latin America its production exceeds barley (about 6.8 million tons vs. 3.7 million tons) (FAOSTAT data, 2014). As it is a gluten-free cereal, recently there has been increased interest in sorghum to substitute gluten containing cereals in the diet of people suffering celiac disease. At this time, the gluten-free diet remains the only available treatment [3]. Thus, sorghum is a growing alternative for food processing and beverages [4]. In this regard, African fermented beverages are developed from sorghum, but compared to barley beer are unsuccessful, since they have some problems such as low-enzymes and high starch gelatinization temperature [5]. Sorghum malting involves three steps: steeping, germination, and drying [6]. It is known that the germination conditions such as temperature and time

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Abbreviations: DB, dry basis; **DP,** diastatic power; **FPC,** free phenolic compounds; **G,** germinated grain; **G1,** one day germinated; **G2,** two days germinated; **G3,** three days germinated; **GA,** gallic acid; **GABA,** γ-aminobutyric acid; **N,** native grain; **PC,** phenolic compounds; **RS,** red sorghum; **S,** soaked grain; **WS,** white sorghum

affect the final quality of the malt [6, 7]. Malting results in mobilization of hydrolytic enzymes such as amylases and proteases, which are essential for the solubilization of starch and proteins in the grains, making them susceptible to fermentation [8].

Germination also increases the synthesis of secondary metabolites such as PC and γ-aminobutyric acid (GABA) [9, 10] as responses to stress generated during soaking and germination. GABA is a four-carbon non-protein amino acid occurring in both plants and animals [11], and plays an important role as neurotransmitter in mammal's brain cells [12]. It provides beneficial effects for human health by decreasing blood pressure, preventing chronic alcohol-relating diseases, and inhibiting cancer cell proliferation [13]. Thus, in addition to increase nutritional quality, grain germination promotes malt bioactivity, generating compounds capable of forming part of beer.

Some authors, mainly in Africa, have studied the effects of temperature and time of germination in the production of sorghum malt suitable for beer raw material [6, 14]. As far as we know, there is no literature related to evaluation of malting sorghum cultivars grown in Latin America, and more specifically in Argentina. It is known that the variety can influence the DP and starch quality [8]. In addition, there are not studies evaluating the influence of germination temperature on the accumulation of PC and GABA in sorghum malting processes. Therefore, the aim of this study was to determine the effect of temperature and germination time on chemical and enzymatic properties of sorghum starchy products from two Argentinean cultivars for obtaining high-quality malt for brewing and to evaluate the accumulation of relevant bioactive compounds due to sorghum typical malting process.

2 Materials and methods

2.1 Raw material

Two cultivars of sorghum (8706 W: white sorghum, WS and 8816: red sorghum, RS) were supplied by Pioneer Company, Pergamino, Buenos Aires, Argentina. The material was carefully cleaned and stored at 4°C. Both samples had good germinative power evaluated for 3 days (more than 90 germinated grains/100 grains) and the moisture content was 13 g/100 g.

2.2 Malting procedures

Sorghum cultivars, RS and WS (600 g), were washed by immersion for 20 min in sodium hypochlorite (1 g available chlorine/100 g), then drained and washed with distilled water. After that, the grains were soaked for 24 h at 25°C in a 1:3 grain-to-solution ratio. The water was changed at 12 h soaking. After that, the samples were washed with sodium hypochlorite (1 g available chlorine/100 g) for 10 min to retard mold growth during germination, then drained, and washed with distilled water. Subsequently, samples of soaked sorghum (90 g) were placed in aluminum plates of 9 cm diameter with a cotton base covered by filter paper, and were allowed to germinate in the dark at 25 or 30°C with 95% relative humidity in an oven (Bioelec®, Santa Fe, Argentina). Samples were collected every 24 h during 3 days. To complete the malting process, the samples were dried in a forced air oven (Bioelec®) at 50°C until less than 10 g/100 g moisture content. The root and sprout portions were manually removed. The grains were ground in a mill (Decalab Fbr®, Córdoba, Argentina) and the flours were stored at 4°C until analysis. Five samples were evaluated: native, N; soaked, S; and germinated for 1–3 days (G1, G2, and G3).

2.3 Diastatic power

Diastatic power (total reducing activity) was determined using the ferricyanide method according to AOAC [15] and results were expressed as °DP.

