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ORIGINAL ARTICLE

Free α-amino acids, γ-Aminobutyric acid (GABA), phenolic compounds and their relationships with antioxidant properties of sorghum malted in different conditions

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Abstract Two cultivars of sorghum were germinated at 25 or 30 °C for 1, 2, or 3 days to investigate the evolution of γ-Aminobutyric acid (GABA), total free phenolic compounds (FPC), hydroxycinnamic acid derivatives, free amino acid (FAA) profile, and antioxidant activity during malting. Results showed time–temperature interaction had significant influence on GABA accumulation, increasing over time at 25 °C, but keeping constant after first day at 30 °C. Free amino acid profile changed during malting with time and temperature, increasing until the third or second day at 25 and 30 °C, respectively. Content of hydroxycinnamic acid derivatives depended on time, temperature, and cultivar; ferulic was the phenolic acid found in greater amount. Pearson correlation analysis suggested malting generated not only FPC responsible for antioxidant activity, but also other bioactive compounds like FAA, particularly sulfur-containing ones. Germination for 3 days at 25 °C was the most suitable condition to obtaining functional sorghum malt.

Keywords Antioxidant activity · GABA · Germination · Hydroxycinnamic acid derivatives · Sorghum

Introduction

Sorghum (*Sorghum bicolor* (L.) Moench) is a cereal for use in food, energy, and industry. It is an important source of proteins, minerals, B group vitamins, and health promoting constituents, such as fibers, antioxidant phenolics and cholesterol-lowering waxes. It belongs to the grass family and it is one of the cereals that has internationally proven cost-effective based on its low production cost and resistance to drought. Thus, it is an important food source in semiarid regions of the world (Ratnavathi et al. 2016). It is in the fifth place in terms of world grain production, after maize, rice, wheat, and barley (FAOSTAT 2014). Since sorghum is a gluten-free cereal, recently there has been increased interest in using it to substitute gluten containing cereals in the diet of people suffering celiac disease.

Germinated cereals have greater nutritional and physiological value than native cereal grains. Phytates are hydrolyzed during germination decreasing it concentration, and total free amino acids (FAA) increase because of the higher proteolytic activity (Baranwal 2017). Cereal malting involves three steps: steeping, germination, and drying. It is known that germination conditions, such as temperature and time, affect final quality of sorghum malt (Okoli et al. 2010).

Sorghum is recognized as a source of phenolic compounds (PC), the most predominant being caffeic acid (CA), ρ-coumaric acid (pCA), ferulic acid (FA), and sinapic acid (SA). All of them are hydroxycinnamic acid derivatives and may provide health benefits. Several studies have demonstrated the relationship between food phenol content and antioxidant activity (Lu et al. 2007). In sorghum and other cereals, the synthesis of secondary metabolites such as phenolics increases during malting as a response to the stress generated during soaking and

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germination (Lu et al. 2007; Baranwal 2017). Thus, in addition to improve the nutritional quality, germination increases the bioactivity of malted flour, which is very important due to the increasing interest in natural products with bio-activity.

On the other hand, studies in barley and rice have shown an increment of γ-Aminobutyric acid (GABA) in soaked and germinated grains (Kihara et al. 2007; Zhang et al. 2014; Lee et al. 2017). GABA is a four-carbon non-protein amino acid occurring in both, plants and animals (Zhang et al. 2014), which play an important role as neurotransmitter in mammal's brain cells (Bouché and Fromm 2004). GABA provides beneficial effects for human health by decreasing blood pressure, preventing chronic alcohol-relating diseases, and inhibiting cancer cell proliferation (Xu and Hu 2014). Other physiological functions such as relaxation, sleeplessness, and depression have been treated with GABA. Furthermore, consumption of GABA-enhanced brown rice can inhibit leukemia cell proliferation (Diana et al. 2014). Typically, GABA levels in plant tissues are low (0.3–20 mg/100 g fresh weight), but they increase several folds in response to many diverse stimuli, including heat shock, mechanical stimulation, hypoxia, and phytohormones (Shelp et al. 1999).

Most of the studies about malting processes have focused on measuring enzymatic activity required for wort production, without searching for the optimum conditions to maximize the development of bioactive compounds. As far as we known, there are not reports neither about the influence of germination parameters on the accumulation of GABA in germinated sorghum, nor the effect of germination temperature on total and individual polyphenol content in aqueous extracts, nor the change in the profile of FAA due to the effects of temperature and germination time. The aims of this study were to determine the effects of steeping, temperature, and germination time on the profile of phenolic acids, GABA content, free amino acids, and antioxidant properties, and to establish the relationships among antioxidant mechanisms and such bioactive compounds from aqueous extracts of sorghum malted in different conditions.

Materials and methods

Raw materials

Two cultivars of sorghum (8706 W: white sorghum, WS; 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.

Chemicals

Diethyl ethoxymethylenemalonate (D94208), α-aminobutyric acid (A1879), GABA (03835), amino acid standard solution (AAS18), 2,20-azino-bis-3-ethylbenzothiazoline-6-sulphonic acid (ABTS) (A1888), 6-hydroxy-2,5,7,8-tetramethylchroman-2-carboxylic acid (Trolox) (238813), β-carotene (C9750), caffeic acid (C0625), trans-ferulic acid (46278), p-coumaric acid (C9008) and trans-sinapic acid (93878) were obtained from Sigma Chemical Co. (St. Louis, MO, USA). All other chemicals and reagent used in the experiments were of analytical grade.

Malting procedures

Samples of RS and WS were conditioned, soaked, and germinated as was descript by Garzón et al. (2016). Briefly, grains (1200 g) were soaked for 24 h at 25 °C in a 1:3 grain-to-solution-ratio. Soaked grains (100 g) were germinated in the dark at 25 or 30 °C with 95% relative humidity in an oven (Bioelec®, Santa Fe, Argentina) during 1, 2, and 3 days.

