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Stability of B vitamins, vitamin E, xanthophylls and flavonoids during germination and maceration of sorghum (*Sorghum bicolor* L.)

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ABSTRACT

The impact of maceration and germination on the concentration of bioactive compounds still needs to be evaluated. The stability of B complex vitamins (thiamine, riboflavin, pyridoxine), vitamin E (α, β, γ, δ tocopherols and tocotrienols), xanthophylls (lutein and zeaxanthin) and flavonoids (3-deoxyanthocyanidins-3-DXAs, flavones and flavanones) was evaluated in sorghum grains subjected to maceration and germination, using High Performance Liquid Chromatography. Maceration and germination reduced thiamine and pyridoxine concentrations (retentions ranging from 3.8 to 50.2%). Riboflavin and Vitamin E concentrations were not affected by maceration. Germination increased riboflavin and reduced vitamin E. 3-DXAs were sensitive to maceration and germination (retentions of 69.6% and 69.9%, respectively). Flavones contents decreased with germination. Our results indicate that, after germination and/or maceration, sorghum had important nutritional and functional value. Thus, its intake, mainly in macerated forms, should be encouraged, since concentrations of riboflavin, vitamin E and flavones were not altered during this processing.

1. Introduction

Interest in sorghum (*Sorghum bicolor* L.) in human consumption has increased worldwide due to its agronomic advantages (drought tolerance, high yield and low production cost) (Carvajal, Corsano, Aguilar, & Avila, 2019) and potential health benefit (anticancer properties, anti-inflammatory, anti-diabetes, anti-radical, risk reduction for cardiovascular diseases and modulation of intestinal microbiota) (Anunciação et al., 2018, 2019; Cardoso, Pinheiro, Martino, & Pinheiro-Santna, 2017). Sorghum stands out compared to other cereals due to its high antioxidant activity, attributed mainly to its phenolic compounds (tannins; anthocyanins; flavones and flavanones), vitamins, especially vitamin E, carotenoids and other bioactive compounds (Awika & Rooney, 2004; Cardoso et al., 2017).

Sorghum is usually used in human food as whole grain or flour. However, before being consumed, sorghum needs to be processed or subjected to different cooking techniques or preparations. Due to their similarity with other cereals, the techniques used for maize and wheat processing can also be applied to obtain food products based on sorghum, with emphasis on maceration, germination and fermentation (Salazar-López, Gonzalez-Aguilar, Rouzaud-Sandez, & Robles-Sanchez, 2018). However, processing can modify its antioxidant profile, including flavonoids, carotenoids and vitamins present in the grain (Cardoso, Pinheiro, da Silva, et al., 2015; Salazar-López et al., 2018). The variety of processing techniques that can be applied to sorghum is important, since it allows the elaboration of several forms of preparation of this cereal, aiming to increase its intake.

Studies that describe the impact of the most used techniques, such as maceration and germination, on the nutritional value of sorghum in countries of Africa and Asia have been carried out. Maceration consists of maintaining the cereal grain under controlled conditions (temperature and humidity variables), in order to promote the development of

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hydrolytic enzymes that are not present in the grain *in natura*. These enzymes are important for the malt manufacturing process, for example. Germination consists of the external growth of the parts of the grain bud and root until appropriate enzymes are produced in adequate quantities, also to produce malt (Lyumugabe, Gros, Nzungize, Bajyana, & Thonart, 2012; Palmer, Etokakpan, & Igyor, 1989).

Studies that evaluated these processes analyzed the composition of macronutrients (Afify, El-Beltagi, El-Salam, & Omran, 2012b), protein digestibility (Afify et al., 2012b), antinutritional factors (Afify et al., 2012b), and concentration of minerals in sorghum (Lestienne, Icard-Vernière, Mouquet, Picq, & Trèche, 2005). Thus, the knowledge about the impact of these processes on the concentration of vitamins (mainly of the B complex), carotenoids and bioactive compounds, including flavonoids, needs to be evaluated.

Thus, this study aimed to evaluate the stability of B vitamins (thiamine, riboflavin and pyridoxine), vitamin E (α, β, γ and δ tocopherols and tocotrienols), xanthophylls (lutein and zeaxanthin) and flavonoids (3-desoxyanthocyanidins, flavones and flavanones) in sorghum grains submitted to maceration and germination.

2. Materials and methods

2.1. Raw material

Sorghum grains from BRS 330 genotype, of red pericarp, belonging to a panel of Embrapa Milho e Sorgo (Brazil) strains were used. Planting was carried out in the Embrapa Milho e Sorgo experimental field, located in Nova Porteirinha, MG (Brazil). After harvesting, the grains were cleaned and stored in a cold chamber (10 ± 2 °C).

2.2. Standards and reagents

The standards of B complex vitamins (thiamine hydrochloride, riboflavin and pyridoxine hydrochloride), xanthophylls (lutein and zeaxanthin) and flavonoids (3-deoxyanthocyanidins: luteolinidin chloride and apigeninidin chloride; flavones: luteolin and apigenin; and flavanones: naringenin and eriodictyol) were purchased from Sigma-Aldrich (St. Louis, MO, USA). The vitamin E standards (α, β, γ and δ-tocopherols and tocotrienols) were obtained from Calbiochem (EMD Biosciences, Inc., USA).