2.4 β-Amylase activity

β-Amylase was determined using the Megazyme kit Betamyl-3 Method: K-BETA 10/10 (©Megazyme International Ireland 2010). One unit of activity was defined as the amount of enzyme, in the presence of an excess of thermostable β-glucosidase, required to release 1 μmol of ρ-nitrophenol from *ρ*-nitrophenyl-β-D-maltotrioside (PNPβ-G3) substrate in one minute under the defined assay conditions, and is termed a Betamyl-3® unit. To facilitate comparison with other studies, the result was expressed in units of Betamyl®/g d.b. (based on *ρ*-nitrophenyl-α-D-maltopentaoside, PNPG5 substrate), considering the following relationship: units of Betamyl® substrate/Betamyl-3® substrate = 58.6.

2.5 Characterization of starchy products

Total starch (g/100 g dry basis, d.b.) was determined according to Tovar et al. [16]. To measure free glucose (g/100 g d.b.) a modified method of Holm et al. [17] without enzymatic hydrolysis step was used. For the colorimetric determination of glucose in both, total starch and free glucose analysis, a kit of glucose oxidase/peroxidase (Wiener Lab, Rosario, Argentina) was used. Absorbance was measured at 505 nm using a spectrophotometer Genesys 5 Milton Roy (Ivyland, USA).

Damaged Starch (g/100 g d.b.) was determined using the AACC Method [18], which determines the percentage of starch granules in flours sensitive to hydrolysis by α-amylase (Sigma A3306).

2.6 Free amino acids

Free amino acids were extracted from 0.05 g of flour samples in 1 mL of distilled water at room temperature. The extract was sonicated for 30 min, centrifuged at 3000 × *g* for 15 min at room temperature, and the supernatant was collected.

Free amino groups were measured using the method of o-phtaldialdehyde [19]. The results were expressed as μEq L-Serine/g d.b.

2.7 Bioactive compounds

Free phenolic compounds (FPC) and GABA were evaluated on N samples of both cultivars (WS and RS), and G3 samples at 25 or 30°C.

Phenolics were extracted as was mentioned for free amino acids. The method of Schanderl [20] using Folin–Ciocalteu reagent was used for FPC quantification. A standard curve with gallic acid (GA) solutions (0–100 mg/L) was used for calibration. The results of FPC were expressed as μg GA/g flour in dry basis (d.b.).

To determine GABA, samples (0.2 g) were extracted with trichloroacetic acid (8 g/100 mL), shaked during 60 min and centrifuged at 3000 × *g* for 10 min. Supernatant (500 μL) was added with 1500 μL of borate buffer (1 mol/L, pH 9). The content of GABA was determined according to Alaiz et al. [21] after derivatization with diethyl ethoxymethylenemalonate (Sigma D94208) using D,L-α-aminobutyric acid (Sigma A1879) as internal standard. The HPLC system consisted in a Perkin Elmer Series 200 pump, with Perkin Elmer 785A UV/vis detector, equipped with a 300 × 3.9 mm i.d. reversed-phase column (Novapack C18, 4 m; Waters). Eluted GABA was detected at 280 nm and expressed as mg/100 g d.b. using a concentration-response curve of 0–325 nmol GABA/mL (Sigma 03835).

2.8 Statistical analysis

All results were expressed as mean ± SD. The data were analyzed by one-way analysis of variance (ANOVA) and by Duncan's multiple range test using the software Statgraphics Centurion XV 15.2.06.

3 Results and discussion

3.1 Diastatic power and β-amylase activity

Figure 1 shows diastatic activity in the two sorghum cultivars germinated at 25 and 30°C for 3 days. The increase of °DP was observed from the second day of germination (G2), reaching the maximum value for each cultivar and germination temperature at the third day. However, the maximum °DP value was obtained at 25°C for RS and at 30°C for WS. At this temperature, no difference was observed between cultivars. Okoli et al. [6] obtained the maximum °DP value at the third day of germination at 30°C, and the value was depended on the Nigerian sorghum varieties analyzed. Agu and Palmer [14] found that germination temperature affected °DP, obtaining higher values when the cultivar was germinated at 30°C (~35 °DP) than 20°C (~28 °DP) on the third day of germination.

