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FULL ARTICLE

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Aptitude of sorghum (*Sorghum bicolor* (L) Moench) hybrids for brewery or bio-functional malted beverages

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Abstract

The potential of twenty-four hybrids of white (WS) and red sorghum (RS) to produce beer or bio-functional malted beverages was studied. Brewery aptitude was evaluated through diastatic power (DP), β-amylase activity, hot water extract (HWE), and Kolbach index using sorghum malted at 30°C; while bio-functional potential was evaluated through GABA, free amino acids (FAA) content, phenolics, and antioxidant capacity of sorghum malted at 25°C. Only eight hybrids showed DP and β-amylase activity appropriate to brewery industry (≥22°DP/g d.b., and ≥60 UBetamyl/g d.b., respectively). White sorghum hybrid presented better potential for brewing. Levels of GABA were higher for malted RS (33.0–60.6 mg/100 g d.b.) than for WS (18.4–48.1 mg/100 g d.b.). GABA content was predicted taking into account the protein content, color, and ether extract of malted hybrids. Red sorghum hybrid presented better potential for bio-functional beverages, and such aptitude could be predicted from composition.

Practical application

Selecting sorghum hybrids for a particular food is crucial to obtain a product with high quality. There are numerous available sorghum hybrids in the market, and the prediction of the best ones for making functional beverages trough simply equations taking into account chemical composition could be a solution of time and resources for the food industry. On the other hand, we checked the best hybrids in regards to its malting potential in a simple manner. This research established white sorghum hybrids were the best raw materials to produce higher enzymatic activity of malted products for sorghum brewery industry, and red sorghum hybrids were the best ones for bio-functional beverages.

KEYWORDS

antioxidant activity, brewery potential, GABA, malted, sorghum

1 | INTRODUCTION

Malting can be defined as the controlled germination of cereals, to ensure desirable physical and biochemical changes within the grain, which is then stabilized by drying (Gupta, Abu-Ghannam, & Gallaghar, 2010). Cereal malting involves three steps: steeping, germination, and drying (Okoli, Okolo, Moneke, & Ire, 2010). The main use of malting is for producing beer, being barley the main cereal used for beer production in Western world. Otherwise, sorghum has been utilized for years in African regions to produce a typical sorghum beer (Lyumugabe, Gros, Nzungize, Bajyana, & Thonart, 2012). Brewery industry has attempted to widen its offer by developing new, innovative products according to increasing demands (Bogdan, & Kordialik-Bogacka, 2017). In this sense, beer

for celiac population is a good alternative to broaden the market, and more studies on sorghum cultivars around the world must to be developed, since malting can be employed as an appropriate pretreatment to improve nutritional, biochemical, and bio-functional properties of cereal grains, through reducing anti-nutrients, such as phytic acid, develop amylase activity, and increasing bioactive compounds, such as γ-aminobutyric-acid (GABA), phenolic acids, and antioxidant activity (Baranwal, 2017; Saleh, Wang, Wang, Yang, & Xiao, 2017).

On the other hand, the production and consumption of functional foods have gained much importance as they provide a health benefit beyond the basic nutritional functions. At present, beverages are by far the most active functional food category because of convenience and possibility to meet consumer demands (Corbo,Bevilacqua, Petruzzi, Casanova, & Sinigaglia, 2014).

It is known the cultivar influences directly biochemical properties of malt (Dicko, Gruppen, Traore, van Berkel, & Voragen, 2005), and a good quality grain is fundamental to produce acceptable food products from malted sorghum. In a previous study, Garzon, Torres, and Drago (2016) found optimal malting conditions to produce sorghum beer (germination at 30°C for 3 days), or biofunctional sorghum beverages (germination at 25°C for 3 days). As far as we know, there is no report about the influence of chemical composition of native or malted sorghum grains on the aptitude to produce functional beverages. The aims of this study were to characterize 24 hybrids of native and malted sorghum by chemical analysis; assess their potential to produce sorghum beer, or their aptitude for producing a bio-functional malted beverage rich in GABA and antioxidant compounds; and to determine the relationship among brewery or bioactive potential with chemical composition.

2 | MATERIALS AND METHODS

2.1 | Chemicals

Diethyl ethoxymethylenemalonate (D94208), α-aminobutyric acid (A1879), GABA (03,835), amino acid standard solution (AAS18), 2,20-azino-bis-3-ethylbenzothiazoline-6-sulphonic acid (ABTS) (A1888), and 6-hydroxy-2,5,7,8-tetramethylchroman-2-carboxylic acid (Trolox) (238813) were obtained from Sigma Chemical Co. (St. Louis, MO, USA). All other chemicals and reagent used in the experiments were of analytical grade.

2.2 | Raw material

Twenty four sorghum hybrids: 14 white (WS) and 10 red (RS) were evaluated. Some of them were donated by INTA Parana and others were from commercial sources. The material was carefully cleaned using a Labofix Brabender (Duisburg, Germany), and stored at 4°C.

2.3 | Methodology

The experiments carried out to determine the brewery and bio-functional potential are showed in Figure 1.

2.4 | Proximate composition

Moisture, fat (ether extract), protein, and ash content were determined according to the AOAC methods (AOAC, 2002). Starch was determined by Ewers method (Mitchel, 1990).

2.5 | Malting procedures

Sorghum samples were conditioned, soaked, and germinated as was described by Garzón et al. (2016). Briefly, grains (300 g) were soaked for 24 hr at 25°C in a 1:3 grain-to-solution-ratio. An aliquot of soaked grains (100 g by triplicate) were germinated in the dark with 95% relative humidity at 30°C to evaluate beer potential, or at 25°C to evaluate bio-functional aptitude, using an force air oven (Bioelec®, Santa Fe, Argentina) for 3 days. Then, germinated samples were dried at 50°C until less than 10 g/100 g moisture content. The root and sprout portions were manually removed. Native (N) and malted at 30 or 25°C grains (M30 and M25, respectively) were ground in a mill (DecalabFbr1, Córdoba, Argentina). The flours obtained were sieved through a 30 mesh screen, and were stored at 4°C until analysis.

