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Mixed sorghum and quinoa flour improves protein quality and increases antioxidant capacity *in vivo*

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ABSTRACT

Quinoa is a pseudocereal that has high protein quality and sorghum has phenolic compounds that give it a higher antioxidant capacity, however, its protein quality is low. Thus, this study aimed to evaluate the effect of the obtaining mixture of sorghum and quinoa flours on protein quality and antioxidant capacity in Wistar rats. The animals were allocated into five groups (n = 8): protein-free, control (casein) and experimental groups: sorghum flour, quinoa flour, and mixed sorghum/quinoa flour. At 21 days of the experiment, animals from the control and experimental groups received sodium fluoride (NaF) water for seven days to induce oxidative stress. The groups fed with sorghum, quinoa, and mixed flour sorghum/quinoa had, respectively, PER values of 0.23, 2.0, 1.5; NPR 1.82, 3.36, 2.88 and TD 60.22, 81.46, 66.82 and weight gain 1.75, 20.84 and 15.92. The total antioxidant capacity of serum increased in sorghum and mixed sorghum/quinoa flour groups, probably due to the higher amount of phenolic compounds. There was no change in the activities of the antioxidant enzymes superoxide dismutase, catalase, and the biomarker malondialdehyde. Thus, the mixed flour of sorghum/quinoa is an excellent alternative for the elaboration of products with better protein quality and high antioxidant capacity.

1. Introduction

The growing consumer interest in "healthy living" has boosted the market for more natural and functional food claims, and producing foods that contain high content of phytochemical and high protein quality that are beneficial to health and have a relatively low cost is considered. Sorghum (*Sorghum bicolor* (L.) *Moench*) is a cereal grown in many countries in Africa, Asia, and Central America due to its adaptability to semi-arid, arid and high-temperature conditions (Awika & Rooney, 2004; Stefoska-Needham, Beck, Johnson, & Tapsell, 2015). Sorghum does not have gluten and has high content of phenolic compounds (phenolic acids, flavonoids and condensed tannins) and high antioxidant capacity, which contribute to the prevention of chronic diseases such as obesity, cardiovascular disease, type 2 diabetes mellitus (DM2), non-fatty liver disease, and cancer (Arbex et al., 2018; Farrar, Hartle, Hargrove, & Greenspan, 2007; Lopes et al., 2018; Stefoska-Needham et al., 2015).

In vivo studies have shown that sorghum has low protein quality due to the structure of its main protein (kafirins), the limiting amino acids such as threonine, tryptophan and lysine, and to interactions with phenolic compounds, such as condensed tannins. These tannins complex with proteins, reducing their digestion and absorption (Khan, Yousif, Johnson, & Gamlath, 2015; Moraes, Natal et al., 2012, Moraes, Queiroz et al., 2012; Stefoska-Needham et al., 2015).

Quinoa (*Chenopodium quinoa Wild*) is a pseudocereal belonging to the Chenopodiaceae family, which originates in the South American Andes. It is known for its genetic diversity, adaptability to different soils and conditions of water scarcity and high salinity (Rojas, Alandia, Irigoyen, & Blajos, 2011). The high protein quality of quinoa, with all essential amino acids and the absence of gluten give it an excellent nutritional value, although its antioxidant activity is lower when compared to sorghum (Filho et al., 2017; Valcárcel-Yamani & Lannes, 2012).

The blend of sorghum and quinoa flour can be a viable combination for the formulation of a variety of food products, due to its nutritional, agronomic and low-cost properties. The mixture of these grains has a

Abbreviations: PER, Protein efficiency ratio; NPR, net protein ratio; TD, True digestibility

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better balance of nutrients and bioactive compounds, providing better protein quality and antioxidant capacity. Thus, this study aimed to evaluate the effect of the mixture of sorghum flour with quinoa flour on the *in vivo* protein quality and antioxidant capacity.

2. Material and methods

2.1. Materials

To obtain the flours, sorghum grains of BRS 305 genotype (high tannin content), with a light brown pericarp, developed by Embrapa Miho and Sorgo, Sete Lagoas, MG, Ago/15 crop were used. Quinoa grains BRS Piabiru genotype were provided by Embrapa Cerrados, Planaltina, DF, crop 2013, lot BSB-003/13. The seeds were manually selected, sieved, to remove impurities and soils, and then stored (Ormifrioa freezer 10 °C).

2.2. Heat treatment and preparation of the flour

For the flour production, sorghum and quinoa grains were submitted to dry heat treatment. Recent studies have shown that the use of this treatment in sorghum flours was more efficient in maintaining antioxidant levels (Cardoso et al., 2014) and resistant starch (Teixeira et al., 2016) compared to the wet heat treatment.

The grains were placed in aluminum trays and exposed to 105 °C in an air circulating oven for 30 min (Moraes, Queiroz et al., 2012). After the heat treatment the grains were ground in a knife mill (C.W. Brabender®, Duisburg, Germany) with a 1.0 mm stainless steel sieve to obtain the flour.

2.3. Proximate composition, antioxidant capacity and total tannin content of the flours before and after the heat treatment

The proximate composition (proteins, lipids, dietary fiber, ash, and moisture) of the flours was determined according to the methods proposed by the AOAC (Association of Official Analytical Chemists, 1998) and the carbohydrate content was estimated by the difference [100 – (ash + protein + lipids + dietary fiber)].

The antioxidant capacity was determined by colorimetric ABTS [2,2′-azino-bis (3-ethylbenzothiazoline) -6-sulfonic acid] method as described by Awika, Rooney, Wu, Prior, and Cisneros-Zevallos (2003).

The determination of the total tannin content was performed by the vanillin/HCl reaction method, according to Burns (1971) with modifications Price, Scoyoc, and Butler (1978).

2.4. In vivo nutritional studies

2.4.1. Diet preparation

Based on the values of the proximate analysis of carbohydrates, proteins, lipids, and ash (AOAC, 2012), casein diet (control) and experimental diets were prepared, whose protein source was sorghum flour, quinoa flour or the mixture thereof in 1:1 ratio, with heat treatment. The composition of the diets was based on AIN-93G, according to Reeves, Nielsen, Fahey, & Fahey Jr., 1993, with changes in protein content between 9 and 10%, for protein quality studies (Moraes, Queiroz et al., 2012). The flours were packed in hermetically sealed polyethylene bags, duly labeled, and stored in a refrigerator at 5 °C. The diets were adjusted to be isocaloric and isoproteic (Table 1).