Analytical grade reagents (VETEC, São Paulo, Brazil) were used for vitamins, xanthophylls and flavonoids extraction. Clara-diastase was purchased from Sigma-Aldrich (St. Louis, MO, USA). For analysis, HPLC grade reagents (methanol, acetonitrile, formic acid, ethyl acetate, acetone, hexane, isopropanol, glacial acetic acid) obtained from Tedia (São Paulo, Brazil) and analytical grade (sodium acetate and sodium heptane sulfonate) obtained from Sigma-Aldrich (St. Louis, MO, USA) were used.

2.3. Processing of sorghum grains

Whole sorghum grains were subjected to different processing, as described below:

(1) Control: 150 g of grains were milled in an analytical hammer mill micro-rotor (MA 090, Marconi®, São Paulo, Brazil), equipped with a sieve aperture of 850 μm;
(2) Maceration: 150 g of grains were soaked in distilled water, with a ratio 1:5 w/v, for 20 h at room temperature (23 ± 2 °C). The soaked water was replaced twice and it was discarded at the end of soaking period (Afify et al., 2012b);
(3) Germination: 150 g of macerated grains as described above were germinated in Petri dishes covered with a moistened paper filter, at room temperature (23 ± 2 °C), for 72 h. The grains were moistened with water twice a day (Afify et al., 2012b).

For storage, the macerated and germinated grains were dried in an oven with forced air circulation (50 °C, 24 h) until they presented moisture lower than 15% (Brasil, 1996). The moisture determination is described on subsection 2.4. Then, the grains were milled with the roots in an analytical hammer mill micro-rotor (MA 090, Marconi®, São Paulo, Brazil), equipped with a sieve aperture of 850 μm.

Flours were packed in plastic polyethylene bags covered with aluminum foil and stored in a freezer (−20 ± 1 °C) until the analysis was completed, which occurred in a maximum of 30 days.

2.4. Determination of moisture

The moisture of the flours obtained from fresh grains and processed grains was determined by gravimetry, after drying in an oven (Nova Etica, 4000, São Paulo, Brazil) at 105 °C, until reaching constant weight (AOAC, 2005).

2.5. Determination of B vitamins, xanthophylls, vitamin E and flavonoids

The analysis was carried out in 4 repetitions. During extraction and analysis, the samples and extracts were protected from light (sunlight and artificial) and oxygen using amber glassware, dark environment, and bottles with nitrogen gas environment.

2.5.1. Extraction and analysis of thiamine and riboflavin

Total thiamine (thiamine, thiamine monophosphate and thiamine pyrophosphate) and total riboflavin (riboflavin, flavin adenine dinucleotide and flavin mononucleotide) were extracted simultaneously based on the methods proposed by Ndaw, Bergaentzle, Aoude-Werner, and Hasselmann (2000) and Arella, Lahely, Bourguignon, and Hasselmann (1996).

Riboflavin and thiamine analyzes were performed in individual runs, using the chromatographic conditions developed by Arella et al. (1996), with modifications: High Performance Liquid Chromatography (HPLC) system (Shimadzu, SCL 10AD VP, Japan) with fluorescence detection (excitation at 366 nm and emission at 435 nm for thiochrome, and excitation at 422 nm and emission at 522 nm for riboflavin; Shimadzu, RF10AXL, Japan); chromatographic column RP-18 Phenomenex Gemini (250 mm × 4.6 mm, 5 μm) coupled to Phenomenex ODS C18 guard column (4 mm × 3 mm); mobile phase composed of methanol: sodium acetate solution 6.25 mM and heptanesulfonic acid 2.5 mM (30:70, v/v); isocratic elution; flow of 0.9 and 1.2 mL/min for thiamine and riboflavin, respectively; running time of 13 and 14 min for thiamine and riboflavin, respectively.

2.5.2. Pyridoxine extraction and analysis

Total pyridoxine (pyridoxol, pyridoxal and pyridoxamine) was extracted according to the method proposed by Ndaw et al. (2000). Five grams of sorghum flour were added to 50 mL of 0.1 M hydrochloric acid solution, and the suspension was incubated in an autoclave (121 °C, 30 min). Then, the suspension was cooled in an ice bath and its pH adjusted to 4.5 with 2.5 M sodium acetate solution. Then, 2.5 mL of 1 M glyoxylic acid solution, 400 μL of 2% ferrous sulfate solution, and 500 mg of clara-diastase were added, and the suspension was incubated in a metabolic bath (37 °C, 18 h). The suspension was completed to 100 mL with distilled water and vacuum filtered through a Büchner funnel. An aliquot of 5 mL was added to 4.5 mL of 0.2 M sodium hydroxide solution containing 0.1 M sodium borohydride, followed by manual stirring (15 s) and resting (5 min). At the end, 0.5 mL of glacial acetic acid was added to the extract.

The analysis was carried out using the conditions developed by Bergaentzlé, Arella, Bourguignon, and Hasselmann (1995), which included: HPLC system with fluorescence detection (excitation at 290 nm and emission at 395 nm); chromatographic column RP-18 Phenomenex Gemini (250 mm × 4.6 mm, 5 μm), equipped with Phenomenex ODS C18 guard column (4 mm × 3 mm); mobile phase composed of

acetonitrile: 0.05 M potassium dihydrogen phosphate solution (4:96, v/v) containing 0.5 × 10–3 M sodium heptanesulfonate and pH adjusted to 2.5 with phosphoric acid; isocratic elution; mobile phase flow of 1 mL/min; running time 15 min.