Then, germinated samples were dried until less than 10 g/100 g moisture content. The following samples were evaluated: native N; soaked S; and germinated for 1–3 days (G1, G2, G3) for both, WS and RS germinated at 25 or 30 °C. Triplicates of native and treated samples were performed for each temperature (25 or 30 °C).

Determination of GABA and free amino acid content (FAA)

To determine FAA content including GABA, sample flours (0.2 g) were extracted 60 min with trichloroacetic acid (8 g/100 mL) at room temperature, and centrifuged 10 min at 3000×*g*. The supernatants were collected and 500 µL were added with 1500 µL of borate buffer (1 mol/L, pH 9). The content of individual FAA and GABA was determined according to Alaiz et al. (1992) after derivatization with diethyl ethoxymethylenemalonate by high-performance liquid chromatography (HPLC), using α-aminobutyric acid 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). A binary gradient was used for elution with a flow of 0.9 mL/min. The solvents used were sodium acetate (25 mmol/L) containing sodium azide (0.02 g/100 mL) pH 6.0 and acetonitrile. Eluted FAA were detected at 280 nm and expressed as mg/100 g d.b using a concentration–response

curve of 0–120, 0–325 and 0–200 μmol/mL for Cys, GABA, and all the others FAA, respectively.

Sample extraction

To measure phenolics and antioxidant activity, 0.05 g of flour samples were extracted with 1 mL distilled water at room temperature (25 °C), sonicated for 30 min, and centrifuged 15 min at 3000×*g*. The extracts were stored at − 20 °C until analysis.

Determination of total free phenolic compounds (FPC)

Total free phenolic compounds (FPC) were quantified according to Schanderl (1970) using Folin–Ciocalteu reagent. A standard curve of gallic acid (GA, 0–100 mg/L) was used for calibration. The results were expressed as μg GA/g flour in dry basis (d.b.).

Phenolic compound profile

An acid hydrolysis for the cleavage of conjugated and condensed soluble phenolic compounds was carried out according to Cian et al. (2012b). Aliquots of 200 μL of water extracts and 100 μL of 6 mol/L HCl were heated for 50 min at 90 °C. After hydrolysis, samples were allowed to cool and filtered through a Millipore 0.45 μm pore size filter. Compounds were separated on a 250 mm × 4.6 mm, 5 μm particle size, Gemini 110A C-18 Phenomenex column. The mobile phase was isocratic, prepared from 16% acetonitrile in acetic acid (1% in water). Ten min of equilibration was required before the next injection. The flow rate was 0.7 mL/min and the analyses were done for 35 min at room temperature (25 °C). Eluted hydroxycinnamic acids (CA, caffeic acid; pCA, p-coumaric acid; FA, ferulic acid, and SA, sinapic acid) were detected at 320 nm and expressed as μg/g d.b. using a concentration response curve of 0–50 μg/mL. Peak identification was performed by comparison of retention times and spectral characteristics with external standards. Data were processed using Shimadzu LC solution software.

Antioxidant properties

Trolox equivalent antioxidant capacity (TEAC)

The scavenging of ABTS was measured according to Cian et al. (2012a). To estimate the TEAC, a concentration–response curve for ABTS inhibition as a function of Trolox standard concentration (0–2.5 mmol/L in 0.01 mmol/L PBS, pH 7.4) was performed. The absorbance reading was taken at 734 nm after 6 min initial mixing, and results were expressed as μmol Trolox/g d.b.

Copper-chelating activity by assay of β-carotene oxidation

Copper-chelating activity (CCA) was determined by the assay of β-carotene oxidation according to Megías et al. (2008), and was calculated using a concentration–response curve for absorbance at 470 nm as a function of concentration of EDTA (ethylenediaminetetraacetic acid) standard solution (20–100 mg/L) in phosphate buffer. Results were expressed as mg EDTA/g d.b.

$$CCA(\%) = 100 - \frac{(Abs\ control(-) - Abs)}{Abs\ control(-) - Abs\ control(+)} \times 100$$

where Abs control (−) is the absorbance of negative control (200 μL β-carotene + 20 μL buffer phosphate); Abs control (+) is the absorbance of positive control (200 μL β-carotene + 10 μL $CuSO_4$ + 10 μL buffer phosphate); and Abs is the absorbance of sample (200 μL β-carotene + 10 μL CuSO4 + 10 μL sample).

Reducing power activity

Reducing power activity (RP) of flour extracts was determined according to Cian et al. (2015a). A standard curve with ascorbic acid (AA, 0–0.05 mg/mL in phosphate buffer) was used. The RP was expressed as mg AA/g d.b.

Statistical analysis

Each experiment was performed at least by duplicate. All results were expressed as mean ± SD. The data were analyzed by one-way analysis of variance (one-way ANOVA), multiple-way analysis of variance (multifactorial ANOVA) to evaluate interaction between variables, Duncan's multiple range test to determine differences, and Pearson correlation test to determine the correlation among means. Statgraphics Centurion XV 15.2.06 software was used.

Results and discussion

GABA content

Previously, Garzón et al. (2016) reported GABA content of two raw cultivars (7.4 mg GABA/100 g d.b.), which increased after steeping in a greater extension for RS than WS (44.3 ± 2.6 vs. 36.3 ± 0.4 mg GABA/100 g d.b., respectively). Kihara et al. (2007) also found steeping incremented GABA levels of two barley varieties in

different way. Water soaking lead to hypoxia in grains due to the limited availability of oxygen, and GABA content may increase rapidly in plant tissues in response to hypoxia (Kihara et al. 2007).