2.4.2. Biological assay

The study protocol was approved by the Ethics Committee of the Veterinary Department of the Federal University of Viçosa, Brazil (Protocol no. 20/2015). A total of 48 male rats (*Rattusnovergicus*, Albinus variety, class Rodentia) of Wistar line, were weaned, at a mean of 21 days old, at the laboratory of the Biological Sciences and Health Center of the Federal University of Viçosa. These animals were housed in individual stainless steel cages and kept under temperature conditions of 22 ± 3 °C, with a photoperiod of 12 h. The animals received distilled water and their respective experimental diets ad libitum.

Table 1
- Composition of the experimental diets. Protein-free (APT), casein (C), sorghum flour BRS 305 (STT), BRS Piabiru quinoa (QTT) and mixed sorghum/quinoa flour (SQTT).

	C	AP	STT	QTT	SQTT
Casein (g)	264.6	–	–	–	–
SorghumTT (g)	–	–	1961.2	–	785.1
QuinoaTT (g)	–	–	–	1309.3	785.1
Maltodextrin (g)	330	330	77.0	330	330
Sucrose (g)	250	250	250	250	250
Soybean oil (g)	175	175	85.6	91.6	89.2
Cellulose (g)	280.1	280.1	–	81.7	49.0
Mineral mix (g)	87.5	87.5	87.5	87.5	87.5
Vitamin mix (g)	25	25	25	25	25
L-cysteine (g)	7.5	7.5	7.5	7.5	7.5
Choline bitartrate (g)	6.3	6.3	6.3	6.3	6.3
Corn starch (g)	1074.10	1338.70	–	311.20	85.30
Total (g)	2500	2500	2500	2500	2500
Caloric Density (Cal/g)	3.65	3.70	3.38	3.52	3.46
Protein (g/100g)	9.36	0	9.41	9.36	9.35
Carbohydrate (g/100g)	66.16	76.74	65.33	65.19	65.24
Lipids (g/100g)	7.0	7.0	7.35	7.20	7.26

The study was divided into two phases: the protein quality (Phase 1) and the end of Phase 1, the oxidative stress induction was started for 7 days to evaluate the antioxidant effect of the experimental diets (phase 2). In the first phase, the animals were divided into five groups: the control groups (casein and protein-free), protein-free group and the experimental groups: sorghum flour BRS 305 (STT), BRS Piabiru quinoa (QTT) and mixed sorghum/quinoa flour (SQTT) in a 1:1 ratio. During the 14 days of the experiment, animals received the diets and deionized water ad libitum (Moraes, Queiroz et al., 2012). The animals were distributed in the groups so that the mean initial weights were similar among groups (57.61 g ± 3.02). All groups received eight animals, except the casein group that received 16 animals, as they were divided into two control groups in phase 2: one group receiving NaF (positive control) and the other with no addition of NaF (negative control). In Phase 2 the animals of the experimental groups, sorghum flour (STT), quinoa flour (QTT) and mixed sorghum/quinoa flour (SQTT) were also given sodium fluoride (NaF) added in drinking water, offered ad libitum for 7 days, at the concentration of 600 ppm (Nabavi et al., 2013; Nabavi, Nabavi, Eslami, & Moghaddam, 2012).

The animals of one control group (protein-free), in Phase 1, were euthanized at 14 days of experimentation and the protein quality indices were evaluated. The remaining groups continued to maintain their respective diets with distilled water ad libitum for 7 days until completing 21 days of experimentation to initiate the induction of oxidative stress for another 7 days, and then, at the end of the experiment, the animals were euthanized.

2.4.3. Protein quality indices

2.4.3.1. Feed efficiency ratio (FER). During the experimental period, animals were weighed on the 1st, 7th and 14th days and the feed efficiency ratio (FER) was determined, which represents the relationship between weight gain (g) and dietary intake of the animals (g).

2.4.3.2. Protein efficiency ratio (PER) and net protein ratio (NPR). The protein efficiency coefficient (PER) was determined using the method modified by Moraes, Queiroz et al., 2012 for 14 days of the experiment, which relates to the weight gain of the test group (g) to its protein intake (g). The relative protein efficiency coefficient (PER-R) was determined to be 100% of the PER result of the standard diet (casein).

The net protein ratio (NPR) was determined according to Bender

and Doell (1957), at 14 days of the experiment, taking into consideration the test group weight gain (g), plus the weight loss of the group with (g), relative to the protein intake of the test group (g). The relative net protein ratio (NPR-R) was determined considering as 100% the NPR result of the standard diet (casein).

2.4.3.3. True digestibility (TD). For determination of digestibility, according to Moraes, Queiroz et al., (2012), the diets were marked with indigo-carmine, in the concentration 0.2%, offered to the animals from the seventh to the tenth day. Marked feces were collected on the eighth day and all feces were marked on the tenth and 11th day, in individual containers for each animal. The feces were dried in an oven at 105 °C for 24 h. Afterward, they were cooled, weighed and crushed in a razor mill to determine the nitrogen content. TD was calculated by measuring the amount of nitrogen ingested (NI) and excreted in feces in the experimental diets (NE) and nitrogen fecal loss of the protein-free diet group (Fk) according to the formula: %TD = NI–(NE–Fk). The relative true digestibility (RTD) was determined based on the casein control group TD (i.e. considering the casein control group TD as 100%).

2.5. In vivo antioxidant potential of the flours submitted to heat treatment

At the end of the experiment (28 days), the animals were anesthetized (isoflurane, Cristália ®) and euthanized by cardiac puncture, to collect blood samples. Blood was collected in a heparinized tube and centrifuged for 10 min at 1006 g for serum collection. The liver was removed, weighed and immediately frozen in liquid nitrogen. All the collected materials were stored in an ultra-freezer at −80 °C until the beginning of the analysis.

2.5.1. Preparation of liver homogenate

Aliquots of 100 mg of liver were weighed into a 1.5 mL Eppendorf microtubes. The tissue was macerated and homogenized with 0.5 mL of 0.1 M sodium phosphate buffer solution, pH 7.4 cooled to 8 °C with the aid of a plastic stick. The homogenate was centrifuged at 13,800 g for 10 min at 4 °C, and the centrifuged supernatant was used. The samples were kept under refrigeration during the analysis.