2.6 | Germination percentage

Germination percentage was determined at three days of germination, at 30 or 25°C. Seed germination was considered when the

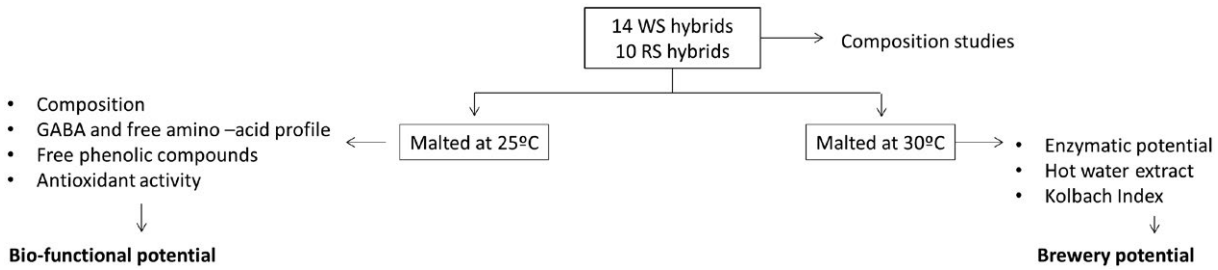

FIGURE 1 Experimental diagram. WS: white sorghum; RS: Red sorghum

emerging radical elongated to 1 mm. Germination percentage was calculated taking random samples of 100 grains by triplicate.

2.7 | Brewery potential of malt (sorghum germinated at 30°C)

2.7.1 | Enzymatic potential

Enzymatic potential of flours from sorghum malted at 30°C was evaluated by the determination of β-amylase, using a Megazyme kit (Betamyl-3 method: K-BETA 10/10). Results were expressed as Ubetamyl/g d.b. In addition, diastatic power (DP) was determined using the ferricyanide method, according to AOAC (2002), and expressed as °DP/g dry basis (d.b.)

2.7.2 | HWE and Kolbach index

Malted flours with better amylase activity and °DP/g d.b. were selected for determining the amount of extract they would generate during maceration, through the hot water extract assay (HWE) according to the European Brewery Convention method (EBC, 1998), with modifications for sorghum flour. Briefly, to 20 g of malted flour, 144 ml of distilled water at 45°C were added. Samples were initially shaken to remove lumps, and incubated at that temperature for 30 min. Subsequently, 60 ml of the supernatant (with enzymatic activities) was removed to prevent enzyme denaturation. The remaining macerate (starchy residue) was incubated at 85°C for 30 min and shaken every 10 min. The residue was then cooled to 50°C in an ice bath, and the supernatant was added. The mixture of supernatant and gelatinized starch was incubated at 65°C for 60 min, shaking every 10 min, and finally brought to 75°C for 10 min. The resulting macerate was cooled to 20°C and the weight was adjusted to 180 g with distilled water. It was thoroughly mixed, and after 30 min decantation it was filtered for 60 min to obtain the sweet wort. Brix was measured with a refractometer (Milwaukee, Italy) for the calculation of HWE. Results were expressed as liter degree/Kg d.b. (°L/Kg).

The Kolbach index (Nk %) was determined to estimate the breakdown of the grain protein matrix during germination. It was calculated by expressing the soluble nitrogen obtained in the wort as a percentage of the total nitrogen of the malt. The content of nitrogen was determined by semimicro-Kjeldalh (EBC, 1998).

2.8 | Bio-functional aptitude of malt (sorghum germinated at 25°C)

2.8.1 | GABA and FAA profile

To determine FAA profile including GABA, M25 flours (0.2 g) were extracted 60 min with trichloroacetic acid (8 g/100 ml) at room temperature, and centrifuged 10 min at 3000xg. The supernatants were collected and 500 µl were added with 1,500 µl of borate buffer (1 mol/L, pH 9). The content of individual FAA and GABA was determined according to Alaiz, Navarro, Girón, and Vioque (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 Shimadzu Series LC-20AT pump, with Shimadzu SPD-M20A diode array detector, equipped with a 300 × 3.9 mm i.d. reversed-phase column (Novapack C18, 4 µm; 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 nmol/mL for Cys, GABA, and all the others FAA, respectively. Total free amino acid (FAA) was calculated as the sum of equivalent of each amino acid. Also the content of amino acid by groups: Sulfur containing amino acids, SCAA (Met and Cys); Polar charge amino acids, PCAA (His, Lys, Arg, Asp, and Glu); Phenolic amino acids, PAA (Thr and Phe) was calculated. Results were expressed as µEq/g d.b.

2.8.2 | Sample extraction

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

2.8.3 | Total free phenolic compounds

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 d.b.

2.8.4 | Phenolic acid profile

An acid hydrolysis for the cleavage of conjugated and condensed soluble phenolic compounds in malted water extracts was carried out according to Cian, Martínez-Augustin, and Drago (2012). 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 minutes 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.

2.8.5 | Antioxidant properties

Trolox equivalent antioxidant capacity

The scavenging of ABTS was measured according to Cian et al. (2012). To estimate the trolox equivalent antioxidant capacity (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.

Reducing power activity

Reducing power activity (RP) of water extracts was determined according to Cian, Garzón, Ancona, Guerrero, and Drago (2015). A standard curve with ascorbic acid (AA, 0–0.05 g/L in phosphate buffer) was used. The RP was expressed as mg AA/g d.b.

2.9 | 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). Duncan's multiple range test is use to determine the differences between samples; and paired sample *t*-test is use to determine the differences between native and malted samples. Multiple regressions were applied to correlate TEAC, GABA, and HWE with proximate composition results, and principal component analysis (PCA) was performed to correlated N and M25 bioactivity capacity. To include the color in PCA, values of 1 and 2 were assigned to WS and RS, respectively. Statgraphics Centurion XV 15.2.06 software was used.

3 | RESULTS AND DISCUSSION

3.1 | Proximate composition and antioxidant activity of native sorghum hybrids

Moisture content of native hybrids was in the range of 12.4%–16.6%. Chemical composition, FPC, and antioxidant activity of native sorghum are presented in Table 1. Ash, protein, fat, and starch were within the ranges reported in the bibliography (Dicko et al., 2005; Jacob, Fidelis, Salaudeen, & Queen, 2013; MoraisCardoso, Pinheiro, Martino, & Pinheiro-Sant'Ana, 2015; Palavecino, Penci, Calderón-Domínguez, & Riobotta, 2016). Average of ash and fat was higher for RS than WS ($p < 0.05$). Analysis of proximate composition of grains plays a crucial role in assessing their nutritional significance. However, grain composition can be affected by factors, such as genotype, climate, soil type, and fertilization (Ebadi et al., 2005).