The GABA content increased after whole malting process (Fig. 1) and it depended on germination temperature, time, and cultivar ($p < 0.05$). The GABA content of sorghum germinated at 25 °C was higher than that obtained at 30 °C ($p < 0.05$), for both cultivars in all germination days, and it was higher for RS. On the third day of germination at 25 °C, GABA level achieved the higher value, and increased 12 and 16-fold for WS and RS, respectively ($p < 0.05$), compared with each native sample. However, germination of WS at 30 °C had less effect in GABA content, achieving a sevenfold increment on G3, while for RS, GABA was not influenced by time, achieving a sevenfold increment for all germination days compared with N-RS. The system of generation of GABA has been attributed to glutamate as substrate, GAD as glutamate decarboxylase to produce GABA, and GABA transaminase (GABA-T) to catalyze the reversible conversion of GABA to succinic semi-aldehyde (Shelp et al. 1999). It seems that GAD activity was raised as the germination go on at 25 °C, but it did not increase with time at 30 °C, and thus GABA was continually produced in the first case, and was accumulated only at the beginning of germination at higher temperature. Zhang et al. (2014) demonstrated that GABA content varied with different germination temperatures (20, 25, 30, 35, and 40 °C) in brown rice. Moreover, several studies found a relationship between germination temperature, time, and cultivar on GABA accumulation in barley (germinated at 15 °C for 24, 48, 72 and 144 h) (Kihara et al. 2007), brown rice (germinated at 28 °C for 12, 24 and 48 h) (Cornejo et al. 2015) and soybean (germinated at 19, 25 and 32 °C for 30, 54, 78 and 102 h) (Xu and Hu 2014).

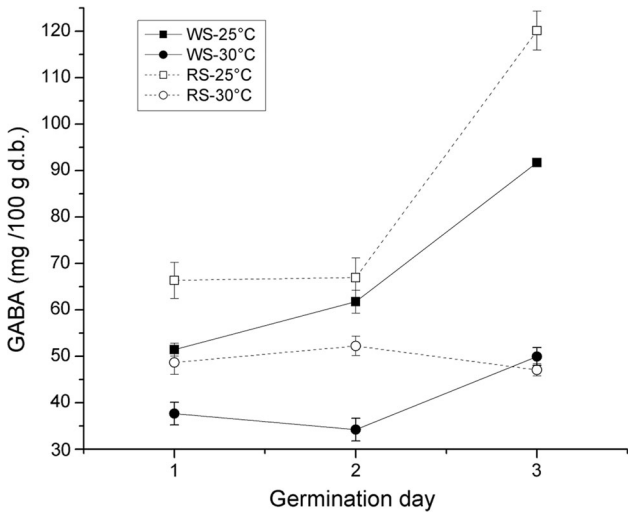

Fig. 1 Accumulation of GABA (mg GABA/100 g d.b.) in WS and RS germinated at 25 and 30 °C during three days

Free amino acid content

Levels of total FAA in native sorghum grains were low (4.1 and 5.3 μEq total FAA/g d.b. for WS and RS, respectively) and incremented threefold after soaking (14.9 and 16.9 μEq total FAA/g d.b. for WS and RS, respectively). As happened with GABA, the content of all amino acids increased during steeping. Kihara et al. (2007) found the value of all FAA of barley increased after 48 h grain imbibition process.

The content of each FAA of germinated samples is showed in Table 1. In general, there was an effect of the interaction time × temperature ($p < 0.05$) for both cultivars. This means that both factors together were relevant on the distribution of FAA during germination. At the first day, there was a higher increase for most amino acids of both cultivars at 30 °C than at 25 °C. Moreover, a tendency of increment as time passed was observed, except for Lys, which showed a different accumulation pattern than the other amino acids. Its content increased at the first day of germination, and then decreased to the third day. Pal et al. (2016) studied changes in amino acid composition after brown rice germination, and found for whole grain that basic amino acid decreased, while acidic amino acid increased after germination. Moreover, Malleshi and Klopfenstein (1998) found higher proportion of Lys in rootlets than in germinated sorghum grains due to migration of a Lys-rich portion of protein from the germ and endosperm.

Additionally, at 30 °C most of FAA increased until the second day of germination, and after that, the level of the individual FAA decreased or was kept constant; while at 25 °C the content generally increased until the third day. The activity of proteases depends on the temperature and time the grains are maintained at that temperature. Aisien et al. (1983) studied the endoprotease activity during germination at 25 °C for 96 h and found that this activity incremented until 72 h and declined by 96 h. However, GABA production during malting indicates that FAA content does not simply result from the cleavage of storage proteins. As GABA is a non-protein amino acid at least some transamination must take place (Taylor 1983).

Total free phenolic compound content

Content of FPC of different sorghum extracts (N, S, G1, G2, and G3) for WS and RS germinated at 25 or 30 °C is showed in Table 2. The level of FPC of N-RS was greater than that of N-WS. It is well known that pigmented