2.5.1.1. Determination of total protein content. For protein determination in liver homogenates, the Bradford (1976) method was employed. 10 µl of a sample of each liver homogenate was diluted 1:5 with distilled water to make 50 µL. Subsequently, 790 µl of distilled water plus 10 µL of the diluted homogenate and 200 µL of the Bradford reagent were placed. For each sample 300 µL were pipetted, 3 times, in *Elisa* plate. After 15 min the absorbance was determined at 595 nm. This protein analysis was performed to express units of activity of antioxidant enzymes and the content of malondialdehydes in relation to protein (mg).

2.5.1.2. Superoxide dismutase. The determination of superoxide dismutase (SOD) activity was performed according to the modified Marklund (1985) method. The technique relies on the ability of the enzyme superoxide dismutase to deplete oxygen, reducing the autooxidation ratio of pyrogallol.

To obtain blanks, 45 µL of buffer solution and 6 µL of 3- [4,5-dimethyl thiazole-2H] −2,5-diphenyl tetrazolium bromide (MTT) were added in triplicate to the wells of the ELISA plate 1.25 mM; for the standard, 30 µl of buffer, 6 µL of MTT and 15 µL of pyrogallol (100 µM) were added; and in the samples 30 µl of supernatant, 99 µl of buffer solution, 6 µl of MTT and 15 µl of pyrogallol were added. Then, with the help of a multichannel automatic pipette, the reaction was stopped with the addition of 150 µL of dimethylsulfoxide (DMSO). Superoxide dismutase activity is represented by U SOD/mg PTN. The plate was read using an ELISA reader (ASYS®, UVM 340) at a wavelength of 570 nm.

2.5.1.3. Catalase. This assay was performed according to the method of Aebi (1984), which is based on the measurement of catalase activity (CAT), in the transformation of hydrogen peroxide (H_2O_2) into water. A blank (10 µL of the sample supernatant 1 mL of phosphate buffer) was made for each sample (10 µL of the sample supernatant 1 mL of the peroxide phosphate buffer solution) to clear the equipment. The reading of each sample was performed in a spectrophotometer at 240 nm at 0, 30, 60 s. A (U) catalase unit is equivalent to the hydrolysis of 1 mol H_2O_2 (E = 39.4 L $mol^{-1}.cm^{-1}$) per minute (Aebi, 1984). Generally, the activity of the enzyme is represented by U catalase/mg of PTN and is calculated by the absorbance at time 0 subtracted from the absorbance at 60 s.

2.5.1.4. Malondialdehyde. The content of malondialdehyde (MDA), formed from the degradation of polyunsaturated fatty acids, is a convenient index to determine the extent of the peroxidation reaction. For determination of MDA, the method Buege and Aust (1978) was used in which 200 µL of the homogenate were mixed with 400 µL of TBA reagent solution (trichloroacetic acid - 15% (w/v) thiobarbituric acid - 0.375% (w/v) and 0.25 M HCl), vortexed for 5 s and incubated in a 90 °C water bath for 40 min. The sample was then cooled on ice for 5 min, and 600 µL of n-butanol was added, shaking for 1–2 min. It was then centrifuged for 10 min at 900 g and the supernatant was counted at 535 nm on Elisa's plate against blanks containing all reagents minus the sample. The final values were calculated using the standard curve, using the N-oxyl-2,2,6,6-tetramethylpiperidine reagent (TEMPO). The results were given as nmol of MDA per milligram of proteins (nmol of MDA/mg PTN).

2.5.2. Serum biochemical variables

Alanine aminotransferase (ALT), aspartate aminotransferase (AST), urea and creatinine were determined in serum samples using commercially available kits (Bioclin).

2.5.3. Total antioxidant capacity in serum

The total antioxidant capacity (TAC) kit protocol (SIGMA®) was used. The substrate working 2,2′-azinobis (3-ethylbenzothiazoline) -6-sulfonic acid solution (ABTS) was prepared: 37.5 µL of 3% hydrogen peroxide solution was added to 15 mL of ABTS, to be used after 20–30 min.

For the standard curve, 10 µL of a Trolox standard (from tubes 1–6) and 20 µL of the myoglobin working solution was used in the respective Elisa plate wells. In these wells 10 µL of the sample and 20 µL of the myoglobin work solution were added.

150 µL of ABTS radical cation working solution were then added to each well, leaving to incubate for 5 min. Then, 100 µL of stop solution was placed in each well. The reading was made at an absorbance at 405 nm. The results were given as mmol equivalent Trolox/L.

2.6. Statistical analysis

The results were submitted to an analysis of variance (ANOVA), and the "F" test was performed, with a probability of 5%. For significance, the Duncan test at 5% probability was used to compare each test group. The t-Student test was also performed for comparisons between the two groups.

The statistical program used was the Statistical Analysis System (SAS), updated version, and licensed to the Federal University of Vicosa.

3. Results and discussion

3.1. Chemical composition and in vivo protein quality of the flours

No significant differences were observed in the chemical composition of sorghum and quinoa flours before and after heat treatment

Table 2
Proximate composition and antioxidant capacity (μmol Trolox Equivalent/g sample) of the whole quinoa and sorghum flours of BRS Piabiru and BRS 305 varieties, on a dry basis (g.100 g -1).

	Quinoa flour		Sorghum flour	
	Raw	HT	Raw	HT
Moisture	11.84 ± 0.51^{a}	5.9 ± 0.47^{b}	12.37* ± 0.26^{A}	9.09 * ± 0.14^{B}
Protein	18.76 ± 1.06^{a}	18.66 ± 0.17^{a}	12.94 ^{ns} ± 0.33^{A}	12.97 ^{ns} ± 0.19^{A}
Lipids	6.44 ± 0.06^{a}	6.76 ± 0.02^{a}	4.33 ± 0.25^{A}	5.01^{ns} ± 0.12^{A}
Ash	3.51 ± 0.12^{a}	3.02 ± 0.09^{a}	1.25 ^{ns} ± 0.08^{A}	1.13 ^{ns} ± 0.04^{A}
Total dietary fiber	11.57 ± 0.42^{a}	12.15 ± 4.31^{a}	14.77 ± 2.78^{a}	14.28 ± 0.69^{a}
Insoluble fiber	8.61 ± 0.05^{a}	8.24 ± 1.73^{a}	13.9 ± 0.05^{a}	13.95 ± 0.77^{a}
Soluble fiber	2.96 ± 0.37^{a}	3.91 ± 2.58^{a}	0.87 ± 2.73^{a}	0.33 ± 0.08^{a}
Carbohydrates	59.72 ± 0.19^{a}	56.41 ± 0.83^{a}	66.64 ± 0.75^{A}	66.61 ± 0.69^{A}
Antioxidant capacity	59.56 ± 2.47^{a}	67.83 ± 0.94^{a}	241.52 ± 65.86^{A}	296.41 ± 25.87^{A}
Condensed tannins	ND	ND	57.16 ± 1.71^{A}	59.53 ± 1.80^{A}

HT = heat treatment.
Means followed by the same letter in the same row, between groups (raw versus HT), do not differ by 5% probability, by the paired *t*-test; ± standard deviation. ND: not detected.