Extracted FPC and antioxidant activity (TEAC and RP) were higher for RS than WS. It is known that RS has a higher content of polyphenols than WS (Dicko et al., 2005), and consequently, higher amounts are extracted in water, thus RS-extracts had higher antioxidant capacity than WS ones. Moreover, a correlation between FPC of water extract with RP or TEAC was observed (r: 0.8577 and 0.8274, respectively). Potential of sorghum as raw material to produce a functional beverage is represented by antioxidant activity (TEAC and RP) and FPC extracted in water. In this regards, antioxidant activity of native grains depends almost exclusively on the phenolic compounds presented in the grain and RS could be a better raw material to produce functional beverage than WS.

3.2 | Brewery potential of malt (sorghum germinated at 30°C)

3.2.1 | Enzymatic potential

Figure 2 shows the results of diastatic power (°DP/g d.b.) and β-amylase activity of the 24 sorghum hybrids, germinated at 30°C (M30 grains). The range of β-amylase activity was 16–102 UBetamyl/g d.b. It was higher than the values reported by Leung (2002), who studied seven sorghum hybrids (15–56 UBetamyl/g d.b.), and the values reported by Beta, Rooney, and Waniska (1995) who studied six sorghum hybrids (37–41 UBetamyl/g d.b.). On the other hand, the average of WS was higher than the average of RS ($p < 0.05$) (65 vs. 47 UBetamyl/g d.b, respectively).

Regarding diastatic power, values ranged 3–51 °DP/g d.b. and like β-amylase, WS showed higher average value than RS (24 vs. 12 °DP/g d.b, respectively). Only 33% of studied cultivars presented a diastatic power higher than 22°DP/g d.b. Although sorghum brewery industry recommend malts with 28°DP/g d.b. or higher (Taylor & Dewar, 1992), eight malted sorghum presenting a value ≥22°DP/g d.b. and ≥60 UBetamyl/g d.b. were selected for analyzing HWE and Nk. The selected hybrids were all WS (NT2 585, NT2 607, NT2 540, NT2 599, NT2 559, NT3 714, NT2 545, and 341x121). The lower activity of sorghum β-amylase compared with barley is one of the disadvantage of using sorghum to brewery industry (Dicko et al., 2005), and because of that there is an increased interest in sorghum hybrids that develop higher activity of this enzyme during germination.

3.2.2 | HWE and Nk

Germination percentage at 30°C (%), HWE, and Nk of the eight selected hybrids are shown in Table 2. All of them presented a good germination percentage at 30°C (91%–99%). The value of HWE indicates the soluble material obtained during mashing. Results ranged 257–289, and the higher HWE values were for hybrids NT2 607, NT2 599, NT2 559, and NT2 545. These values were similar to those reported in barley (289 °L/Kg), and in sorghum (268 °L/Kg) by Igyor, Ogbonna, and Palmer (2001).

Regarding Nk values, there were significant differences among hybrids ($p < 0.05$). Hybrids NT2 599 and NT2 545 presented an insufficient disintegration; therefore, they could not be used for beer production. Nk indicates proteolytic activity both, during malting

TABLE 1 Proximate composition, free phenolic compounds (FPC), and antioxidant activity (TEAC and RP) of white (WS) and red (RS) native (N) sorghum hybrids