2.5.3. Extraction and analysis of vitamin E

The extraction and analysis of vitamin E components (α, β, γ and δ-tocopherols and tocotrienols) were performed according to Pinheiro-Sant'Ana et al. (2011). Five grams of sorghum flour was added to 4 mL of heated ultrapure water (80 ± 1 °C); 10 mL of isopropanol; 1.0 mL of hexane containing 0.05% BHT, 5 g of anhydrous sodium sulfate and 25 mL of extraction solvent mixture (hexane: ethyl acetate, 85:15, v/v). The suspension was homogenized using a micro grinder for 1 min and vacuum filtered on a Buchner funnel using filter paper, maintaining the residue in the extraction tube. The extraction step was repeated, adding to the residue 5 mL of isopropanol and 30 mL of the solvent mixture, with subsequent homogenization and vacuum filtration. Then, the extract was concentrated in a rotary evaporator (70 ± 1 °C, 2 min), transferred to a volumetric flask and the volume completed to 25 mL with solvent mixture.

Aliquots of 5 mL of the extract were dried under nitrogen gas, redissolved in 2 mL of HPLC grade hexane and filtered using filter units (0.45 μm). The analyzes were performed by injecting 20 μL of the extracts. The following chromatographic conditions were used: HPLC system (Shimadzu, SCL 10AD VP, Japan); fluorescence detector (290 nm excitation and 330 nm emission; Shimadzu, RF10AXL); Phenomenex Luna Si100 column (250 × 4 mm, 5 μm) coupled Si100 Phenomenex guard column (4 × 3 mm). The mobile phase was composed by hexane: isopropanol: glacial acetic acid (98.9:0.6:0.5 v/v/v); flow rate of 1.0 mL/min and run time of 22 min.

2.5.4. Xanthophylls extraction and analysis

The xanthophylls (lutein and zeaxanthin) were extracted according to Rodriguez, Raymundo, Lee, Simpson, and Chichester (1976). Two and a half grams of sorghum flour were homogenized in 15 mL of cooled acetone, for 5 min, using a microgrinder. The suspension was vacuum filtered in a Büchner funnel with filter paper, keeping the residue in the extraction tube. Then, the extraction procedure was repeated by adding 15 mL of cooled acetone to the residue, with subsequent homogenization and vacuum filtration.

Subsequently, the carotenoids were partitioned from acetone to petroleum ether. The filtrate was transferred, in two fractions, to a separatory funnel containing 20 mL of cooled petroleum ether. After the transfer of each fraction, distilled water was added for phase separation (carotenoids-petroleum ether and acetone–water), with the lower phase (acetone–water) being discarded. Anhydrous sodium sulfate was added to the ether extract to remove the water residue. Then, the extract was transferred to a 25 mL volumetric flask, the volume being made up with petroleum ether.

For analysis, 5 mL of extract were evaporated under nitrogen gas flow and recovered in 1.0 mL of hexane: isopropanol (90:10 v/v) (Panfili et al., 2004). Then the extract was filtered in polyesterafluorethylene (PTFE) filtering units (0.45 μm). Carotenoid analyzes were performed using the chromatographic conditions developed by Panfili et al. (2004), with modifications: HPLC system, diode array detector (DAD) with detection at 450 nm; chromatographic column Phenomenex Luna Si100 (250 mm × 4 mm, 5 μm) coupled to Phenomenex Si100 guard column (4 mm × 3 mm); mobile phase composed of hexane: isopropanol (95: 5, v/v); isocratic elution; mobile phase flow 1.3 mL/min, run time 13 min.

2.5.5. Extraction and analysis of flavonoids

For extraction, 1 g of sorghum flour was added to 10 mL of 1% methanol / HCl (v: v) and stirred in a metabolic bath (Marconi, Brazil) for 2 h at 180 rpm. Then, the suspension was centrifuged (FANEM centrifuge, Excelsa Baby II, Brazil) at 2790 g, for 5 min, with the supernatant collected and its volume completed to 20 mL with acidified methanol. Subsequently, the extract was packed in an amber bottle and stored at −18 ± 1 °C until analysis (Dykes, Seitz, Rooney, & Rooney, 2009), which occurred within 2 h.

The method proposed by Yang and Ooraikul (2001) and modified by Cardoso et al. (2014) was used to identify and quantify 3-DXAs (luteolinidin - LUT, apigeninidin - API, 7-methoxy-apigeninidin - 7-MeO-API and 5-methoxy-luteolinidin - 5-MeO-LUT), flavones (luteolin and apigenin) and flavanones (naringenin and eriodictyol) from sorghum. The analyzes were performed in a HPLC system (Shimadzu, SCL 10AT VP, Japan) equipped with DAD (Shimadzu, SPD-M10A, Japan), quaternary pump for high pressure gradient (Shimadzu, LC-10AT VP, Japan), auto-injector with 500 μL loop (Shimadzu, SIL-10AF, Japan), and mobile phase degasing system with helium gas (Shimadzu, DGU-2 A, Japan).

The chromatographic conditions used included: Kinetex C-18 column (150 mm × 4.6 mm, 5 μm) equipped with C-18 guard column (4 mm × 3 mm) (Phenomenex, Torrance, CA), column temperature 35 °C, injection volume of 15 μL, spectrum scanning from 200 to 700 nm with detection at 480 nm for 3-deoxyanthocyanidins, 360 nm for flavones and 280 nm for flavanones. The mobile phase was composed of 2% formic acid in ultrapure water (line A) and 2% formic acid in acetonitrile (line B). The elution gradient for B and the flow gradient was described by Cardoso et al. (2014).