(Table 2), although studies show that processing (extrusion, cooking, dry heat, and moist heat) can affect the chemical composition and antioxidant profile (Brady, Ho, Rosen, Sang, & Karwe, 2007; Cardoso et al., 2014). The heat treatment also did not affect the antioxidant capacity in the grains and the sorghum had a higher antioxidant capacity than the quinoa, which can be attributed to the high content of condensed tannins present in the genotype BRS 305 (Table 2) (Moraes, Natal et al., 2012).

The chemical compositions of whole flours evaluated with and without heat treatment corroborate the values described in the literature (Borges, Bonomo, Paula, Oliveira, & Cesário, 2010). The protein content of quinoa is higher than that of sorghum. Therefore, quinoa can be considered a food with high protein content, higher than that found in cereals (Filho et al., 2017).

The initial weight of the animals did not differ between the groups, indicating the homogeneity of the experiment (Fig. 1A). The weight gain of the sorghum fed animals was lower than that of the other groups and could be associated with lower feed intake and sorghum phenolic compounds (Fig. 1A), such as tannins, which decrease protein digestibility and can reduce palatability, giving it an astringent taste (Makkar, 2003; Neilson, Giddins, & Richards, 1986). Sorghum also has a limitation on essential amino acids that limits its protein quality and thus the weight gain of the animals (Moraes, Queiroz et al., 2012).

The group fed sorghum/quinoa (SQTT) and quinoa (QTT) did not present differences in weight gain (Fig. 1A). The addition of quinoa to sorghum may have improved protein quality (Alves, Rocha, & Gomes, 2008), the palatability and consequently the food consumption, which reflected in a weight gain similar that with quinoa flour (Fig. 1B and C). The mixing of flours can increase their appeal and commercialization, due to the low price of sorghum and its phenolic compounds, besides the high protein quality present in quinoa.

Among the experimental groups, the STT group had an ingested nitrogen value lower than that of the QTT and SQTT groups (Table 3). In spite of this, fecal nitrogen excretion was higher in animals fed the STT and SQTT diets, compared to casein and quinoa control. No difference was observed for nitrogen excreted in feces in the control group and in the QTT group. The high excretion of fecal nitrogen observed in the SQTT and STT groups is possibly due to the presence of tannins, which may have complexed with the proteins, impairing their digestion and absorption. Al-Mamary, Al-Habori, Al-Aghbari, and Al-Obeidi (2001), reported a high amount of fecal nitrogen in animals fed with tannin-rich sorghum, in addition to the reduction in body weight gain due to the interaction between tannins and intestinal mucus glycoproteins.

Higher digestibility of the quinoa protein was found, compared to the digestibility of the other groups (STT and SQTT) (Table 3), due to the presence of all the essential amino acids in the quinoa protein (Repo-Carrasco, Espinoza, & Jacobsen, 2003; Watanabe et al., 2014). On the other hand, digestibility of sorghum proteins is affected by condensed tannins, which demonstrates that sorghum is not indicated as a good source of protein, but its presence in the diet increases its antioxidant potential (Khan et al., 2015; Stefoska-Needham et al., 2015) and combined with quinoa, improves the protein quality of the diet, as shown in Table 3. Ranhotra et al. (1993) and Mendes, Oliveira, Costa, Pires, and Hoffmam (2009) obtained a digestibility for quinoa of 85.95 and 84.3%, respectively, and Moraes, Queiroz et al., (2012) found a digestibility of 57.6% for sorghum, values similar to those obtained in the present study.

The values of PER, NPR, PER-R, and NPR-R of the sorghum whole meal group were lower than the other groups tested (Table 4). Similar values were found by Moraes, Queiroz et al., (2012) who evaluated the protein quality of different sorghum genotypes. According to Friedman and Gumbmann (1986), a PER value lower than 1.5 represents a low-quality protein, in the case of sorghum, due to the presence of the tannins that complex with the proteins, preventing their digestion and absorption (Dunn, Yang, Girard, Bean, & Awika, 2015; Moraes, Queiroz et al., 2012). In addition, they have low levels of essential amino acids, such as lysine, tryptophan, and threonine (Badi, Pedersen, Monowar, & Eggum, 1990; Stefoska-Needham et al., 2015) which impairs the growth and development of these animals, thus affecting PER and NPR values.

The QTT and SQTT groups showed significant differences for PER and NPR values. This means that the QTT is efficient to promote weight gain with PER values above 2.0, considered high-quality protein. The SQTT diet represents a protein of medium quality, according to the PER values between 1.5 and 2.0 (Friedman & Gumbmann, 1986). The combination of these raw materials resulted in improved protein quality due to the contribution of quinoa to the sorghum limiting amino acids. No difference was observed in feces moisture among the different test groups (Table 5), possibly associated with the type of fibers present in sorghum and quinoa, which differed making them superior to the control group (cellulose). Freitas, Motta, Amâncio, Neto, and Morais (2004) evaluated the effect of the polysaccharide of soybean in relation to the cellulose on the weight and fecal moisture, finding a greater dry weight in the feces of the animals of the cellulose group. They concluded that the fermentation of non-cellulosic polysaccharides is more efficient than the fermentation of the cellulose, less being degraded in the intestinal tract.