Table 1 Content of free amino acids of sorghum germinated at 25 or 30 °C

FAA (mg/100 g d.b.)	25 °C			30 °C		
	G1	G2	G3	G1	G2	G3
White Sorghum						
Asp + Glu*	$27.93^{a} \pm 1.66$	$61.77^{c} \pm 0.67$	$79.21^{d} \pm 2.85$	$54.86^{b} \pm 2.86$	$55.91^{bc} \pm 2.51$	$84.43^{d} \pm 1.01$
Ser*	$14.69^{a} \pm 0.12$	$60.85^{b} \pm 2.85$	$79.42^{c} \pm 1.14$	$20.63^{a} \pm 1.14$	$75.76^{c} \pm 2.29$	$79.64^{c} \pm 3.71$
His*	$26.83^{a} \pm 0.57$	$34.04^{cd} \pm 0.53$	$37.88^{d} \pm 3.08$	$32.43^{bcd} \pm 2.55$	$51.61^{e} \pm 4.21$	$30.37^{bc} \pm 0.14$
Gly*	$20.00^{a} \pm 0.43$	$29.26^{bc} \pm 0.28$	$39.20^{d} \pm 0.74$	$29.01^{b} \pm 1.49$	$32.30^{c} \pm 1.49$	$37.13^{d} \pm 1.59$
Thr*	$27.42^{a} \pm 0.25$	$45.68^{b} \pm 3.66$	$54.06^{c} \pm 0.94$	$39.84^{b} \pm 3.66$	$61.79^{d} \pm 2.71$	$56.20^{cd} \pm 0.07$
Arg*	$40.95^{a} \pm 0.64$	$42.27^{a} \pm 1.45$	$53.34^{b} \pm 3.46$	$54.90^{b} \pm 5.49$	$76.71^{c} \pm 6.86$	$62.84^{b} \pm 3.36$
Ala*	$47.19^{a} \pm 1.72$	$91.74^{cd} \pm 1.56$	$97.98^{d} \pm 2.36$	$68.70^{b} \pm 2.36$	$70.19^{b} \pm 4.36$	$87.84^{c} \pm 1.79$
Pro***	$14.59^{a} \pm 0.32$	$31.80^{c} \pm 1.38$	$22.82^{b} \pm 0.25$	$15.11^{a} \pm 1.38$	$30.18^{c} \pm 2.34$	$23.79^{b} \pm 1.87$
Tyr*	$26.02^{a} \pm 0.60$	$47.09^{b} \pm 0.68$	$62.90^{c} \pm 7.50$	$39.34^{b} \pm 4.56$	$85.64^{d} \pm 1.30$	$58.50^{c} \pm 2.84$
Val*	$39.35^{a} \pm 1.42$	$45.53^{a} \pm 2.34$	$56.68^{b} \pm 3.25$	$55.87^{b} \pm 1.42$	$73.42^{c} \pm 7.24$	$57.54^{b} \pm 1.77$
Met*	$4.16^{a} \pm 0.12$	$11.26^{b} \pm 1.19$	$16.35^{c} \pm 0.66$	$14.92^{c} \pm 0.49$	$22.22^{e} \pm 0.50$	$18.61^{d} \pm 0.63$
Cys*	$0.93^{a} \pm 0.09$	$5.01^{bc} \pm 0.66$	$6.81^{d} \pm 0.25$	$3.84^{b} \pm 0.09$	$9.98^{e} \pm 0.66$	$6.35^{cd} \pm 0.69$
Ile*	$18.71^{a} \pm 0.50$	$29.89^{b} \pm 0.78$	$41.94^{c} \pm 0.33$	$29.74^{b} \pm 0.91$	$41.34^{c} \pm 2.64$	$39.83^{c} \pm 0.45$
Leu*	$31.84^{a} \pm 0.94$	$64.21^{b} \pm 2.21$	$114.70^{d} \pm 1.30$	$77.61^{c} \pm 2.21$	$110.35^{d} \pm 8.26$	$105.78^{d} \pm 2.86$
Phe*	$22.51^{a} \pm 0.42$	$44.19^{b} \pm 2.32$	$62.06^{c} \pm 1.16$	$42.65^{b} \pm 1.22$	$92.32^{d} \pm 7.68$	$61.99^{c} \pm 4.65$
Lys*	$29.71^{a} \pm 0.58$	$31.47^{a} \pm 1.80$	$32.90^{a} \pm 2.04$	$44.02^{b} \pm 1.80$	$32.71^{a} \pm 0.60$	$33.77^{a} \pm 0.97$
Ratio	7.34	12.77	15.98	11.98	16.79	15.60
Red Sorghum						
Asp + Glu*	$20.21^{a} \pm 1.72$	$51.26^{b} \pm 1.58$	$62.54^{bc} \pm 1.94$	$66.88^{c} \pm 2.02$	$59.76^{bc} \pm 2.51$	$98.30^{d} \pm 0.08$
Ser***	$17.82^{a} \pm 1.62$	$64.38^{b} \pm 3.64$	$79.84^{c} \pm 2.08$	$13.68^{a} \pm 0.45$	$59.95^{b} \pm 2.03$	$77.92^{c} \pm 2.08$
His**	$16.21^{Aa} \pm 0.57$	$43.63^{Ab} \pm 1.85$	$52.10^{Ac} \pm 2.54$	$22.91^{Ba} \pm 1.39$	$48.42^{Bb} \pm 0.95$	$60.89^{Bc} \pm 4.87$
Gly*	$17.11^{a} \pm 0.01$	$30.34^{b} \pm 1.48$	$73.60^{e} \pm 5.27$	$23.67^{ab} \pm 0.06$	$45.10^{c} \pm 0.81$	$63.84^{d} \pm 4.27$
Thr*	$24.34^{a} \pm 0.18$	$38.89^{c} \pm 0.13$	$78.00^{f} \pm 0.13$	$31.50^{b} \pm 0.35$	$59.00^{d} \pm 1.74$	$68.85^{e} \pm 0.48$
Arg**	$28.05^{Aa} \pm 4.07$	$40.85^{Ab} \pm 1.30$	$85.19^{Ac} \pm 7.56$	$46.84^{Ba} \pm 0.39$	$60.89^{Bb} \pm 7.74$	$84.96^{Bc} \pm 5.24$
Ala*	$41.41^{a} \pm 0.94$	$77.78^{c} \pm 1.03$	$124.15^{e} \pm 2.10$	$53.23^{b} \pm 2.10$	$86.27^{d} \pm 6.96$	$78.58^{cd} \pm 1.11$
Pro**	$8.18^{Aa} \pm 0.37$	$19.87^{Ab} \pm 1.19$	$42.81^{Ac} \pm 2.28$	$12.79^{Ba} \pm 0.42$	$21.32^{Bb} \pm 1.87$	$47.80^{Bc} \pm 0.20$
Tyr*	$13.42^{a} \pm 1.22$	$43.10^{c} \pm 0.59$	$123.97^{e} \pm 6.50$	$29.60^{b} \pm 0.25$	$53.29^{c} \pm 6.64$	$110.65^{d} \pm 6.50$
Val*	$2.84^{a} \pm 0.11$	$45.06^{c} \pm 0.66$	$74.69^{f} \pm 2.38$	$38.69^{b} \pm 1.92$	$67.55^{e} \pm 2.34$	$56.67^{d} \pm 2.38$
Met*	$3.75^{a} \pm 0.14$	$8.77^{b} \pm 0.33$	$22.99^{cd} \pm 2.43$	$12.11^{b} \pm 0.49$	$19.91^{c} \pm 2.08$	$23.64^{d} \pm 1.11$
Cys*	ND	$2.15^{a} \pm 0.03$	$9.04^{c} \pm 0.68$	$1.86^{a} \pm 0.09$	$4.00^{b} \pm 0.06$	$5.06^{b} \pm 0.13$
Ile*	$13.43^{a} \pm 0.92$	$25.39^{b} \pm 0.12$	$48.22^{d} \pm 5.22$	$22.60^{b} \pm 0.91$	$38.25^{c} \pm 3.52$	$34.22^{c} \pm 0.41$
Leu*	$21.08^{a} \pm 0.90$	$48.89^{b} \pm 0.24$	$137.13^{d} \pm 14.91$	$56.21^{b} \pm 1.47$	$99.80^{c} \pm 10.77$	$90.57^{c} \pm 2.98$
Phe*	$15.28^{a} \pm 1.22$	$39.10^{b} \pm 0.93$	$119.76^{e} \pm 12.24$	$30.66^{b} \pm 0.91$	$67.06^{c} \pm 1.99$	$95.84^{d} \pm 1.14$
Lys*	$26.98^{b} \pm 1.70$	$27.63^{b} \pm 1.31$	$19.16^{a} \pm 1.86$	$39.58^{c} \pm 1.49$	$48.20^{d} \pm 3.42$	$23.17^{ab} \pm 3.65$
Ratio	4.05	9.04	17.04	7.04	12.33	14.52