2.5.6. Identification and quantification of complex B vitamins, xanthophylls, vitamin E and flavonoids

All compounds were identified by co-chromatography and by comparing the retention time of the authentic commercial standards with the components of interest in the samples. In addition, xanthophylls and flavonoids were identified by comparing the absorption spectra of the standards and peaks of interest in the samples, using DAD.

The compounds were quantified using analytical curves constructed from the injection, in duplicate, of six different concentrations of standard solutions (Table 1).

The total concentrations of thiamine, riboflavin and pyridoxine were expressed in mg/100 g of sorghum (wet and dry matter). The total concentrations of xanthophylls (sum of lutein + zeaxanthin) and vitamin E (sum of α, β, γ and δ tocopherols and tocotrienols) and their isolated components were expressed in μg/100 g (wet and dry matter). The total concentration of flavonoids (sum of 3-DXAs, flavones and flavanones) and their isolated components was expressed in μg/g of sample (wet and dry matter).

The α-tocopherol equivalent was calculated using the equation: (α-tocopherol × 1.0) + (β-tocopherol × 0.5) + (γ-tocopherol × 0.1) + (δ-tocopherol × 0.03) + (α-tocotrienol × 0.3) + (β-tocotrienol × 0.05) (U. S. Institute of Medicine, 2000).

2.6. Validation of methodologies

Tests were carried out to validate the methodologies used for the analysis of thiamine, riboflavin and pyridoxine. The minimum and maximum concentration of vitamin B complex standards for the construction of analytical curves, regression equations and their respective regression coefficients (R2), detection limits (LD), limits of quantification (LQ) and recovery obtained for the quantification of compounds are described on Table 1.

The other methodologies used for the analysis of xanthophylls, vitamin E and flavonoids were previously validated for sorghum analysis (Cardoso et al., 2014; Cardoso, Pinheiro, da Silva, et al., 2015).

2.6.1. Recovery

Vitamin recovery tests were performed by adding standards to the samples in the proportion of 20 to 100% of the original average concentration observed. The recovery percentages were obtained from the following equation:

$$R = [Q(O + S) - Q(O)/Q(S)] \times 100$$

Table 1
Minimum and maximum concentration of vitamin B complex standards for the construction of analytical curves, regression equations and their respective regression coefficients (R2), detection limits (LD), limits of quantification (LQ) and recovery obtained for the quantification of compounds.

Vitamin	Minimum and maximum concentration of standards (ng)	Regression equation	R^2	LD (ng/mL)	LQ (ng/mL)	Recovery (%)
Thiamine	0.08–4.35	y = 2038489.93x + 108766.32	1.00	0.03	0.35	114.60
Riboflavin	0.21–21.30	y = 248925x – 26,032	1.00	0.001	0.01	92.10
Pyridoxine	0.17–13.05	y = 339167x – 36,329	0.9936	0.03	0.33	99.30

where

R: percentage of recovery;
Q (O + S): vitamin concentration in the sample added with a standard;
Q (O): vitamin concentration in the sample;
Q (S): weight of the standard added to the sample.

2.6.2. Linearity

The linearity range was determined by the injection, in duplicate, of six increasing concentrations of the standard solutions, using the same chromatographic conditions used for the analysis of the samples. The data obtained for the peak areas were used for linear regression analysis (Lanças, 2004) (Table 1).

2.6.3. Limits of detection and quantification

The detection limit (LD) was determined by successive dilutions of the thiamine, riboflavin and pyridoxine standards, followed by the determination of the smallest detectable amount, as being three times the mean baseline noise amplitude ($S/R \geq 3$). The limit of quantification (LQ) was 10 times the LD (Catharino, Godoy, & Lima-Pallone, 2006).

2.7. Calculation of the true retention of bioactive compounds

The true retention of vitamins, xanthophylls and flavonoids was evaluated in sorghum grains submitted to the different types of processing, by calculating the percentage of real retention (% RR), using the equation of Murphy, Criner, and Gray (1975).

2.8. Experimental design and statistical data analysis

The data on the concentration of nutrients and bioactive compounds in sorghum were evaluated using a completely randomized design, with 4 repetitions. Data were assessed for normality using the Shapiro-Wilk test. Then, they were submitted to One-Way ANOVA. When necessary, the data means were subjected to Duncan's test for multiple comparison of means. Statistical analyzes were performed using the SPSS software, adopting a significance level (α) of 5%.

3. Results and discussion

3.1. Validation of methods for analysis of B complex vitamins

The recovery percentages for thiamine, riboflavin and pyridoxine were, on average: 114.6%; 92.1% and 99.3%, respectively (Table 1). These values were considered as adequate percentages of retention and reflected excellent efficiency of the extraction processes (adequate cleaning process in the extraction steps, low levels of losses of compounds during their extraction and low level of errors).

Similarly, the linearity coefficients obtained were excellent and reflected excellent linearity of the data, since they were very close to 1. The limits of detection and quantification showed very low values, which indicate a greater sensitivity of the methods used for the quantification of compounds present in small quantities in food matrices.

3.2. B complex vitamins

The three B vitamins analyzed in our study were identified in the sorghum samples not submitted to processing (control samples), in macerated and in germinated samples (Fig. 1C–E; Table 2). The concentration of total riboflavin was similar, pyridoxine was lower, and thiamine was twice higher than that observed in other sorghum genotypes (0.06 mg/100 g; 0.33 mg/100 g; and 0.33 mg/100 g, respectively) (U. S. Department of Agriculture. (2016) (2016) (2016), 2016). The divergence between the results obtained and those reported in the literature may be due to genetic variability and cultivation conditions as soil and climate, which can affect the concentration and the profile of vitamins in sorghum (Ochanda, Akoth, Mwasaru, Kagwiria, & Mathooko, 2010).