3.2. *In vivo* antioxidant potential of flours

In this study, no change in oxidative stress was observed in animals

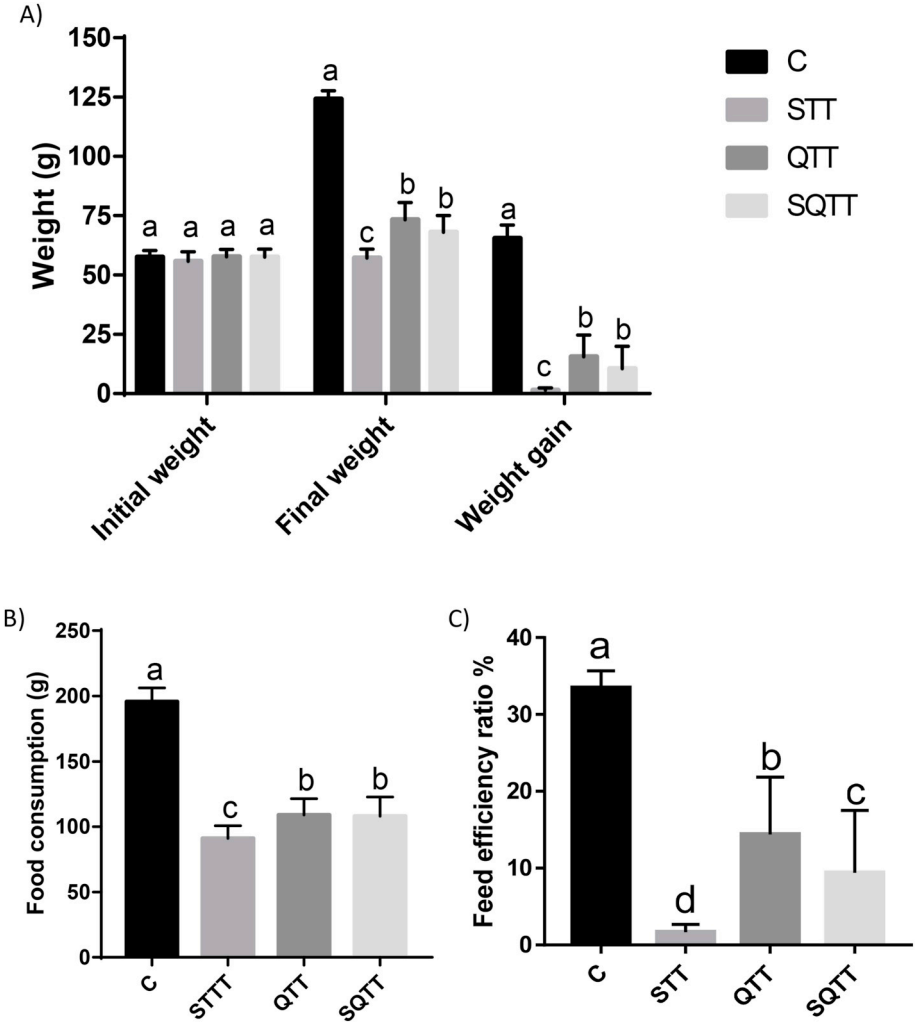

Fig. 1. Weight (A), food consumption (B) and feed efficiency ratio (C) of Wistar rats fed sorghum, quinoa and mixed sorghum/quinoa flours submitted to heat treatment. Casein (C), sorghum flour BRS 305 (STT), BRS Piabiru quinoa flour (QTT) and mixed flour sorghum/quinoa (SQTT). Means followed by the same letter do not differ by 5% probability, by the Duncan test.

Table 3
Nitrogen ingested in the diet (NI), nitrogen excreted in feces (NE), true digestibility (TD) and relative true digestibility (RTD) of animals fed diets with casein (C), sorghum flour BRS 305 STT), quinoa flour BRS Piabiru (QTT) and mixed flour sorghum/quinoa (SQTT), subjected to heat treatment. Means followed by the same letter in the same column do not differ by 5% probability, by the Duncan test; ± standard deviation.

Diet	NI	NE	TD	RTD
C	0.62 ± 0.03^{a}	0.05 ± 0.01^{b}	92.65 ± 1.90^{a}	100*
STT	0.28 ± 0.04^{c}	0.13 ± 0.04^{a}	60.22 ± 6.60^{d}	61.24 ± 9.11^{c}
QTT	0.38 ± 0.04^{b}	0.08 ± 0.01^{b}	81.46 ± 2.64^{b}	87.93 ± 2.85^{a}
SQTT	0.34 ± 0.06^{b}	0.12 ± 0.02^{a}	66.82 ± 2.44^{c}	72.12 ± 2.64^{b}

* It was considered to be 100 for the casein control group.

Table 4
Protein efficiency ratio (PER), relative protein efficiency ratio (PER-R), net protein ratio (NPR) and relative net protein ratio (NPR-R) of diets with casein (C), sorghum flour BRS 305 (STT), quinoa flour BRS Piabiru (QTT) and mixed flour sorghum/quinoa (SQTT), subjected to heat treatment.

Diets	PER	PER-R (%)	NPR	NPR-R (%)
C	3.58 ± 0.24^{a}	100*	4.31 ± 0.24^{a}	100*
STT	0.23 ± 0.05^{d}	5.77 ± 3.08^{c}	1.82 ± 0.17^{d}	42.31 ± 3.90^{c}
QTT	2.02 ± 0.56^{b}	56.46 ± 15.64^{a}	3.36 ± 0.51^{b}	77.82 ± 11.90^{a}
SQTT	1.52 ± 0.52^{c}	42.58 ± 14.62^{a}	2.88 ± 0.36^{c}	66.85 ± 8.47^{b}

Means followed by the same letter in the same column do not differ by 5% probability, by the Duncan test; ± standard deviation.
* PER-R and NPR-R were determined considering 100% for the standard diet (casein).

receiving NaF by the MDA measurement (Fig. 2A). This result is unexpected because NaF did not induce oxidative stress and metabolic disorders. Our study was based on the methodology of S. F. Nabavi et al. (2013) and S. M. Nabavi et al. (2012), in which we used the same dosage and intervention time, but we did not observe the induction of oxidative stress. NaF may have altered the taste of water, reflecting lower water intake. Low water intake may compromise food intake (Boyle, Lorenzen, Compton, & Watts, 2012; García-Luna, Amaya, Alvarez-Salas, & Gortari, 2010) and in fact, this was observed. The average consumption of animals (in g.animal-1) prior to treatment was 166.21, 44.84, 68.83, 68.64 for the positive control, sorghum, quinoa, and mixed flour sorghum/quinoa, respectively, and increased to 70.77, 23.10, 32.29, 21.59, one week after NaF intake.