N hybrids	Protein (g/100 g)	Ash (g/100 g)	Fat (g/100 g)	Starch (g/100 g)	FPC (mg GA/g)	TEAC (μmol Trolox/g)	RP (mg AA/g)
WS							
ECR G96	10.9 ± 0.2	1.6 ± 0.4	3.4 ± 0.1	77.1 ± 1.3	0.96 ± 0.02	42.4 ± 0.2	55.0 ± 0.7
NT2 585	11.6 ± 0.2	2.2 ± 0.0	4.4 ± 0.0	73.6 ± 0.6	1.18 ± 0.05	35.9 ± 0.1	55.8 ± 0.6
NT2 607	12.7 ± 0.0	1.7 ± 0.0	3.5 ± 0.0	73.0 ± 0.8	0.90 ± 0.00	40.0 ± 0.3	53.8 ± 1.3
NT2 540	12.3 ± 0.1	1.7 ± 0.0	3.5 ± 0.1	71.0 ± 1.4	0.86 ± 0.02	34.1 ± 0.3	47.1 ± 0.5
ECR G75	11.8 ± 0.3	2.3 ± 0.1	3.9 ± 0.2	68.6 ± 1.0	1.26 ± 0.02	45.8 ± 0.6	66.9 ± 2.1
ECR G150	11.2 ± 0.1	2.4 ± 0.1	4.3 ± 0.1	76.1 ± 0.8	1.37 ± 0.02	49.7 ± 1.1	64.6 ± 1.0
NT2 599	10.3 ± 0.0	2.0 ± 0.0	4.5 ± 0.2	81.0 ± 1.5	1.00 ± 0.02	31.1 ± 0.6	56.8 ± 0.8
NT2 559	11.5 ± 0.1	1.7 ± 0.1	3.3 ± 0.0	75.6 ± 1.5	0.93 ± 0.03	28.9 ± 0.2	52.2 ± 1.2
NT3 714	11.6 ± 0.2	2.1 ± 0.1	4.3 ± 0.0	77.1 ± 1.1	1.08 ± 0.03	31.4 ± 0.6	59.5 ± 0.7
NT3 726	10.5 ± 0.0	1.9 ± 0.0	4.1 ± 0.1	75.5 ± 0.7	1.31 ± 0.06	37.3 ± 1.0	66.4 ± 0.7
NT2 621	11.0 ± 0.0	1.9 ± 0.0	3.7 ± 0.1	75.7 ± 0.8	1.18 ± 0.01	33.8 ± 0.8	57.7 ± 1.1
NT2 545	10.1 ± 0.2	1.4 ± 0.0	2.4 ± 0.0	76.8 ± 0.3	1.01 ± 0.05	36.1 ± 0.3	63.7 ± 1.6
Jowar Food	8.8 ± 0.2	2.3 ± 0.0	4.7 ± 0.1	76.0 ± 0.7	1.14 ± 0.02	36.1 ± 0.5	46.5 ± 0.4
341x121	9.8 ± 0.3	2.7 ± 0.1	5.2 ± 0.2	73.3 ± 0.6	1.11 ± 0.02	27.7 ± 0.2	48.4 ± 1.3
WS average	11.1 ± 1.0	2.0 ± 0.3*	3.9 ± 0.7*	75.1 ± 3.0	1.09 ± 0.16*	36.5 ± 6.3*	56.7 ± 6.9*
RS							
Gaiman	9.2 ± 0.2	2.0 ± 0.0	4.2 ± 0.0	74.4 ± 0.5	1.42 ± 0.05	43.9 ± 0.7	75.0 ± 0.7
MS 105	10.3 ± 0.1	2.5 ± 0.0	5.4 ± 0.2	72.1 ± 0.6	1.82 ± 0.05	54.6 ± 0.5	82.4 ± 0.2
G2 166	12.8 ± 0.1	2.4 ± 0.1	7.4 ± 0.2	66.3 ± 0.5	1.95 ± 0.05	51.5 ± 1.2	79.8 ± 1.2
G3 119	11.3 ± 0.3	3.5 ± 0.1	7.4 ± 0.3	69.7 ± 0.3	2.10 ± 0.06	60.4 ± 1.1	90.9 ± 1.1
G4 154	12.6 ± 0.1	3.5 ± 0.1	7.7 ± 0.1	67.5 ± 0.5	1.92 ± 0.07	56.3 ± 0.0	88.8 ± 0.7
G5 109	7.9 ± 0.2	1.9 ± 0.0	4.2 ± 0.1	82.1 ± 0.5	1.23 ± 0.03	35.2 ± 1.1	66.1 ± 0.4
P83G19	7.5 ± 0.3	2.1 ± 0.0	4.1 ± 0.0	82.0 ± 0.1	1.16 ± 0.05	38.6 ± 0.3	43.8 ± 0.8
P84G62	7.8 ± 0.0	2.0 ± 0.2	4.0 ± 0.1	80.4 ± 0.4	1.12 ± 0.06	45.2 ± 0.5	47.5 ± 0.1
VDH 305	8.5 ± 0.0	2.7 ± 0.1	5.5 ± 0.1	72.3 ± 0.4	1.50 ± 0.07	56.8 ± 1.4	57.6 ± 0.4
DK 51	8.7 ± 0.0	2.6 ± 0.1	5.9 ± 0.2	70.8 ± 0.4	1.38 ± 0.06	43.9 ± 1.0	56.2 ± 1.0
RS average	9.6 ± 1.9	2.5 ± 0.6	5.6 ± 1.5	73.8 ± 5.8	1.56 ± 0.36	48.6 ± 8.5	68.8 ± 17.0

Notes. Results were expressed as Mean ± *SD* in dry base. *significant difference between WS and RS average (p-value < 0.05). GA: gallic acid; TEAC: Trolox equivalente antioxidant capacity; RP: reducing power; AA: ascorbic acid.

and mashing procedures. It is represented by the ratio of soluble nitrogen obtained in wort and total nitrogen of malt (Hassani, Zarnkow, & Becker, 2013). A value of Nk lower than 35% represent insufficient hydrolysis of protein matrix; values between 35% and 41% indicate a good disintegration; values between 41% and 45%, a very good disintegration, and values higher than 45% represent an excessive disintegration that could cause beer alterations (Ratnavathi, Patil, & Chavan, 2016). Taking into account hybrids with higher HWE and appropriate Nk values, hybrids NT2 607 and NT2 559 had aptitude for brewery industry.

In order to study the relationship among indicators of brewing potential and composition of native grains, a multiple regression was performed. However, no valid relationship was observed. Thus, to predict sorghum aptitude for brewing, DP, HWE, and Nk should be analyzed.

3.3 | Bio-functional aptitude of malt (sorghum germinated at 25°C)

3.3.1 | Changes in chemical composition

Evaluating the changes in nutritional components after germination can help to understand the worth of malted sorghum. Moisture content of malted hybrids ranged 6.7%–9.0%. Ash, protein, fat (ether extract), and starch were determined over the 24 hybrids malted at 25°C (Table 3). Ash content, ether extract, and starch decreased

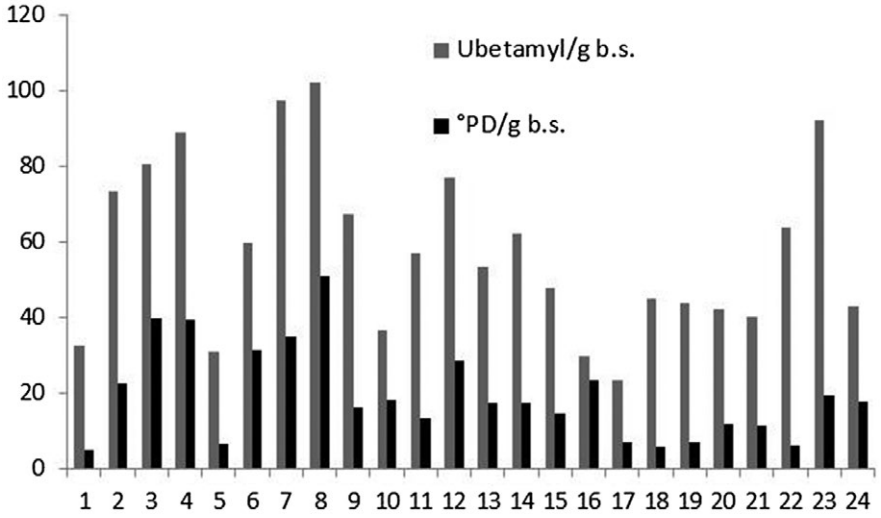

FIGURE 2 Enzymatic potential of the 24 malted sorghum hybrids

TABLE 2 Germination percentages (G), hot water extract (HWE) and Kolbach Index (Nk) of high enzymatic potential sorghum hybrids malted at 30°C (M30)