Maceration increased the concentration of riboflavin in the grains by 40% when compared to the control ($p < 0.05$). This increase was similar to that obtained by other authors in sorghum macerated for three days (increase from 44.2% to 82%) (Malleshi & Klopfenstein, 1998; Ochanda et al., 2010). During maceration, germination begins, which results in biochemical, textural and physiological transformations in the grains, including the synthesis of some B vitamins, such as riboflavin (Chavan, Kadam, & Beuchat, 1989; Ochanda et al., 2010). In contrast, the concentration of total thiamine and total pyridoxine decreased (retentions of 60.8% and 30.1%, respectively) after maceration ($p < 0.05$), possibly due to the leaching of these compounds in water (Afify, El-Beltagi, El-Salam, & Omran, 2012a). Some authors reported that sorghum maceration reduced or did not affect the concentration of both vitamins (Malleshi & Klopfenstein, 1998; Ochanda et al., 2010).

In view of this differentiated behavior of the B vitamins, it is suggested that there were also losses in the concentrations of riboflavin after maceration, but these losses were not significant to the point of being lower than the concentrations of the control sorghum (since its concentration in the macerated sorghum was still greater than that of raw grain). Thus, it is suggested that there was a greater synthesis of riboflavin by the sorghum grains during the germination process, when compared to the other B vitamins.

After germination, only the riboflavin concentration increased significantly when compared to the control. This result was compatible with that reported by other authors, who observed an increase in the concentration of riboflavin and an absence of changes in the concentration of other B vitamins (Ochanda et al., 2010). The germinated grains showed an increase of at least 70% in the retention of riboflavin and pyridoxine in relation to the macerates, which corroborates the possible synthesis of these vitamins during the germination verified by Chavan et al. (1989).

3.3. Vitamin E

The concentration of vitamin E in the control sorghum varied within the range reported in the literature (Afify et al., 2012a; Cardoso, Pinheiro, da Silva, et al., 2015) (Table 3). The sorghum used in our study showed six vitamin E compounds, with a prevalence of γ-tocotrienol (66.5% of the total vitamin E) and tocotrienols in relation to tocopherols (ratio of tocopherols/tocotrienols = 0.23). The vitamin E profile verified in the present study differed from that reported by Cardoso, Pinheiro, da Silva, et al. (2015), who observed the prevalence of tocopherols and γ-tocopherol in 97 sorghum genotypes. It is noteworthy that sorghum

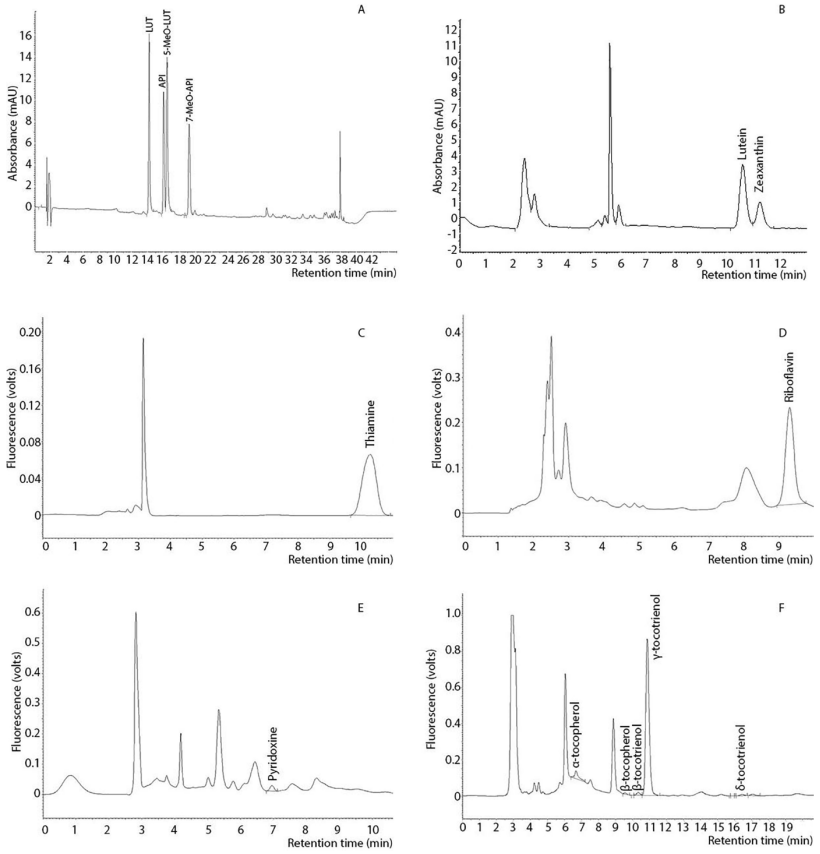

Fig. 1.