The total antioxidant capacity in the serum was higher for the STT and SQTT diets (Fig. 2B), which may be due to the presence of condensed tannins, anthocyanins and phenolic acids in sorghum (Iglesia, Milagro, Campión, Boqué, & Martínez, 2010; Moraes, Natal et al., 2012; Stefoska-Needham et al., 2015). The increase of exogenous antioxidants offered by sorghum increased the total antioxidant capacity and

Table 5
Moisture in feces (MF), wet feces (FU) and dry feces (FS) of animals fed diets with casein (C), sorghum flour BRS 305 (STT), quinoa flour BRS Piabiru (QTT) and mixed flour sorghum/quinoa (SQTT), subjected to heat treatment.

Diets	MF (g)	FU (g)	FS (g)
C	12.84 ± 3.46[b]	6.79 ± 0.50[a]	5.92 ± 0.57[a]
STT	20.48 ± 1.33[a]	3.72 ± 0.90[b]	2.95 ± 0.69[b]
QTT	20.47 ± 3.46[a]	3.53 ± 0.69[b]	2.90 ± 0.48[b]
SQTT	22.20 ± 3.17[a]	3.89 ± 0.72[b]	3.02 ± 0.55[b]

Means followed by the same letter in the same column do not differ by 5% probability, by the Duncan test; ± standard deviation.

therefore did not demand an increase in the endogenous antioxidant defenses as measured by the SOD and CAT enzymes. As NaF intake did not induce oxidative stress, the activities of these enzymes were also not altered in the quinoa and control groups (Fig. 2C and D).

The increase in antioxidant capacity was also observed in the study of Khan et al. (2015) which evaluated the effect of sorghum pasta on oxidative stress markers in healthy individuals, observing an increase in the enzymes and antioxidant capacity for red sorghum. Although, in our study, the quinoa group did not demonstrate an increase in the antioxidant capacity in relation to the NaF group subjected to stress, Pasko, Zagrodzki, Bartoń, Chłopicka, and Gorinstein (2010) evaluated the effect of the diet supplemented with quinoa seeds on oxidative status in plasma and rat tissues and concluded that administration of quinoa protected the plasma against lipid peroxidation.

Plasma levels of hepatic enzymes (AST and ALT) and renal function markers (creatinine and urea) increased in sorghum and quinoa-based groups compared to controls (Table 6). Although these increases are within the recommended range, with the exception of AST, since it is more susceptible to variation (Ramaiah, 2007), it is suggested that the lower intake of water and food, together with the greater loss of moisture in the feces (Table 5), observed for the experimental groups, caused dehydration in the animals and promoted the increase of these values (Roncal-Jimenez, Lanaspa, Jensen, Sanchez-Lozada, & Johnson, 2015).

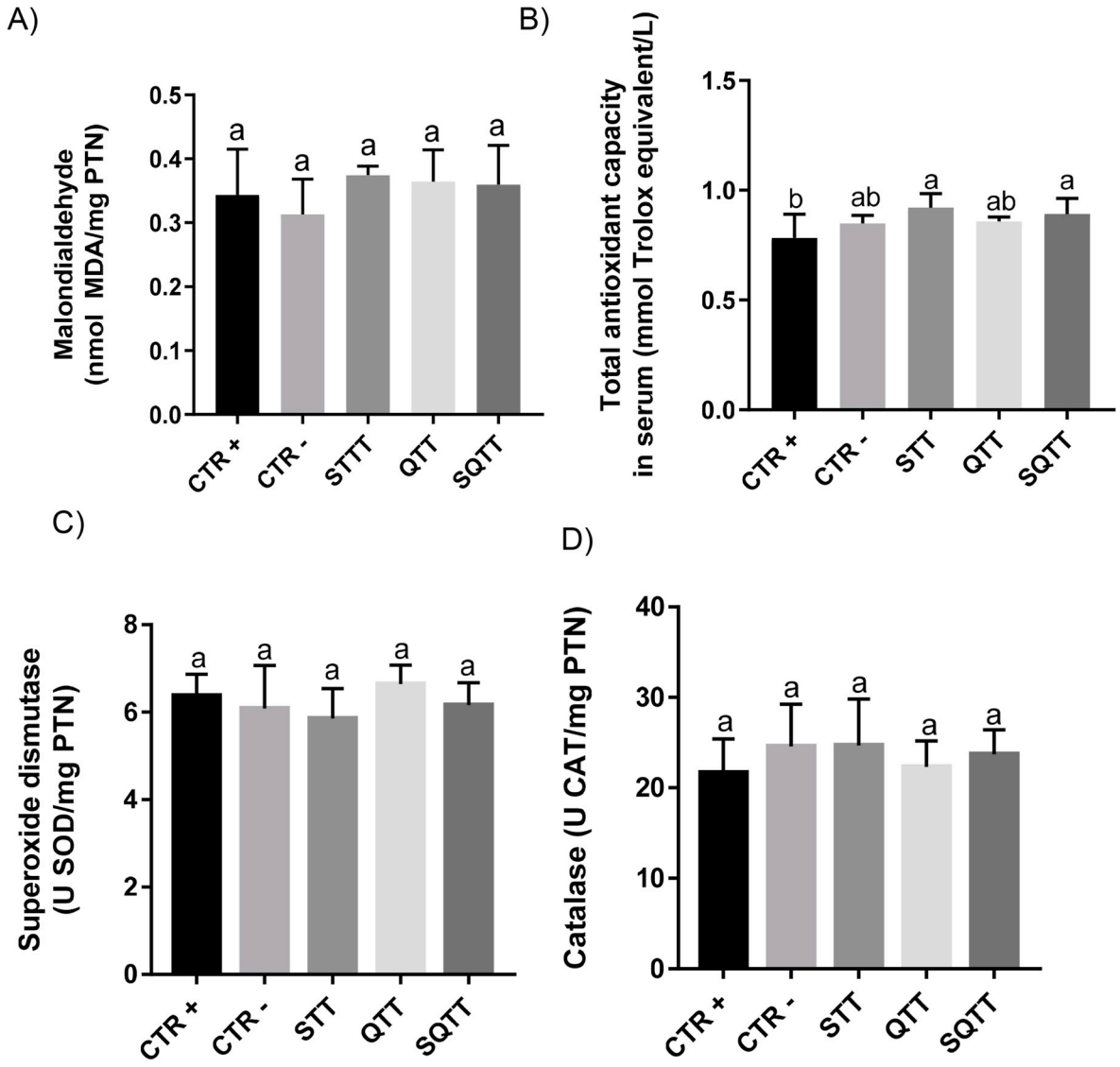

Fig. 2. The effect of sorghum, quinoa and mixed sorghum/quinoa flours ingested with sodium fluoride (NaF) for seven days on the contents of malondialdehyde (A), total antioxidant capacity in serum (B), superoxide dismutase (SOD) (C) and catalase (CAT) (D). Casein control diet with NaF (CTR +), casein control diet without NaF (CTR -) and sorghum flour diets BRS 305 (STT), BRS Piabiru quinoa flour (QTT) and mixed flour sorghum/quinoa (SQTT), ingested with NaF. Means followed by the same letter do not differ by 5% probability, by the Duncan test.