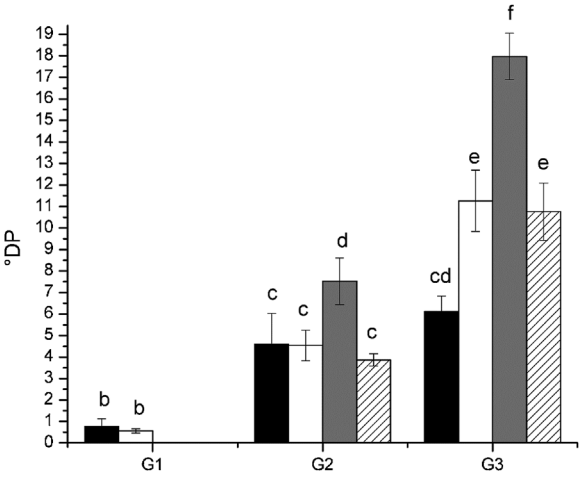

Figure 1. Diastatic power (°DP) in dry weight of WS and RS germinated at 25 and 30°C, during 3 days. References: black bar, WS 25°C; white bar, WS 30°C; gray bar, RS 25°C; striped bar, RS 30°C. Different superscripts show significant difference ($p < 0.05$) between samples.

On the third day, °DP values were lower than the minimum value specified for sorghum malt to sorghum brewery (28 °DP) [22]. However, the values obtained here have also been found in other studies [23, 24], and it is important to note that the cultivar influences on °DP value.

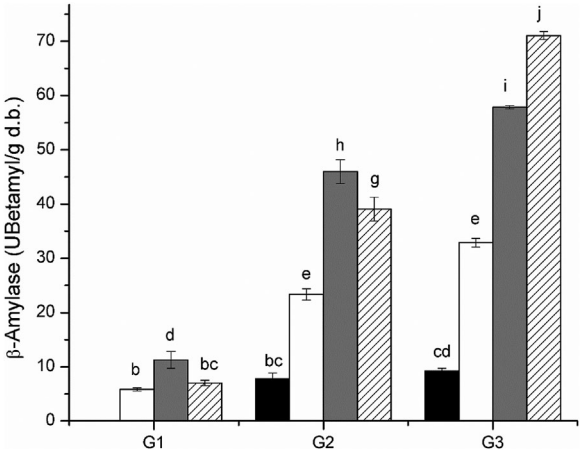

Figure 2. β-Amylase activity (Ubetamyl/g d.b.) of WS and RS germinated at 25 and 30°C during 3 days. References: black bar, WS 25°C; white bar, WS 30°C; gray bar, RS 25°C; striped bar, RS 30°C. Different superscripts show significant difference ($p < 0.05$) between samples.

β-Amylase activity of sorghum cultivars germinated at 25 and 30°C is shown in Fig. 2. Germination at 30°C significantly improved the activity of β-amylase of WS respect to germination at 25°C. For both temperatures, RS had higher activity than WS. Moreover, the maximum activity was on the third day of germination. The values for RS were 71.06 ± 0.74 and 57.86 ± 0.30 UBetamyl/g d.b. at 30 or 25°C, respectively. Also, Agu and Palmer [25] found β-amylase activity of sorghum varieties increased significantly when the grains were malted at 30°C, and they suggested that amylase activity was higher in red sorghum varieties than in white ones.

The values of β-amylase for RS were higher than those reported in other studies from 3 to 5 days of germination [4, 23], indicating that this variety germinated at 30°C could have a good saccharifying activity in the process of brewing.

3.2 Changes in total starch, damaged starch, and free glucose contents

The values of total starch for WS (63.8 ± 0.2 g/100 g d.b.) and RS (64.6 ± 0.4 g/100 g d.b.) native grains (N) were in agree with those reported by Leung [8], who studied starch content in seven varieties of sorghum and reported it was in a range of 62.3–78.6 g/100 g.

After 3 days of malting, both sorghum cultivars showed a decrease in total starch (Fig. 3). Total starch content decreased further along the germination time for RS, faster at 25°C, and RS values were lower than WS (50 vs. 56 g/100 g d.b.) in both, 25 or 30°C, reaching a loss of starch of 14 and 24 g/100 g d.b., respectively. This was related to higher β-amylase activity on RS. Total starch content had a strong correlation with β-amylase activity (total starch = 59.81–0.15 × β-amylase; *r*: −0.900), and there was a statistically significant relationship between the variables (*p*-value < 0.05). The loss of starch reached for WS on G3 (14 g/100 g d.b.) was similar to the percentage of loss of barley malt starch [26].