Table 2
Effect of maceration and germination on the concentration (mg/100 g) and real retention (%) of B complex vitamins in sorghum.[A,B]

Vitamin	Control		Maceration			Germination		
	Concentration in Fresh matter	Concentration in Dry matter	Concentration in Fresh matter	Concentration in Dry matter	RR	Concentration in Fresh matter	Concentration in Dry matter	RR
Thiamine	0.70 ± 0.04	0.79 ± 0.04 [a]	0.46 ± 0.09	0.48 ± 0.09[b]	60.8	0.41 ± 0.05	0.45 ± 0.05[b]	57.1
Riboflavin	0.04 ± 0.00	0.05 ± 0.00[b]	0.06 ± 0.01	0.07 ± 0.01[b]	140.0	0.18 ± 0.06	0.19 ± 0.06 [a]	380.2
Pyridoxine	0.09 ± 0.02	0.10 ± 0.02 [a]	0.03 ± 0.00	0.03 ± 0.00[b]	30.1	0.04 ± 0.00	0.05 ± 0.00[b]	50.3

[A] The results were expressed as the average of 4 repetitions ± standard deviation.
[B] Means followed by the same letter in the lines for concentration on a dry matter are not statistically different at 5% probability by the Duncan test. Real retention = [(concentration of the compound in the processed grain × weight of the processed grain)/(concentration of the compound in the grain before processing × weight of the grain before processing)] × 100. RR = Real Retention.

has great genetic variability and in the vitamin E profile (Cardoso, Pinheiro, da Silva, et al., 2015), which justifies these divergences.

Few studies are available on the concentration and profile of vitamin E in macerated and germinated sorghum (Afify et al., 2012a). The total concentration of this vitamin and the equivalent of α-tocopherol in sorghum were not altered by maceration ($p > 0.05$), although this

Table 3
Effect of maceration and germination on the concentration (μg/100 g) and real retention (%) of vitamin E and xanthophylls in sorghum.[A,B]

Vitamins	Control		Maceration			Germination		
	Concentration in Fresh matter	Concentration in Dry matter	Concentration in Fresh matter	Concentration in Dry matter	RR	Concentration in Fresh matter	Concentration in Dry matter	RR
Total Vitamin E	1950.2 ± 179.2	2216.4 ± 179.2 [a]	2462.1 ± 517.8	2573.0 ± 517.8 [a]	116.1	1078.5 ± 77.1	1180.6 ± 77.1[b]	53.3
α-tocopherol	281.8 ± 36.5	320.3 ± 36.5 [a]	244.0 ± 29.6	255.0 ± 29.6 [a]	79.6	226.5 ± 52.8	248.0 ± 52.8 [a]	77.4
α-tocotrienol	250.2 ± 29.7	284.3 ± 29.7 [a]	132.0 ± 16.5	137.9 ± 16.5[b]	48.5	49.9 ± 11.4	54.6 ± 11.4[c]	19.2
β-tocopherol	11.3 ± 17.8	12.8 ± 17.8[c]	573.9 ± 291.5	599.8 ± 291.5 [ab]	85.9	223.8 ± 19.2	245.0 ± 19.2 [bc]	191.41
γ-tocopherol	81.0 ± 16.9	92.0 ± 16.9 [a]	0.0 ± 0.0	0.0 ± 0.0[b]	0.0	0.0 ± 0.0	0.0 ± 0.0[b]	0.0
β-tocotrienol	0.0 ± 0.0	0.0 ± 0.0[b]	0.0 ± 0.0	0.0 ± 0.0[b]	0.0	89.2 ± 16.3	97.6 ± 16.3 [a]	0.0
γ-tocotrienol	1296.4 ± 122.4	1473.4 ± 122.4 [a]	1367.2 ± 204.3	1428.8 ± 204.3 [a]	96.9	475.2 ± 29.3	520.2 ± 29.3[b]	35.3
δ-tocoferol	0.0 ± 0.0	0.0 ± 0.0[b]	20.2 ± 1.4	21.1 ± 1.4 [a]	00	0.0 ± 0.0	0.0 ± 0.0[b]	0.0
δ-tocotrienol	29.6 ± 3.3	33.6 ± 3.3 [a]	17.9 ± 2.7	18.7 ± 2.7[b]	55.7	14.6 ± 0.7	16.0 ± 0.7[b]	289.5
α-T Equiv.	370.6 ± 35.8	421.2 ± 35.8[b]	511.7 ± 43.8	534.7 ± 43.8 [a]	126.9	360.4 ± 49.3	394.5 ± 49.3[b]	93.7
Xanthophylls (sum)	27.2 ± 8.4	30.9 ± 8.4 [a]	35.0 ± 6.9	36.6 ± 6.9[b]	118.8	27.4 ± 23.0	30.0 ± 23.0 [a]	97.0
Lutein	12.2 ± 8.9	13.9 ± 8.9 [a]	12.8 ± 5.5	13.4 ± 5.5 [a]	521.6	13.1 ± 4.8	14.3 ± 4.8 [a]	102.8
Zeaxanthin	16.7 ± 4.1	19.0 ± 4.1 [a]	22.2 ± 4.8	23.2 ± 4.8 [a]	122.1	14.3 ± 78.2	15.6 ± 78.2 [a]	82.1

[A] Results were expressed as the average of 4 repetitions ± standard deviation.
[B] Means followed by the same letter on the lines are not statistically different at 5% probability by the Duncan test. α-T Equiv.: equivalents of α-tocopherol. Real retention = [(concentration of the compound in the processed grain × weight of the processed grain)/(concentration of the compound in the grain before processing × weight of the grain before processing)] × 100.

processing increased the concentrations of β and δ-tocopherols and reduced those of δ-tocotrienols and γ-tocopherol ($p < 0.05$). Afify et al. (2012a), when evaluating the effect of maceration on sorghum α-tocopherol, found a reduction in the concentration of this compound, which differs from the present study, in which maintenance was verified. At the end of the maceration, the grains showed a higher concentration of α-tocopherol equivalents, which resulted in an increase in the grain's potential to supply an individual's nutritional vitamin needs.