Table 6
Effect of sorghum and quinoa flour ingested with sodium fluoride (NaF) on the biochemical markers of the renal and hepatic function of the animals for seven days.

C	Creatinine (mg/dl)	Ureia (mg/dl)	AST (U/l)	ALT (U/l)
CTR +	0.35 ± 0.04[c]	7.5 ± 0.84[b]	118.86 ± 9.92[b]	36.43 ± 4.35[c]
CTR -	0.37 ± 0.03[c]	8.83 ± 2.48[b]	121.8 ± 8.32[b]	32.22 ± 6.20[c]
STT	0.55 ± 0.07[a]	16.2 ± 2.28[a]	177 ± 10.38[a]	44.29 ± 5.74[b]
QTT	0.43 ± 0.03[b]	15.75 ± 3.86[a]	172 ± 17.68[a]	52.17 ± 8.91[a]
SQTT	0.53 ± 0.06[a]	18.80 ± 4.97[a]	164 ± 30.69[a]	50.5 ± 7.62[ab]
Reference[a]	0.2–0.8	15.0–21.0	39.0–92.0	17.0–50.0

Casein control diet with NaF (CTR +), casein control diet without NaF (CTRL -) and sorghum diets BRS 305 flour (STT), BRS Piabiru quinoa flour (QTT) and mixed flour sorghum/quinoa (SQTT), ingested with NaF.

[a] Martino, Peluzio, Costa, Rodriguez, and Gontijo (2013). The averages followed by the same letter in the same column do not differ by 5% probability, by the Duncan test; ± standard deviation; ALT = alanine aminotransferase; AST = aspartate aminotransferase.

4. Conclusions

The blend of quinoa and sorghum flours is an alternative for the preparation of mixed flour with good protein quality, high antioxidant capacity, better palatability, and more affordable price. In addition, it provided an increase in exogenous antioxidants, demonstrated by the increase of the total antioxidant capacity, maintaining the endogenous antioxidant enzymes and the biochemical variables of renal and hepatic function. Thus, the mixed sorghum/quinoa flour presents a promising effect on the protein quality and increased antioxidant capacity of foods, demonstrating a protective effect in the body.

CRediT authorship contribution statement

Oscar David Medina Martinez: Formal analysis, Investigation, Writing - original draft, Visualization. **Renata Celi Lopes Toledo:** Formal analysis, Investigation, Methodology. **Valéria Aparecida Vieira Queiroz:** Methodology, Writing - review & editing. **Mônica Ribeiro Pirozi:** Methodology, Writing - review & editing, Supervision. **Hércia Stampini Duarte Martino:** Conceptualization, Methodology, Writing - review & editing, Supervision. **Frederico Augusto Ribeiro de Barros:** Conceptualization, Methodology, Validation, Resources, Writing - review & editing, Supervision, Project administration, Funding acquisition.

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References

Aebi, H. (1984). Catalase in vitro. *Methods in Enzymology, 105*, 121–126. https://doi.org/10.1016/S0076-6879(84)05016-3.

Al-Mamary, M., Al-Habori, M., Al-Aghbari, A., & Al-Obeidi, A. (2001). In vivo effects of dietary sorghum tannins on rabbit digestive enzymes and mineral absorption. *Nutrition Research, 21*(10), 1393–1401. https://doi.org/10.1016/S0271-5317(01)00334-7.

Alves, L. F., Rocha, M. S., & Gomes, C. C. F. (2008). Avaliação da qualidade protéica da quinua real (Chenopodium quinoa Willd) através de métodos biológicos. *E-Scientia, 1*(1), 1–22.

Arbex, P. M., Moreira, M. E. de C., Toledo, R. C. L., Cardoso, L. de M., Pinheiro-Sant'ana, H. M., Benjamin, L. dos A., & Martino, H. S. D. (2018). Extruded sorghum flour (Sorghum bicolor L.) modulate adiposity and inflammation in high fat diet-induced obese rats. *Journal of Functional Foods, 42*, 346–355. https://doi.org/10.1016/j.jff.2018.01.010 May 2017.

Awika, J. M., & Rooney, L. W. (2004). Sorghum phytochemicals and their potential impact on human health. *Science, 65*, 1199–1221. https://doi.org/10.1016/j.phytochem.2004.04.001.

Awika, J. M., Rooney, L. W., Wu, X., Prior, R. L., & Cisneros-Zevallos, L. (2003). Screening methods to measure antioxidant activity of sorghum (sorghum bicolor) and sorghum products. *Journal of Agricultural and Food Chemistry, 51*(23), 6657–6662. https://doi.org/10.1021/jf034790i.

Badi, S., Pedersen, B., Monowar, L., & Eggum, B. O. (1990). The nutritive value of new and traditional sorghum and millet foods from Sudan. *Plant Foods for Human Nutrition, 40*(1), 5–19. https://doi.org/10.1007/BF02193775.

Bender, A., & Doell, B. (1957). Note on the determination of net protein utilization by carcass analysis. *British Journal of Nutrition, 11*(2), 138–139.

Borges, J. T., Bonomo, R. C., Paula, C. D., Oliveira, L. C., & Cesário, M. C. (2010). Physicochemical and nutritional characteristics and uses of quinoa (*Chenopodium quinoa Willd.*). *Temas Agrarios, 15*(1), 9–23.

Boyle, C. N., Lorenzen, S. M., Compton, D., & Watts, A. G. (2012). Dehydration-anorexia derives from a reduction in meal size, but not meal number. *Physiology & Behavior, 105*(2), 305–314. https://doi.org/10.1016/j.physbeh.2011.08.005.

Bradford, M. M. (1976). A rapid and sensitive method for the quantitation of microgram quantities of protein utilizing the principle of protein-dye binding. *Analytical Biochemistry, 72*(1–2), 248–254. https://doi.org/10.1016/0003-2697(76)90527-3.