FAA free amino acid, *Ratio* total FAA of G sample (μEq g^{-1}d.b)/total FAA of N sample (μEq/g d.b). Values with the same superscript letter in a row are not significantly different ($p < 0.05$), *ND* not detected

*Significantly interaction between time and temperature ($p < 0.05$); **principal effect of time and temperature ($p < 0.05$), capital superscript letter for time, small superscript letter for temperature; ***principal effect only for time ($p < 0.05$)

sorghum has more content of phenolics than not pigmented ones.

Steeping decreased 31 and 38% FPC compared with N-grains for WS and RS, respectively. Also, phenolics of two barley varieties decreased after steeping, probably due to the leaching of phenolic compounds located in pericarp and testa (Lu et al. 2007). The levels of FPC increased gradually from G1 to G3 due to the synthesis of secondary metabolites. Also, the increase of protease activities

Table 2 Total free phenolic compounds (FPC) of native (N), soaked (S), and germinated sorghum cultivars at 1, 2, or 3 days of germination (G1, G2, G3)

Sample	FPC (μg GA/g d.b.)			
	White Sorghum		Red Sorghum	
	25 °C	30 °C	25 °C	30 °C
N	$1095.6^{cd} \pm 26.5$		$1227.4^{e} \pm 34.7$	
S	$753.4^{a} \pm 26.5$		$757.4^{a} \pm 46.8$	
G1	$1104.9^{cd} \pm 56.8$	$1129.1^{d} \pm 21.5$	$1081.2^{c} \pm 13.6$	$923.2^{b} \pm 15.5$
G2	$1396.0^{fg} \pm 49.8$	$1542.9^{h} \pm 23.6$	$1406.6^{g} \pm 12.9$	$1357.7^{f} \pm 9.6$
G3	$1703.7^{j} \pm 78.1$	$1699.9^{j} \pm 37.9$	$1642.0^{i} \pm 49.4$	$1577.5^{h} \pm 23.1$

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

(Aisien et al. 1983) decreases polyphenol-protein interactions facilitating FPC extraction (Cian et al. 2015a).

Red cultivar germinated at 25 °C presented higher FPC than at 30 °C in all germination days, while temperature did not impact on FPC for WS. Dicko et al. (2005) found germination decreased or increased phenolic content according to sorghum cultivar, and Garzón et al. (2016) reported germination temperature influenced on final content of FPC depending on cultivar.

Phenolic compound profile

The content of hydroxycinnamic acid derivatives of different sorghum extracts (N, S, G1, G2, and G3) for WS and RS germinated at 25 or 30 °C is showed in Table 3.

Water extracts of native samples presented contents of FA, CA, and pCA around 26–28, 11–17, and 7–9 μg/g d.b., respectively, but SA was not detected. As in other cereals, phenolic acids of sorghum are mostly concentrated in the bran, and mainly exist in bound forms, ferulic acid being the most abundant bound phenolic in sorghum (Awika and Rooney 2004). Ferulic acid ranges 120.5–173.5 μg/g, while other phenolics are in small amounts in sorghum varieties, such as pCA (41.9–71.9 μg/g), CA (13.6–20.8 μg/g), and SA (41.4–78.6 μg/g) (Morais-Cardoso et al. 2015). According to those values, water extracts of native sorghum represent about 20, 14, and 90% of total FA, pCA, and CA, respectively, while SA was not detected.