M30 hybrids	G (%)	HWE (°L/Kg)	Nk
NT2 585	99 ± 1	273.8 ± 4.8^{bc}	44.1 ± 0.3^{e}
NT2 607	96 ± 3	283.8 ± 2.5^{de}	34.6 ± 1.1^{ab}
NT2 540	97 ± 1	278.8 ± 2.4^{cd}	35.7 ± 0.4^{b}
NT2 599	97 ± 1	288.9 ± 2.3^{e}	34.3 ± 0.1^{a}
NT2 559	97 ± 1	282.2 ± 2.4^{cde}	38.1 ± 0.9^{c}
NT3 714	97 ± 1	267.0 ± 4.8^{b}	41.5 ± 1.0^{d}
NT2 545	91 ± 7	287.2 ± 4.7^{de}	33.3 ± 0.1^{a}
341x121	98 ± 1	256.9 ± 4.8^{a}	43.6 ± 0.3^{e}

Notes. Results were expressed as Mean ± *SD*. Values with different superscript letter are significantly different ($p < 0.05$).

significantly after malting ($p < 0.05$), with a mean reduction of 24%, 34%, and 5%, respectively, while protein content did not change after malting process. Ratnavathi et al. (2016) also reported crude protein content of three sorghum cultivars kept constant after germination. Ash decreased as result of mineral losses and degradation of outer bran layer. Fat and starch decreased due to hydrolysis by increased lipolytic and amylolytic enzyme activities during germination (Singh, Sharma, & Singh, 2017).

3.3.2 | Bio-functional properties

Table 4 shows the germination percentage, FPC, phenolic acids (CA, pCA, and FA), antioxidant activity (TEAC, RP), FAA, GABA, Lys, and FAA groups of sorghum hybrids malted at 25°C (M25). Except for ECR G75, all hybrids had a good germination percentage (83%–99%) and there was no significant difference between WS and RS grains. The content of water extractable FPC and antioxidant activity in M25 hybrids were opposite compared with native ones, since the corresponding average measured for WS was higher than for RS ($p < 0.05$). Part of water soluble FPC leach during steeping, since phenolics are primary located in pericarp and testa, thus water-extractable FPC decrease respect to native grains. However, after germination, water-extractable FPC increased (Garzón et al., 2016). Dicko et al. (2005) found germination decreased or increased total phenolic content according to sorghum cultivar. In this sense, water-extractable FPC depend on hybrid color, WS germinated at 25°C being better to produce an antioxidant beverage than malted RS. Regarding phenolic acid profile, sinapic acid was detected only in 1 (NT2 585 hybrid) of 14 WS cultivars (2.03 ± 0.24 μg/g d.b.), and in 4 of 10 RS hybrids (1.53 ± 0.05, 3.97 ± 0.15, 1.14 ± 0.01, and 3.14 ± 0.24 μg/g d.b., for G3 119, P84G62, VDH 305, and DK51, respectively). However, there were not differences in CA, pCA, and FA content between WS and RS water extracts (Table 4). Ferulic acid was the most abundant phenolic acid in malted water extracts. According to this, several studies reported FA as the principal phenolic compound in sorghum grains (Chiremba, Taylor, Rooney, & Beta, 2012; Dicko et al., 2005).

On the other hand, the correlation between FPC and RP was higher for native hybrids than for M25 hybrids (r: 0.8577 vs. 0.7883, for N and M25, respectively). Germination generates FAA and short peptides having antioxidant activity (Xu, & Hu, 2014) that, together with FPC, could be responsible of RP activity.

The average of total FAA in M25 hybrids was higher for WS than RS, while GABA and Lys had an opposite trend being higher for RS than WS ($p < 0.05$). The enzymes produced during germination lead to the hydrolysis of protein with release of amino acids (Baranwal, 2017). In this sense, protease activity in WS was higher than that of RS in this malting condition. Levels of GABA reported here (18.4–60.6 mg/100 g d.b.) were within the range used in GABA-enriched functional foods (Diana, Quílez, & Rafecas, 2014). GABA is the main inhibitory neurotransmitter in the human cortex and in recent years it has become widely available as a food supplement (Boonstra et al., 2015).

There were not significant differences between WS and RS regarding average content of SCAA and PCAA, while PAA was higher

TABLE 3 Proximate composition of white (WS) and red (RS) sorghum hybrids malted at 25°C (M25)

M25 hybrids	Protein (g/100 g)	Ash (g/100 g)	Fat (g/100 g)	Starch (g/100 g)
WS				
ECR G96	10.5 ± 0.2	1.7 ± 0.1	2.9 ± 0.2	71.7 ± 0.3
NT2 585	12.1 ± 0.0	1.6 ± 0.1	2.3 ± 0.0	73.6 ± 0.4
NT2 607	11.9 ± 0.4	1.4 ± 0.0	2.1 ± 0.0	68.5 ± 0.4
NT2 540	11.2 ± 0.2	1.5 ± 0.0	2.3 ± 0.1	71.7 ± 0.6
ECR G75	11.9 ± 0.1	1.5 ± 0.1	3.9 ± 0.1	67.9 ± 0.3
ECR G150	11.0 ± 0.3	1.7 ± 0.0	2.3 ± 0.0	67.3 ± 0.7
NT2 599	11.6 ± 0.1	1.5 ± 0.2	2.5 ± 0.2	72.6 ± 0.3
NT2 559	12.3 ± 0.2	1.7 ± 0.1	2.1 ± 0.1	72.5 ± 0.1
NT3 714	12.6 ± 0.1	1.8 ± 0.0	2.5 ± 0.1	71.1 ± 0.6
NT3 726	12.2 ± 0.0	1.6 ± 0.1	2.6 ± 0.0	71.5 ± 0.3
NT2 621	12.0 ± 0.2	1.6 ± 0.1	2.5 ± 0.2	68.3 ± 0.5
NT2 545	12.1 ± 0.1	1.7 ± 0.0	2.4 ± 0.1	68.0 ± 0.2
Jowar Food	8.4 ± 0.3	1.6 ± 0.3	2.6 ± 0.0	76.2 ± 0.4
341x121	10.2 ± 0.5	1.7 ± 0.1	3.2 ± 0.1	69.0 ± 0.4
WS average	11.4 ± 1.1	1.6 ± 0.1	2.6 ± 0.5*	70.8 ± 2.4
RS				
Gaiman	10.9 ± 0.0	1.6 ± 0.0	3.1 ± 0.2	72.9 ± 0.5
MS 105	10.4 ± 0.4	1.7 ± 0.2	2.9 ± 0.3	70.7 ± 0.6
G2 166	12.3 ± 0.0	1.7 ± 0.1	3.5 ± 0.0	66.5 ± 0.3
G3 119	11.8 ± 0.3	1.6 ± 0.1	3.7 ± 0.1	69.5 ± 0.5
G4 154	13.2 ± 0.1	1.8 ± 0.0	3.4 ± 0.1	67.8 ± 0.3
G5 109	9.5 ± 0.3	1.5 ± 0.1	3.0 ± 0.0	73.0 ± 0.3
P83G19	8.3 ± 0.0	1.6 ± 0.0	2.7 ± 0.1	71.0 ± 0.6
P84G62	8.5 ± 0.0	1.5 ± 0.0	2.7 ± 0.1	75.2 ± 0.2
VDH 305	8.2 ± 0.2	1.2 ± 0.3	3.1 ± 0.2	72.3 ± 0.3
DK 51	8.6 ± 0.1	1.6 ± 0.0	2.9 ± 0.1	70.4 ± 0.5
RS average	10.2 ± 1.8	1.6 ± 0.2	3.1 ± 0.3	72.6 ± 2.3