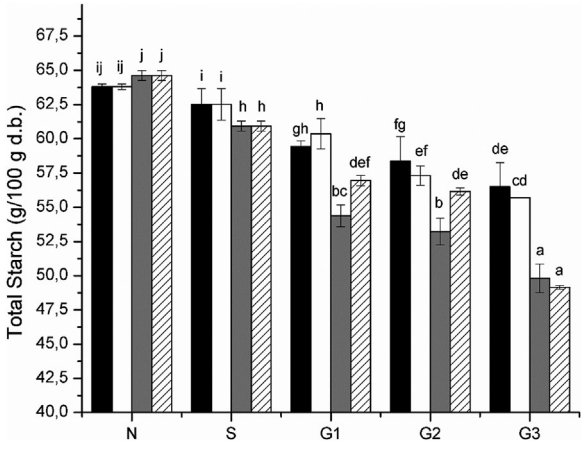

Figure 3. Changes in starch content (g/100 g d.b.) in dry weight of WS and RS germinated at 25 and 30°C during 3 days. References: black bar, WS 25°C; white bar, WS 30°C; gray bar, RS 25°C; striped bar, RS 30°C. Different superscripts show significant difference ($p < 0.05$) between samples.

Table 1. Damaged starch content (g/100 g d.b.) of native (N), soaked (S), and germinated sorghum cultivars at 1, 2, or 3 days of germination (G1, G2, and G3)

	WS		RS	
Sample	25°C	30°C	25°C	30°C
N	9.6 ± 1.2^{gh}	9.6 ± 1.2^{gh}	13.3 ± 1.3^{i}	13.3 ± 1.3^{i}
S	4.4 ± 0.5^{a}	4.4 ± 0.5^{a}	5.1 ± 0.5^{ab}	5.1 ± 0.5^{ab}
G1	5.2 ± 0.5^{ab}	7.1 ± 0.0^{cde}	5.5 ± 0.1^{abc}	6.3 ± 0.3^{bcd}
G2	6.9 ± 0.0^{cde}	8.2 ± 1.2^{efg}	7.9 ± 1.1^{def}	8.4 ± 0.7^{efgh}
G3	9.2 ± 0.7^{fgh}	9.0 ± 0.6^{fgh}	9.4 ± 0.5^{fgh}	10.0 ± 0.4^{h}

Values are means ± SD; d.b.: dry basis; values with the same superscript letter are not significantly different ($p < 0.05$).

Damaged starch content (g/100 g d.b.) is shown in Table 1. It decreased significantly after steeping due to a loss of starch in the soaking water. Starch granules are easily damaged by pressure, shear, or strain such as that applied by grinding procedures, and is particularly notable during flour milling. A normal starch granule is resistant to the action of digestive enzymes, but damaged starch has lost much of this resistance [27]. After soaking and during germination time, starch granules become partially digested, and the intergranular protein matrix is modified, allowing the starch granules becoming free [28].

Damaged starch content increased as germination time passed (Table 1) reaching its maximum value at the third day. Differences between varieties and germination temperatures were not found at the end of germination.

Unlike what happens with total starch content, free glucose increased as germination time, achieving maximum values on the third day, for both cultivars and germination temperatures (Table 2). Sorghum germinated at 30°C had the higher content of glucose on the third day. Furthermore, a negative correlation between total starch and glucose

Table 2. Free glucose content (g/100 g d.b.) of native (N), soaked (S), and germinated sorghum cultivars at 1, 2, or 3 days of germination (G1, G2, and G3)

	WS		RS	
Sample	25°C	30°C	25°C	30°C
N	0.11 ± 0.05^{a}	0.11 ± 0.05^{a}	0.10 ± 0.01^{a}	0.10 ± 0.01^{a}
S	0.46 ± 0.09^{a}	0.46 ± 0.09^{a}	0.49 ± 0.09^{a}	0.49 ± 0.09^{a}
G1	2.15 ± 0.27^{c}	2.22 ± 0.09^{cd}	1.96 ± 0.41^{c}	1.48 ± 0.18^{b}
G2	2.89 ± 0.07^{e}	3.95 ± 0.12^{gh}	2.54 ± 0.01^{de}	3.43 ± 0.28^{f}
G3	3.81 ± 0.12^{g}	4.56 ± 0.01^{i}	4.13 ± 0.19^{gh}	4.32 ± 0.10^{hi}

Values are means ± SD; d.b.: dry basis; values with the same superscript letter are not significantly different ($p < 0.05$).

amount (total starch = 62.97–2.29 × glucose; *r*: −0.789) suggests that starch loss is caused by hydrolysis, resulting in higher amount of glucose (p-value < 0.05). The results were higher than those reported by Agu and Palmer [25] who evaluated glucose content of sorghum malt extract germinated at 30°C for 3 days, but lower than those reported by Okoli et al. [6] to the 4 days of germination of three varieties of sorghum.