After soaking, pCA and FA decreased, in accordance to FPC, but CA increased, and SA was not detected. It is possible that during this process, a balance between CA leaching and synthesis due to stress conditions is produced. Afify et al. (2012) found a reduction in pCA, FA, and CA during sorghum soaking, due to leaching of phenols in the soaking medium, but the reduction of CA was smaller than FA and pCA (1, 65, and 44%, respectively).

During germination, phenolic content was depended on germination temperature, time, and cultivar ($p < 0.05$). Sinapic acid (SA) was not detected in water extracts of sorghum germinated at 30 °C, but it was detected in the following samples germinated at 25 °C: G2-WS and G1, G2, and G3-RS (2.3 ± 0.1, 3.3 ± 0.5, 5.1 ± 0.3, and 3.2 ± 0.1 μg/g d.b., respectively).

The higher values of CA, pCA, and FA content were obtained for WS on the second day of germination at 25 °C (G2-WS sample). On the other hand, at 30 °C, the higher content of CA and pCA was observed on G1; while FA content presented a profile similar to FPC, the higher content being achieved at G3. Growing conditions, such as temperature, are known to affect phenolic acid content in sorghum.

Germination incremented the amount of phenolics extracted in aqueous medium. This indicates the potential bioactive compounds that could be present in a malted sorghum beverage.

Antioxidant properties

Different mechanisms can be implied in antioxidant activity of phytochemicals (Dicko et al. 2005). Thus, evaluation of antioxidant properties of sorghum samples was performed using three different methods. The capacity of bioactive compounds to scavenge free radicals was monitored by ABTS assay. Copper chelating assay evaluate chelating agents which may inhibit radical generation by stabilizing transition metals, and reducing power may serve as an indicator of electron donor compounds (Zhao et al. 2008).

Figure 2 shows the results obtained with these three antioxidant assays. In all of them (with the exception of CCA for RS) there was a decrease in antioxidant activity after steeping, which was in accordance with the reduction of FPC after soaking. Moreover, the antioxidant properties increased from G1 to G3. In this sense, Lu et al. (2007) evaluated ABTS, Fe^{2+} chelating activity, and reducing power during barley malting, and also found a reduction of

Table 3 Cinnamic acid derivatives (μg/g d.b.) of native (N), soaked (S), and germinated sorghum cultivars at 1, 2, or 3 days of germination (G1, G2, G3)

Sample	Caffeic acid (μg/g d.b.)				p-Coumaric acid (μg/g d.b.)				Ferulic acid (μg/g d.b.)			
	WS		RS		WS		RS		WS		RS	
	25 °C	30 °C	25 °C	30 °C	25 °C	30 °C	25 °C	30 °C	25 °C	30 °C	25 °C	30 °C
N	$11.3^{a} \pm 0.5$		$17.2^{b} \pm 0.3$		$7.1^{c} \pm 0.1$		$8.9^{e} \pm 0.2$		$26.0^{de} \pm 0.4$		$28.4^{f} \pm 0.6$	
S	$19.5^{c} \pm 0.0$		$22.2^{d} \pm 0.5$		$7.5^{c} \pm 0.1$		$6.5^{b} \pm 0.5$		$18.3^{b} \pm 0.4$		$17.7^{ab} \pm 1.2$	
G1	$22.0^{d} \pm 0.9$	$22.8^{d} \pm 0.3$	$24.8^{e} \pm 0.3$	$23.1^{d} \pm 0.5$	$8.8^{e} \pm 0.0$	$7.3^{c} \pm 0.1$	$8.3^{d} \pm 0.3$	$5.0^{a} \pm 0.1$	$29.5^{fg} \pm 1.0$	$22.4^{c} \pm 0.0$	$34.8^{h} \pm 0.5$	$16.8^{a} \pm 0.1$
G2	$28.9^{g} \pm 0.4$	$20.0^{c} \pm 1.0$	$27.3^{f} \pm 0.3$	$17.9^{b} \pm 1.3$	$10.7^{g} \pm 0.2$	$7.1^{c} \pm 0.0$	$10.2^{f} \pm 0.2$	$5.1^{a} \pm 0.2$	$44.0^{k} \pm 0.5$	$30.3^{g} \pm 1.5$	$40.1^{j} \pm 0.5$	$25.2^{d} \pm 0.2$
G3	$19.5^{c} \pm 0.3$	$12.3^{a} \pm 0.3$	$20.6^{c} \pm 0.4$	$20.0^{c} \pm 1.1$	$8.4^{de} \pm 0.1$	$6.0^{b} \pm 0.0$	$8.6^{de} \pm 0.0$	$6.4^{b} \pm 0.2$	$27.0^{e} \pm 0.1$	$29.3^{fg} \pm 0.2$	$38.1^{i} \pm 0.4$	$35.7^{h} \pm 0.2$

Values are mean ± SD; d.b., dry basis; values with the same superscript letter for each phenolic acid are not significantly different ($p < 0.05$)