Notes. Results were expressed as Mean ± *SD* in dry base. *significant difference between WS and RS average (p-value < 0.05).

for WS than RS, according to the higher AAO of M25-WS hybrids. It is known that sulfur containing (SCAA; Met and Cys), polar-charge (PCAA, His, Lys, Arg, Asp, Glu) and phenolic amino acids (PAA, Tyr and Phe) contribute to antioxidant activity (Nimalaratne, Lopes-Lutz, Schieber, & Wu, 2011; Triantis, Yannakopoulou, Nikokavoura, Dimotikali, & Papadopoulos, 2007).

In order to study the relationship among bio-functional properties of the N and M25 grains and the ability of producing a bio-functional beverage, a PCA was performed. It showed that two components might explain 71% of cases. Figure 3 shows that protein of N and M25 are represented by the second component with a direct relationship, and GABA, CA, and pCA with an inverse one. Moreover, an indirect correlation between GABA and proteins was found (r = −0.6775). The different amounts of GABA found among hybrids are mainly caused by their genetic constitution and their ability to synthesize GABA in grains (Karladee, & Suryong, 2012), and this genetic difference is maybe related with protein content. On the other hand, a direct correlation between GABA and CA (r = 0.5029), and pCA (r = 0.6242) was found. In this sense, phenolic acids and GABA are secondary metabolites which act in stress conditions in plants (Boonstra et al., 2015; Salazar-López, González-Aguilar, Rouzaud-Sández, & Robles-Sánchez, 2018). Thus, sorghum hybrids could produce different secondary metabolites and have diverse response to stress during germination.

The first component of PCA directly represents FPC, antioxidant properties, and color of N hybrids, and inversely total FAA and groups of FAA (SCAA and PCAA), FA, FPC and antioxidant activity of M25 hybrids. In this sense, as mentioned above, antioxidant properties of aqueous extract of native grains are higher in RS, but for M25 grain extracts there is an indirect correlation with color. Dicko et al. (2005) reported that after germination of sorghum varieties, the correlation between antioxidant activity and phenolics was weaker than that for native grains, due to during germination there is a synthesis of other antioxidant compounds like vitamin C, antioxidant peptides, and free amino acids.

The choice of sorghum hybrids to produce malt for bio-functional beverage is critical. In this sense, the possibility to predict

TABLE 4 Germination percentage (G), total free phenolic compounds (FPC), caffeic acid (CA), p-coumaric acid (pCA), ferulic acid (FA), antioxidant activity (TEAC and RP), total free amino-acids (FAA), GABA, Lys and free amino acids groups: Sulfur-containing (SCAA), Polar charge (PCAA) and Phenolic amino acids (PAA) of white (WS) and red (RS) malted at 25°C (M25) sorghum hybrids