It is important to increase the amount of fermentable sugars and starch accessibility to enzymatic attack to make the process of obtaining wort and fermentation more effective [29]. Thus, the analysis of sorghum malt including enzyme activity, starchy products, and free glucose, allows estimate the extent of enzymatic action on starch granules.

3.3 Free amino acid content

Figure 4 shows the increase of the content of free amino acids during germination. The highest values for both varieties were found at 30°C on the third day of germination. RS showed higher values than WS (145.7 ± 3.7 vs. 125.9 ± 2.3 μEq L-Serine/g d.b. at 30°C, respectively). These results suggest that protease activity was modified significantly by germination temperature, achieving greater activity at 30°C. Kano et al. [26] also observed a higher content of free amino acids in a variety of sorghum germinated at 30°C compared to 20°C.

Free amino acids are produced by the hydrolysis of grain endosperm storage proteins by endogenous proteinase and peptidase enzymes [4]. It is important to evaluate free amino acid content because malt provides amino acids, small peptides, and larger polypeptides, and yeasts are only capable of assimilating simple amino acids and peptides, but not protein [6]. However, this leads not just to the assimilable nitrogenous materials, but also the liberation of the starch granules embedded in the protein matrix, thus promoting extract yield and wort fermentability [29]. In this sense, to study the correlation between increased free amino acids and starchy components a multivariate analysis was used. Correlation equations and coefficients (r) obtained for free amino acids versus total starch, damaged starch or free glucose were: total starch = 64.94–0.09 × free amino acids (*r*: −0.762); damaged starch = 2.49 + 0.05 × free amino acids (r: 0.941); and free glucose = -0.97 + 0.04 × free amino acids (*r*: 0.963), respectively, indicating a strong linear correlation between variables. In all cases, p-values less than 0.05 were obtained. This results suggested that hydrolysis of protein matrix effectively influenced the release of starch granules, which were more accessible to amylolytic action, increasing the damaged starch content and free glucose.

3.4 Bioactive compounds

Since samples germinated 3 days had higher enzymatic activity and free amino acid content, bioactive compounds (FPC and GABA) were evaluated on G3 samples and N as controls.

The content of FPC of N-RS was higher than that of N-WS (Fig. 5). It is well known that pigmented sorghum has more content of FPC than not pigmented ones [30]. However, the values increased after malting process being higher for WS. This could be due to the leaching of FPC during steeping, since phenolics are primary located in the pericarp and testa. In this sense, Lu et al. [9] reported a decreased in PC after steeping during barley malting. When the germination started, the extractable FPC increased at the same rate, both in WS and RS due to the synthesis of secondary metabolites [10], hydrolysis of proteins [31] and degradation of cell walls

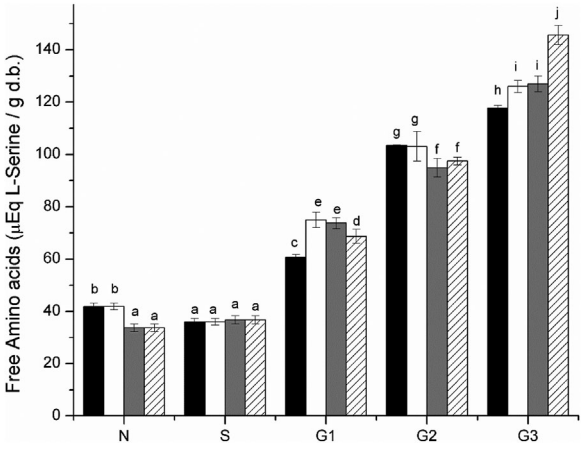

Figure 4. Changes in free amino-acids content (μEq L-serine/g d.b.) of WS and RS germinated at 25 and 30°C during 3 days. References: black bar, WS 25°C; white bar, WS 30°C; gray bar, RS 25°C; striped bar, RS 30°C. Different superscripts show significant difference ($p < 0.05$) between samples.