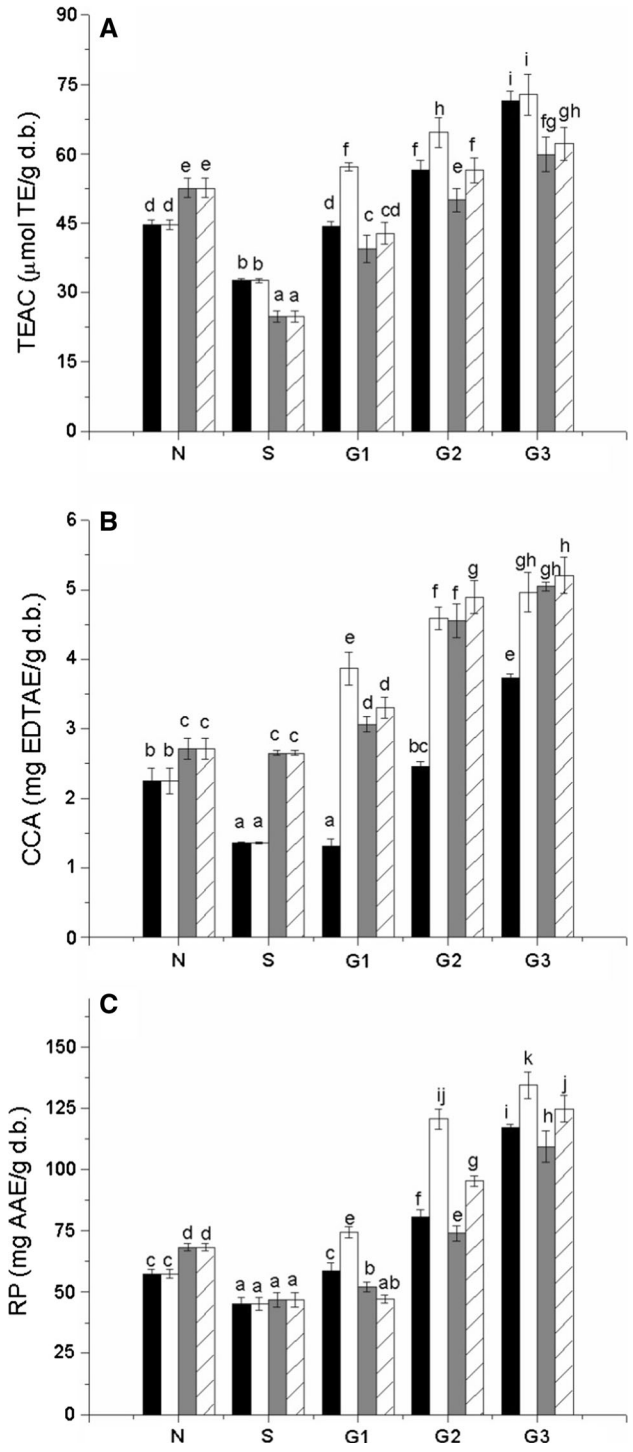

Fig. 2 Effect of germination conditions on antioxidant activities of two sorghum cultivars (WS and RS). **a** Trolox equivalent antioxidant capacity (TEAC). **b** Copper chelating activity (CCA). **c** Reducing power activity (RP). References: black bar, WS 25 °C; white bar, WS 30 °C; gray bar, RS 25 °C; striped bar, RS 30 °C. Different superscripts show significant difference ($p < 0.05$) between samples

antioxidant activity after steeping, and an increase during germination.

The TEAC obtained for WS after whole process was higher than that of RS at both temperatures, probably due TEAC was higher after steeping (Fig. 2a). Lu et al. (2007) observed less ABTS activity of one germinated barley cultivar in accordance with the lesser TEAC after soaking step. On the other hand, TEAC values for sorghum malted for 3 days at 25 or 30 °C (60–73 µmol Trolox/g d.b.) were in the range of those obtained by Dicko et al. (2005) over 50 varieties of red and white sorghum germinated at 27 °C for 3 days (20–70 µmolTrolox/g d.b.), and were higher than fourteen typical malting barley varieties (11–13 µmolTrolox/g d.b.) reported by Zhao et al. (2008).

Contrary to what happened with TEAC, the value of CCA after steeping was higher for RS than for WS (Fig. 2b), suggesting a different nature of bioactive compounds having these activities. Moreover, WS germinated at 25 °C presented less CCA compared with the other three samples (WS at 30 °C and RS at both temperatures), which did not present differences among them at G3 (5 mg EDTA/g d.b.). Malting resulted in 90% increase of metal chelating activity for WS at 30 °C and for RS at both temperatures, and 66% for WS at 25 °C.

Figure 2c shows higher reducing power (RP) for both sorghum cultivars germinated at 30 °C. Considering the same temperature, it was higher for WS than RS. Moreover, the values after the whole process for both temperatures and cultivars (110–135 mg AA/g d.b.) were much higher than RP of malted barley (3 mg AA/g d.b.) (Lu et al. 2007). Therefore, sorghum malting could improve flavor stability of beer and other foods by inhibiting lipid peroxidation processes.

The water extracts obtained from the different samples presented different antioxidant activity results, reflecting differences in the nature of compounds responding to different mechanism evaluated by antioxidant assays.

Correlation between antioxidant activity mechanisms and bioactive compounds

Phenolic compounds have been reported to be responsible for the antioxidant activities of grains (Lu et al. 2007). However, germination generates FAA and short peptides (Taylor 1983) having antioxidant activity (Wu et al. 2003; Sarmadi and Ismail 2010). In order to clarify whether specific bioactive compounds produced by malting make a contribution to antioxidant activity measured by different methods, all data obtained from two cultivars of malted sorghum samples were used to analyze correlations among antioxidant activity assays and total FPC, CA, pCA, FA, GABA, and FAA belonging to a specific group: sulfur-containing amino acids (SCAA, Met and Cys), phenolic amino acids (PAA, Tyr and Phe) and charged amino acids (CAA, Lys, Arg, His, Asp and Glu).