M25 hybrids	G (%)	FPC (mg GA/g)	CA (μg/g)	pCA (μg/g)	FA (μg/g)	TEAC (μmol Trolox/g)	RP (mg AA/g)	FAA (μEq/g)	GABA (mg/100 g)	Lys (mg/100 g)	SCAA (μEq/g)	PCAA (μEq/g)	PAA (μEq/g)
WS													
ECR G96	83 ± 1	1.60 ± 0.06	18.6 ± 1.8	6.3 ± 0.7	29.7 ± 2.2	52.7 ± 0.5	139.0 ± 2.5	75.8 ± 2.4	37.2 ± 0.8	43.2 ± 0.9	1.7 ± 0.0	12.4 ± 0.5	9.9 ± 0.2
NT2 585	94 ± 5	1.99 ± 0.07	28.3 ± 0.6	11.8 ± 0.5	50.6 ± 1.8	62.2 ± 1.6	191.6 ± 2.2	118.9 ± 11.4	38.8 ± 4.1	26.4 ± 1.0	2.5 ± 0.1	13.0 ± 0.8	19.5 ± 1.8
NT2 607	98 ± 3	2.06 ± 0.05	29.1 ± 1.5	12.1 ± 0.5	63.3 ± 2.3	63.7 ± 1.6	231.7 ± 4.3	140.1 ± 7.8	38.0 ± 3.3	27.9 ± 3.0	2.9 ± 0.1	17.8 ± 1.1	21.8 ± 0.8
NT2 540	95 ± 7	1.99 ± 0.04	27.9 ± 0.3	10.6 ± 0.4	62.1 ± 1.8	69.1 ± 1.2	290.7 ± 5.2	168.6 ± 11.8	45.7 ± 3.5	34.1 ± 2.2	4.6 ± 0.3	23.1 ± 0.8	27.5 ± 2.0
ECR G75	33 ± 3	1.56 ± 0.07	8.5 ± 0.1	2.3 ± 0.1	17.8 ± 0.7	56.3 ± 1.3	202.9 ± 1.6	67.1 ± 2.2	18.4 ± 0.1	21.3 ± 1.5	1.6 ± 0.0	9.1 ± 0.7	9.6 ± 0.2
ECR G150	95 ± 1	1.84 ± 0.07	17.9 ± 0.5	10.1 ± 0.6	43.5 ± 2.2	67.2 ± 1.1	189.8 ± 2.0	87.7 ± 3.0	48.1 ± 5.3	37.8 ± 0.7	2.2 ± 0.1	12.6 ± 0.4	12.6 ± 0.5
NT2 599	99 ± 1	1.52 ± 0.00	24.6 ± 5.0	10.9 ± 0.1	56.7 ± 1.8	63.4 ± 1.5	196.1 ± 0.1	87.0 ± 2.4	28.3 ± 1.8	26.1 ± 0.1	2.2 ± 0.0	11.2 ± 0.4	13.3 ± 0.3
NT2 559	93 ± 4	2.08 ± 0.08	39.9 ± 1.4	12.0 ± 0.6	65.6 ± 1.0	70.2 ± 0.4	220.6 ± 0.6	144.7 ± 4.9	36.2 ± 4.0	24.5 ± 1.1	3.8 ± 0.4	18.6 ± 0.3	24.1 ± 0.3
NT3 714	95 ± 4	2.02 ± 0.06	19.3 ± 0.5	7.2 ± 0.2	35.7 ± 1.5	67.5 ± 0.3	217.6 ± 1.0	82.2 ± 4.3	27.9 ± 0.7	25.0 ± 0.3	1.8 ± 0.1	10.2 ± 0.2	12.6 ± 0.6
NT3 726	96 ± 5	2.02 ± 0.09	19.9 ± 0.6	10.3 ± 0.3	51.9 ± 1.2	65.6 ± 0.1	204.7 ± 0.9	82.9 ± 1.7	27.1 ± 1.5	26.3 ± 0.2	1.9 ± 0.2	10.7 ± 0.2	12.4 ± 0.6
NT2 621	98 ± 1	1.99 ± 0.08	20.2 ± 1.9	10.7 ± 0.6	51.9 ± 4.3	65.3 ± 0.9	178.4 ± 2.5	104.3 ± 2.3	33.2 ± 0.5	27.6 ± 1.1	2.1 ± 0.2	13.1 ± 0.2	16.7 ± 0.5
NT2 545	95 ± 4	2.06 ± 0.07	28.1 ± 2.3	10.6 ± 0.2	57.1 ± 1.2	69.8 ± 0.1	229.7 ± 3.6	161.2 ± 13.9	41.3 ± 3.8	33.8 ± 1.2	3.6 ± 0.4	20.4 ± 1.4	24.9 ± 2.2
Jowar Food	93 ± 1	1.38 ± 0.03	27.8 ± 0.7	10.0 ± 0.6	46.0 ± 0.9	52.3 ± 0.5	72.7 ± 1.1	57.1 ± 2.8	40.1 ± 3.5	38.9 ± 3.4	1.6 ± 0.1	10.8 ± 0.7	7.1 ± 0.4
341x121	90 ± 5	1.60 ± 0.09	28.9 ± 0.8	10.8 ± 0.3	53.9 ± 0.9	53.7 ± 0.6	70.2 ± 0.7	69.7 ± 3.2	25.9 ± 2.0	33.5 ± 2.8	2.1 ± 0.0	10.7 ± 0.4	9.1 ± 0.3
WS average	90 ± 16	1.84 ± 0.25*	24.2 ± 7.4	9.7 ± 2.7	49.0 ± 13.5	62.8 ± 6.4*	188.3 ± 59.7*	103.4 ± 36.8*	34.7 ± 8.3*	30.5 ± 6.4*	2.5 ± 0.9	13.8 ± 4.3	15.8 ± 6.6*
RS													
Gaiman	98 ± 1	1.57 ± 0.04	20.9 ± 1.6	7.8 ± 0.2	35.7 ± 1.5	56.8 ± 1.1	181.8 ± 0.8	71.1 ± 5.2	44.2 ± 3.4	34.3 ± 2.0	1.5 ± 0.2	10.4 ± 0.4	9.0 ± 0.5
MS 105	97 ± 4	1.77 ± 0.09	26.8 ± 1.5	13.3 ± 0.1	48.8 ± 2.6	58.4 ± 1.0	141.4 ± 2.8	67.8 ± 1.6	48.3 ± 1.4	31.6 ± 1.0	1.4 ± 0.0	10.9 ± 0.3	9.3 ± 0.3
G2 166	87 ± 5	1.60 ± 0.06	24.6 ± 0.4	10.9 ± 0.3	41.3 ± 1.0	53.7 ± 0.3	150.9 ± 1.0	78.3 ± 3.8	33.1 ± 1.7	40.8 ± 1.9	1.8 ± 0.1	12.2 ± 0.7	10.4 ± 0.4
G3 119	95 ± 1	1.68 ± 0.03	31.3 ± 0.9	10.2 ± 0.1	33.9 ± 1.3	55.4 ± 0.4	134.6 ± 0.8	67.8 ± 1.2	43.6 ± 0.2	45.7 ± 0.1	1.6 ± 0.0	11.0 ± 0.2	8.6 ± 0.2
G4 154	93 ± 1	1.74 ± 0.07	20.3 ± 0.5	8.1 ± 0.1	27.9 ± 0.5	60.2 ± 1.1	187.1 ± 1.9	94.2 ± 7.1	33.0 ± 1.9	25.4 ± 1.5	2.2 ± 0.0	12.8 ± 0.4	12.1 ± 0.1
G5 109	93 ± 1	1.56 ± 0.02	26.2 ± 0.2	10.6 ± 0.3	37.9 ± 1.1	49.9 ± 0.7	120.9 ± 1.0	60.8 ± 3.2	42.6 ± 2.7	43.4 ± 0.3	1.4 ± 0.2	10.8 ± 0.2	7.3 ± 0.8
P83G19	99 ± 1	1.40 ± 0.07	31.4 ± 0.3	13.2 ± 0.4	50.4 ± 1.5	45.9 ± 1.1	77.2 ± 0.9	66.1 ± 3.9	57.2 ± 2.0	39.4 ± 2.9	1.9 ± 0.1	11.5 ± 0.7	8.0 ± 0.6
P84G62	99 ± 1	1.45 ± 0.08	44.6 ± 1.8	16.9 ± 0.6	54.9 ± 0.5	46.6 ± 1.1	80.5 ± 0.3	64.6 ± 2.8	52.1 ± 4.3	39.2 ± 3.6	2.0 ± 0.2	11.3 ± 0.7	7.7 ± 0.4
VDH 305	97 ± 1	1.60 ± 0.05	26.3 ± 0.8	12.0 ± 0.2	44.6 ± 0.6	53.4 ± 0.1	68.9 ± 0.7	86.9 ± 4.6	60.6 ± 0.2	51.1 ± 0.7	3.0 ± 0.1	15.3 ± 0.4	11.7 ± 0.8
DK 51	95 ± 4	1.31 ± 0.04	30.8 ± 0.3	14.0 ± 0.3	49.4 ± 1.3	44.2 ± 0.4	68.4 ± 1.1	64.2 ± 2.1	49.7 ± 3.7	39.7 ± 1.0	1.9 ± 0.2	11.1 ± 0.4	7.3 ± 0.2
RS average	95 ± 4	1.57 ± 0.15	28.3 ± 6.9	11.7 ± 2.8	42.5 ± 8.6	52.4 ± 5.5	121.2 ± 45.5	72.2 ± 10.9	46.5 ± 9.1	39.1 ± 7.3	1.9 ± 0.5	11.7 ± 1.4	9.2 ± 1.7