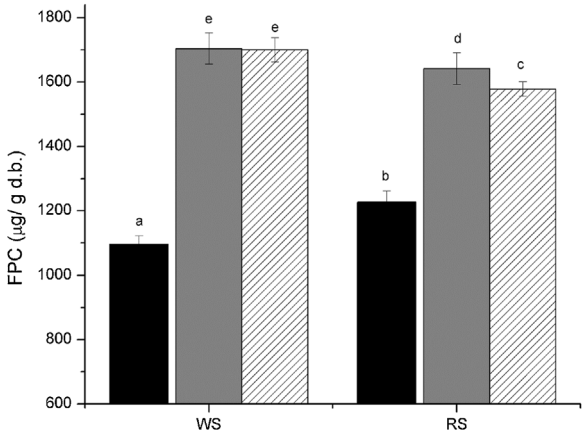

Figure 5. Accumulation of free phenolic compounds (FPC) (μg GA/ g d.b.) at third day of germination of WS and RS germinated at 25 and 30°C. References: black bar, native sorghum (N); gray bar, $T = 25$°C; striped bar, $T = 30$°C. Different superscripts show significant difference ($p < 0.05$) between samples.

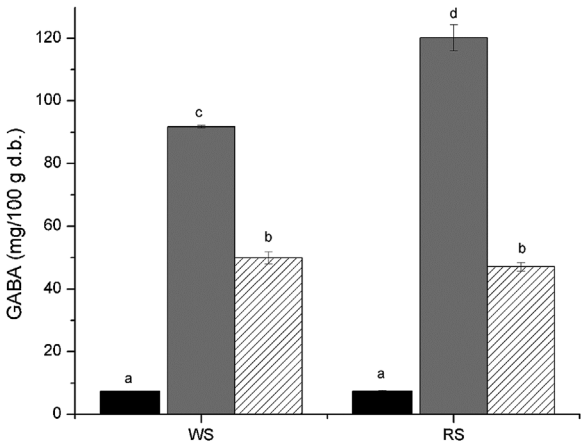

Figure 6. Accumulation of γ-aminobutyric acid (GABA) (mg/100 g d.b.) at third day of germination of WS and RS germinated at 25 and 30°C. References: black bar, native sorghum (N); gray bar, $T = 25$°C; striped bar, $T = 30$°C. Different superscripts show significant difference ($p < 0.05$) between samples.

during germination [32]. On the other hand, germination temperature had no effect in the case of WS, but RS germinated at 25°C presented higher FPC than RS germinated at 30°C. Dicko et al. [33] found germination decreased or increased phenolic content according to sorghum cultivar. Thus, these results could depend on analyzed cultivar.

Figure 6 shows the content of GABA for germinated and not germinated WS and RS samples. There was no difference between cultivars for N samples. The values obtained (7.4 mg/100 g d.b.) were similar to those reported for native barley [34] and brown rice [35]. The level of GABA increased after malting processes. For both cultivars, germination at 25°C allowed higher accumulation of GABA, achieving the maximum value for RS. Influence of cultivar on accumulation of GABA during germination also was found for two barley varieties [34]. Also, Xu et al. [13] observed different GABA levels in soybean germinated at different temperatures. Nevertheless, the range of GABA content obtained for both conditions and cultivars (47.1–120.1 mg/100 g d.b.) was higher to that reported in germinated barley (25.7–89.4 mg/100 g d.b.) by Kihara et al. [34] and in germinated soybean (14.6–23.8 mg/100 g d.b.) [13].

4 Conclusions

Germination at 30°C for 3 days were the most suitable conditions for obtaining a good sorghum malt with good enzymatic activity and high content of free amino acids and fermentable sugars, subtracts required for alcoholic fermentation. Taking into account the higher values of diastatic power, β-amylase activity, and free amino acids, RS was better than WS as starchy material for brewing.

One disadvantage of sorghum is the high starch gelatinization temperature. Therefore, damaged starch content can be an indicator to select malt containing higher amount of starch susceptible to the attack of enzymes to produce fermentable sugars.

Germinated sorghum grains had higher levels of FPC and GABA than native ones, and the content of these compounds depended on germination temperature and cultivar. Malted sorghum (besides of its enzymatic activity and free amino acids constituents) may become a good source of bioactive compounds that could be part of the end product. More studies will be needed to analyze the levels of these bioactive compounds after fermentation to obtain beer.

It is important to remark that the cultivar evaluated significantly influences on the characteristics of the malt, and to make this study on more and new varieties increases the chances of finding an optimal malt for beer production that achieves a high quality and bio-functional product to increase the spectrum of functional beverages for celiac growing population and the general consumer.

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