TEAC and RP had a strong positive correlation with each other (r = 0.9199), but poor correlation with CCA (r = 0.6770 and 0.7804, respectively). This suggests that compounds having TEAC or RP activity were not the same than those with CCA. Lu et al. (2007) studied TEAC, RP, and CCA during barley malting and did not found a significant correlation between CCA and the other assays.

The antioxidant assays showed a positive correlation ($p < 0.05$) with FPC, SCAA, PAA, or CAA, but in a different way. The higher correlation coefficient for TEAC and RP was with FPC (r = 0.9329 and 0.9308, respectively). Regarding relationships with specifics groups of amino acids, correlations were higher for RP than TEAC, particularly for SCAA, which are sensitive to oxidation, and may represent an antioxidant defense (Bourdon et al. 2005). Furthermore, it has been found that sulfhydryl group present in sulfur amino acids interacts directly with free radicals by exerting a reducing effect (Sarmadi and Ismail 2010). Moreover, Cian et al. (2015b) found a direct relationship between RP and Met and Cys which provide the redox couple –SH/S–S. Also, phenolic amino acid (PAA) group had high correlation with RP. The aromatic nature of their structures is considered to be the major factor contributing to antioxidant activity (Nimalaratne et al. 2011).

Regarding CCA, the higher correlation coefficient was with SCAA (r = 0.8342). In this sense, Bourdon et al. (2005) found a direct correlation between metal chelating activity and Met and Cys. Moreover, Zhao et al. (2008) suggested that phenolic compounds in malting barley might be weak chelators of ferrous ions, thus FAA would be the main compounds implied in CCA.

Additionally, GABA did not correlate with antioxidant activity of malted sorghum, although it is a compound with many others functional properties (Diana et al. 2014).

On the other hand, as GABA, the studied phenolics (caffeic acid, p-coumaric acid and ferulic acid) did not correlate with antioxidant activity of malted sorghum. It is important to note that the sum of these three compounds for each sample represents about 4% of total FPC. Although the Folin–Cioclateau method measures constituents other than phenolics and its specificity is poor (Kamath et al. 2004), sorghum aqueous extract could contain another phenolics different than hydroxycinnamic acids derivatives, like hydroxybenzoic acid derivatives or flavonoids (Morais-Cardoso et al. 2015), which could be responsible in part of antioxidant activity in sorghum aqueous extracts. Similarly, Maillard and Berset (1995) found no correlation between antioxidant activity and phenolic content, since other compounds were found to be responsible for the antioxidant activity. Kamath et al. (2004) reported that various sorghum products possess high antioxidant activity. Moreover, Dicko et al. (2005) studied the effect of germination on phenolic compounds and antioxidant activity in 50 sorghum cultivars and found a weaker correlation between antioxidant activity and phenolic compounds after germination than before germination. Therefore, results indicated that the evolution of antioxidant activity during malting not only might be attributed to FPC, but also to other bioactive compounds generated during germination, like FAA, which could be extracted in aqueous medium and could represent potential bioactive compounds in malted sorghum beverages.

Bio-functionality of malted sorghum flours and their potential application

There has been an increased interest in utilization of GABA as a bioactive plant component. The maximum GABA values obtained here for all studied conditions and sorghum cultivars (47.1–120.1 mg/100 g d.b.) were high and within the range used in GABA-enriched functional foods (Diana et al. 2014). Thus, malted sorghum could be an interesting alternative for healthy foods and beverages production that might be effective in blood pressure regulation and in the recovery of alcohol related symptoms.

On the other hand, the content of branched-chain amino acids (BCAA, Val, Leu, Ile) was higher in RS germinated for 3 days at 25 °C than at 30 °C (20.5 vs. 14.3 μEq/g b.s., respectively). The BCAA are unique among amino acids since they are primarily metabolized extra-hepatically in skeletal muscle, serving as both, an important energy substrate during exercise and stress period, and as precursor for the synthesis of other amino acids and proteins. Also, BCAA improve nitrogen retention and protein synthesis in patients with liver failure (Platell et al. 2000). Thus, this amino acid group is used as food ingredient and for synthesis of pharmaceuticals (Pátek 2007). Then, it is possible to achieve an increase in BCAA content to produce a BCAA-enriched food taking into account red sorghum germination temperature.

In addition, malting including steeping, germination, and kilning had significant effects on total FPC and particular phenolics, as well as antioxidant activities of sorghum samples, which could improve flour flavor stability by well manipulating malting technology. The maximum values of FPC and antioxidant activities were observed at third day of germination for both temperatures and cultivars, being greater for WS (except for CCA). Nowadays, phenolic compounds are generally regarded as desirable components of human food, as some phenolic compounds protect against neurological disorders and exert anti-carcinogenic, anti-mutagenic, and cardio-protective effects linked to their free-radical scavenging activities (Dicko et al. 2005).

Conclusion

Germination temperature and time influenced together on accumulation of GABA, phenolics, antioxidant activity, and free amino acid profile. Malting process not only increased antioxidant activity due to an increment of total free phenolic compounds, but also due to free amino acids, specially sulfur containing ones (Met and Cys).

Germination at 25 °C for 3 days was the most suitable condition for obtaining sorghum malt with high content of GABA, total phenolic compounds, and good antioxidant activity. Taking into account the higher value of GABA, and the low difference between antioxidant activity, RS was better raw material for making functional malt than WS.

Ferulic acid was the major hydroxycinnamic acid derivative extracted in aqueous medium, while caffeic acid was almost totally extracted with this method. More studies are needed to determine other phenolics in aqueous extracts to know the potential phenolic composition of malted sorghum beverages.

These results contribute to determine germination conditions for production of GABA-enriched sorghum malt with good antioxidant activity, to make new functional foods and beverages for celiac population and the general consumer.

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