Notes. Results were expressed as Mean ± *SD* in dry base. *significant difference between WS and RS average (p-value < 0.05). GA: gallic acid; TEAC: Trolox equivalente antioxidant capacity; RP: reducing power; AA: ascorbic acid; SCAA: Met and Cys; PCAA: His, Lys, Arg, Asp and Glu; PAA: Tyr and Phe.

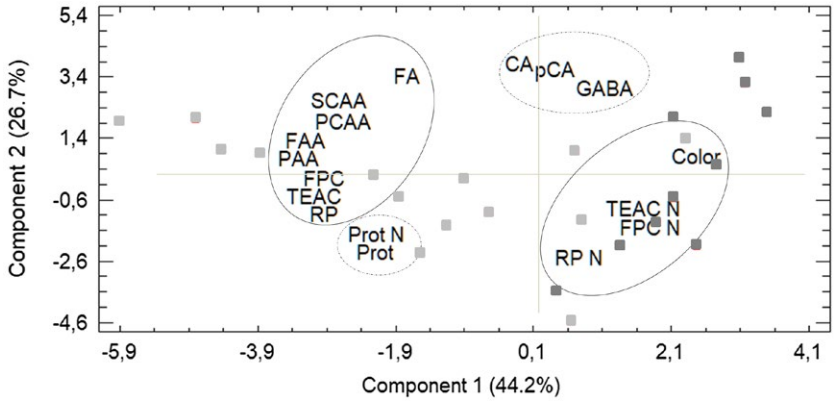

FIGURE 3 Weights of the components of composition and antioxidant activity for native (N) and malted grains. Protein content (Prot N and Prot, respectively), free phenolic compound (FPC N and FPC, respectively), trolox equivalent antioxidant capacity (TEAC N and TEAC, respectively), reducing power (RP N and RP, respectively); color of the testa. Only for malted sorghum hybrids: coumaric acid (CA), p-coumaric acid (pCA), ferulic acid (FA), GABA, free amino acids (FAA), groups of free amino acids: Sulfur containing amino acids (SCAA), polar charge amino acids (PCAA), and phenolic amino-acids (PAA). Gray points are for white sorghum hybrids, dark-gray points are for red sorghum hybrids

antioxidant activity and content of GABA in a simply manner could be a solution of time and resources for the food industry. In order to predict TEAC activity and GABA content in M25 sorghum hybrids, multiple regressions were applied. It was found that using G%, protein content and ether extract of native (N) grains as variables, the following correlation (R^2 = 0.72) was found:

$$\text{TEAC} = 13.0769 + 0.189463 \times \text{G\%} + 3.98062 \times \text{Protein N} - 2.9843 \times \text{Ether extract N}$$

Thus, through the determination of germination percentage, ether extract, and protein of native sorghum, it is possible to predict for 72% of cases its antioxidant properties (TEAC) of sorghum hybrids malted at 25°C.

On the other hand, the determination of GABA require expensive equipment and reagents, and considering the increasing interest in GABA-enriched foods due to its health benefits (Boonstra et al., 2015; Poojary et al., 2017), prediction of this component in a simply manner could help to select sorghum hybrids with good malting aptitude and GABA production. The following correlation was found:

$$\text{GABA} = 91.0039 - 11.5516 \times \text{Ether extract M25} + 13.1583 \times \text{Color} - 3.60193 \times \text{Protein M25}\ (R^2 = 0.78).$$

In this case, taking into account the color (assigning 1 to WS and 2 to RS), and ether extract and protein content of M25 hybrids, it is possible to predict GABA content that will have 78% of hybrids after malting. If the value is greater or equal to 36 mg GABA /100 g d.b., this hybrid could have aptitude for producing a GABA-enriched food.

4 | CONCLUSIONS

White sorghum hybrids presented better potential for brewing. However, few sorghum hybrids could be destined to brewery industry. Since, no valid relationship among indicators of brewing potential and composition of native grains was observed, to predict sorghum aptitude for brewing, DP, HWE, and Nk should be analyzed.

Antioxidant activity and FPC extracted in water after malting differed with those of native extracts of sorghum. Malting produced soluble antioxidant compounds different than phenolics, which increment antioxidant activity of malted products. Also, ferulic acid was the most abundant phenolic compound in malted water extracts. On the other hand, high GABA levels found in malted red sorghum indicate that malting at 25°C could be a good strategy to produce a GABA-enriched food.

Differences in composition and bioactive potential of sorghum hybrids required compositional studies to select the best cultivar for a particular food product. These results contribute to determine the aptitude of sorghum hybrids for producing bio-functional beverages from simple equations. In this way, it is possible to estimate the antioxidant potential of malted grains from the composition of native sorghum in the 72% of cases, and the GABA content from the composition of malted hybrids in the 78% of cases, allowing saving resources and reagents.

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