Germination reduced the total concentration of vitamin E, tocotrienols (α, γ and δ-tocotrienols) and γ-tocopherol, however, increased β-tocopherol and β-tocotrienol ($p < 0.05$). The increase in the concentration of total vitamin E and its compounds was observed after rice germination (Ng, Huang, Chen, & Su, 2013). It should be noted that rice has different chemical and metabolic characteristics from those of sorghum, which may justify the different behavior during germination. The mechanisms involved in altering the concentration and profile of vitamin E in sorghum during germination are poorly understood. However, it is possible, although it did not influence the total concentration of vitamin E, that some of its compounds were synthesized, aiming to supply the nutrient needs during grain development (Chavan et al., 1989).

3.4. Xanthophylls

The concentration and profile of xanthophylls (lutein and zeaxanthin) in sorghum (Fig. 1B) were similar to that observed by other authors (Cardoso, Pinheiro, da Silva, et al., 2015). Both processes did not affect the total concentration and the carotenoid profile in the grain ($p > 0.05$).

Studies on lutein and zeaxanthin concentrations in macerated and germinated sorghum grains are still scarce and need to be the object of future studies. Although the effects of maceration on the concentration of lutein and zeaxanthin in sorghum have not been evaluated, they are the most prevalent carotenoids (xanthophylls) in this cereal. Unlike our study, Afify et al. (2012a) found that maceration reduced the concentration of β-carotene in three sorghum varieties.

The behavior of carotenoids during germination seems to differ depending on the compound and the type of cereal analyzed. In a study with sorghum, Chavan et al. (1989) found an increase in the concentration of provitamin A. Unlike the present study, Yang and Ooraikul (2001) found an increase in the concentration of carotenoids during the germination of wheat grains, which was proportional to the germination time of the grains. In addition to the aforementioned factors, the characteristics of the maceration and grain germination techniques can also contribute to the variability of the results, such as time of immersion in water, time of germination, temperature and water:cereal ratio. In addition, carotenoids are fat-soluble compounds, which would hinder their possible losses only through the process of immersion in water (maceration).

3.5. Flavonoids

3.5.1. 3-Deoxyanthocyanidins

The control grains showed the four 3-DXAs analyzed (Fig. 1A; Table 4), with prevalence of LUT and 5-MeO-LUT (26.7% and 29.4% of the total of 3-DXAs, respectively). Total 3-DXAs of the raw grains represented 61.8% of the total of flavonoids (3-DXAs, flavones and flavanones). These results were similar to those observed by other authors (Cardoso, Pinheiro, da Silva, et al., 2015).

Maceration and germination reduced the total concentration of 3-DXAs and their forms from 11.7 to 43.7%, when compared to the control sorghum ($p < 0.05$). After maceration, LUT and 5-MeO-LUT remained the majority 3-DXAs. The reduction in total 3-DXAs in sorghum grains after germination was also verified by Dicko, Gruppen, Traoré, van Berkel, and Voragen (2005), when determined by spectrometry. In addition, Afify et al. (2012a), when analyzing three macerated white sorghum varieties, also found a reduction in flavonoid concentrations, with 3-DXAs showing losses of 21.9–28.3%.

In general, the reduction in the concentration of the 3-DXAs forms occurred equally. However, after the grain germination, there was a reduction in the concentration of LUT and 5-MeO-LUT and an increase in API and 5-MeO-API, when compared to macerated grains. Specifically, in this study, we should consider the possibility of converting forms derived from luteolinidin into forms derived from apigeninidin, as well as synthesis and degradation of these compounds independently (Cardoso, Pinheiro, da Silva, et al., 2015).

The mechanisms involved in changes in the 3-DXAs profile during sorghum maceration and germination are poorly understood and, therefore, need to be studied. Several mechanisms have been proposed to explain these effects of malting, which includes a stage of maceration followed by germination, in phenolic compounds. The leaching of phenolic compounds during maceration and germination of cereals has been proposed as one of the main reasons for the reduction of phenolic compounds (Afify et al., 2012a; Chavan et al., 1989). The aqueous environment during these processes facilitates the solubilization of phenolic compounds, which subsequently leach into the water (Taylor &

Table 4
Effect of maceration and germination on the concentration and real retention of flavonoids (μg / 100 g) (%) in sorghum.[A,B]

Flavonoids	Control		Maceration			Germination		
	Concentration in Fresh matter	Concentration in Dry matter	Concentration in Fresh matter	Concentration in Dry matter	RR	Concentration in Fresh matter	Concentration in Dry matter	RR
Total 3-DXAs	72.78 ± 3.73	82.71 ± 3.73 [a]	55.13 ± 3.42	57.61 ± 3.42[b]	69.6	52.86 ± 7.62	57.86 ± 7.62[b]	69.9
Luteolinidin	19.44 ± 3.64	22.09 ± 3.64 [a]	13.75 ± 3.21	14.37 ± 3.21[b]	65.3	11.56 ± 1.61	12.66 ± 1.61[b]	57.3
Apigeninidin	16.08 ± 2.31	18.28 ± 2.31 [a]	10.77 ± 1.30	11.25 ± 1.30[b]	61.5	12.74 ± 1.96	13.95 ± 1.96[b]	76.3
5-MeO-Luteolinidin	21.37 ± 1.62	24.29 ± 1.62 [a]	17.24 ± 1.94	18.02 ± 1.94[b]	74.2	12.78 ± 1.78	13.99 ± 1.78[c]	57.5
7-MeO-Apigeninidin	15.86 ± 1.19	18.02 ± 1.19 [a]	13.36 ± 1.60	13.96 ± 1.60[c]	77.4	14.54 ± 1.37	15.92 ± 1.37[b]	88.3
Total Flavones	86.41 ± 4.95	98.21 ± 4.95 [ab]	105.85 ± 22.48	110.62 ± 22.48 [a]	112.6	11.45 ± 1.30	12.53 ± 1.30[b]	20.2
Luteolin	55.94 ± 6.31	63.58 ± 6.31 [a]	66.69 ± 8.97	69.69 ± 8.97 [a]	109.6	7.09 ± 0.99	7.76 ± 0.99[b]	12.2
Apigenin	30.47 ± 1.36	34.63 ± 1.36 [a]	39.16 ± 3.56	40.92 ± 3.56 [a]	118.1	4.36 ± 0.82	4.77 ± 0.82[b]	13.7
Total Flavanones	362.55 ± 22.50	412.03 ± 22.50[b]	356.62 ± 15.91	372.68 ± 15.91 [a]	90.4	100.27 ± 8.92	109.76 ± 8.92[c]	26.6
Eriodyctiol	213.01 ± 7.40	242.08 ± 7.40 [a]	182.25 ± 3.48	190.46 ± 3.48 [a]	78.6	75.77 ± 1.11	82.94 ± 1.11[b]	34.3
Naringenin	149.53 ± 9.65	169.94 ± 9.65 [a]	174.37 ± 3.06	182.22 ± 3.06 [a]	107.3	20.73 ± 2.23	22.69 ± 2.23[b]	13.3

[A] Results were expressed as the average of 4 repetitions ± standard deviation.
[B] Means followed by the same letter on the lines are not statistically different at 5% probability by the Duncan test. Real retention = [(concentration of the compound in the processed grain × weight of the processed grain)/(concentration of the compound in the grain before processing × weight of the grain before processing)] × 100.

Duodu, 2015).

Another proposed mechanism by which grain maceration and germination can decrease sorghum phenolic compounds is the entry of these compounds into the endosperm together with water soaked during processing (Towo, Svanberg, & Ndossi, 2003). Within the endosperm, phenolic compounds can bind with proteins and other macromolecules (mainly starch) and become less extractable (Towo et al., 2003).

3.5.2. *Flavones and flavanones*

The total concentration and flavone profile observed in control grains, with a prevalence of luteolin (Table 4) was similar to that reported in the literature (Afify et al., 2012a; Cardoso, Pinheiro, de Carvalho, et al., 2015). The total concentration of flavones and their isolated forms were not altered by maceration, which differed from that reported by Afify et al. (2012a), who observed reductions of these compounds in three sorghum varieties. In contrast, in our study, germination reduced flavone concentrations (20.2% retention), which also differed from that found by Dykes et al. (2009), who observed that the germination of different sorghum varieties did not influence the total concentration of flavones and their isolated forms.

The investigated flavanones (eriodictyol and naringenin) were identified in control and processed sorghum grains (Table 3), with a prevalence of eriodictyol (on average, 58.8% of the total). The prevalence of eriodictyol differs from that observed by Dykes et al. (2009) and is similar to that reported by (Cardoso, Pinheiro, de Carvalho, et al., 2015). It should be considered that the concentration of flavanones can be influenced by the color of the grain pericarp, which could cause discrepancies in the values of flavanones found in different sorghum varieties (Dykes, Peterson, Rooney, & Rooney, 2011).

Few studies have evaluated the effects of maceration and germination on sorghum flavanones. Maceration maintained the total concentration of flavanones and their isolated forms, while germination reduced these compounds between 73 and 87%. Unlike the present study, reductions greater than 45.3% in the total of flavanones and their compounds isolated in sorghum were also verified by Afify et al. (2012a) in three sorghum varieties submitted to maceration. Regarding germination, the reduction in the concentration of flavanones in sorghum is compatible with that described by Dykes et al. (2009). The mechanisms for changing the concentrations of flavones and flavanones may include the leaching of these compounds into the maceration water and migration into the grain, followed by binding to macromolecules, as previously discussed for 3-DXAs (Taylor & Duodu, 2015; Towo et al., 2003).

4. Conclusion

To date, this is the first study to assess the impact of sorghum maceration and germination on the concentration of vitamins (mainly B complex), xanthophylls and bioactive compounds. Maceration resulted in greater stability of vitamins, xanthophylls and flavonoids, while, in general, germination resulted in less retention of the analyzed compounds. Our results indicate that, even after germination and, especially, after maceration, sorghum showed an important nutritional value. Our results suggest that ingestion of sorghum, especially in macerated form, should be stimulated.

CRediT authorship contribution statement

Soraia Silva Pinheiro: Conceptualization, Methodology, Investigation, Writing - original draft. **Pamella Cristine Anunciação:** Conceptualization, Writing - review & editing. **Leandro de Morais Cardoso:** Conceptualization, Methodology, Writing - review & editing, Supervision. **Ceres Mattos Della Lucia:** Conceptualization, Writing - review & editing, Supervision. **Carlos Wanderlei Piler de Carvalho:** Methodology, Resources, Writing - review & editing, Supervision. **Valéria Aparecida Vieira Queiroz:** Resources, Writing - review & editing, Funding acquisition. **Helena Maria Pinheiro Sant'Ana:** Conceptualization, Methodology, Resources, Writing - review & editing, Supervision, Project administration, Funding acquisition.

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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