Brady, K., Ho, C. T., Rosen, R. T., Sang, S., & Karwe, M. V. (2007). Effects of processing on the nutraceutical profile of quinoa. *Food Chemistry, 100*(3), 1209–1216. https://doi.org/10.1016/j.foodchem.2005.12.001.

Buege, J. A., & Aust, S. D. (1978). Microsomal lipid peroxidation. *Methods in Enzymology, 52*(C), 302–310. https://doi.org/10.1016/S0076-6879(78)52032-6.

Burns, R. E. (1971). Method for estimation of tannin in grain sorghum. *Agronomy Journal, 63*, 511–512. https://doi.org/10.2134/agronj1971.00021962006300030050x.

Cardoso, L. de M., Montini, T. A., Pinheiro, S. S., Pinheiro-Sant'Ana, H. M., Martino, H. S. D., & Moreira, A. V. B. (2014). Effects of processing with dry heat and wet heat on the antioxidant profile of sorghum. *Food Chemistry, 152*, 210–217. https://doi.org/10.1016/j.foodchem.2013.11.106.

Dunn, K. L., Yang, L., Girard, A., Bean, S., & Awika, J. M. (2015). Interaction of sorghum tannins with wheat proteins and effect on in vitro starch and protein digestibility in a baked product matrix. *Journal of Agricultural and Food Chemistry, 63*(4), 1234–1241. https://doi.org/10.1021/jf504112z.

Farrar, J. L., Hartle, D. K., Hargrove, J. L., & Greenspan, P. (2007). A novel nutraceutical property of select sorghum (sorghum bicolor) brans: Inhibition of protein glycation. *Phytotherapy Research, 22*, 1052–1056. https://doi.org/10.1002/ptr.

Filho, A. M. M., Pirozi, M. R., Borges, J. T. D. S., Pinheiro Sant'Ana, H. M., Chaves, J. B. P., & Coimbra, J. S. D. R. (2017). Quinoa: Nutritional, functional, and antinutritional aspects. *Critical Reviews in Food Science and Nutrition*. https://doi.org/10.1080/10408398.2014.1001811.

Freitas, K. C., Motta, M. E. F. a, Amâncio, O. M. S., Neto, U. F., & Morais, M. B. (2004). The effect of soy polysaccharide fiber on fecal weight and moisture in growing rats. *Jornal de Pediatria, 80*(3), 183–188.

Friedman, M., & Gumbmann, M. R. (1986). Nutritional improvement of soy flour through inactivation of trypsin inhibitors by sodium sulfite. *Journal of Food Science, 51*(5), 1239–1241. https://doi.org/10.1111/j.1365-2621.1986.tb13094.x.

García-Luna, C., Amaya, M. I., Alvarez-Salas, E., & Gortari, P. de (2010). Prepro-orexin and feeding-related peptide receptor expression in dehydration-induced anorexia. *Regulatory Peptides, 159*(1–3), 54–60. https://doi.org/10.1016/j.regpep.2009.09.011.

Iglesia, R. de la, Milagro, F. I., Campión, J., Boqué, N., & Martínez, J. A. (2010). Healthy properties of proanthocyanidins. *BioFactors, 36*, 159–168.

Khan, I., Yousif, A. M., Johnson, S. K., & Gamlath, S. (2015). Acute effect of sorghum flour-containing pasta on plasma total polyphenols, antioxidant capacity and oxidative stress markers in healthy subjects: A randomised controlled trial. *Clinical Nutrition, 34*(3), 415–421. https://doi.org/10.1016/j.clnu.2014.08.005.

Lopes, R. de C. S. O., de Lima, S. L. S., da Silva, B. P., Toledo, R. C. L., Moreira, M. E. de C., Anunciação, P. C., ... Martino, H. S. D. (2018). Evaluation of the health benefits of consumption of extruded tannin sorghum with unfermented probiotic milk in individuals with chronic kidney disease. *Food Research International, 107*(March), 629–638. https://doi.org/10.1016/j.foodres.2018.03.004.

Makkar, H. P. (2003). Effects and fate of tannins in ruminant animals, adaptation to tannins, and strategies to overcome detrimental effects of feeding tannin-rich feeds. *Small Ruminant Research, 49*(3), 241–256. https://doi.org/10.1016/S0921-4488(03)00142-1.

Marklund, S. (1985). Pyrogallol autoxidation. In G. RA (Ed.). *Handbook of methods for oxygen radical research* (pp. 243–247). CRC Press.

Martino, H. S. D., Peluzio, M. do C. G., Costa, N. M. B., Rodriguez, F. C., & Gontijo, L. N. (2013). *Nutrição experimental (Editora UF)*.

Mendes, F. Q., Oliveira, M. G. A., Costa, N. M. B., Pires, C. V., & Hoffmam, Z. B. (2009). Protein quality of foods, including different soybean varieties. *Alimentos e Nutrição Araraquara, 20*(1), 77–86 ISSN 0103-4235.

Moraes, É. A., Natal, D. I. G., Queiroz, V. A. V., Schaffert, R. E., Cecon, P. R., de Paula, S. O., ... Martino, H. S. D. (2012). Sorghum genotype may reduce low-grade inflammatory response and oxidative stress and maintains jejunum morphology of rats fed a hyperlipidic diet. *Food Research International, 49*(1), 553–559. https://doi.org/10.1016/j.foodres.2012.07.029.

Moraes, É. A., Queiroz, V. A. V., Shaffert, R. E., Costa, N. M. B., Nelson, J. D., & Ribeiro, S. M. R. (2012). *In vivo* protein quality of new sorghum genotypes for human consumption. *Food Chemistry, 134*(3), 1549–1555. https://doi.org/10.1016/j.foodchem.2012.03.079.

Nabavi, S. M., Nabavi, S. F., Eslami, S., & Moghaddam, A. H. (2012). In vivo protective effects of quercetin against sodium fluoride-induced oxidative stress in the hepatic tissue. *Food Chemistry, 132*(2), 931–935. https://doi.org/10.1016/j.foodchem.2011.11.070.

Nabavi, S. F., Nabavi, S. M., Habtemariam, S., Moghaddam, A. H., Antoni Sureda, M. J., & Latifi, A. M. (2013). Hepatoprotective effect of gallic acid isolated from *Peltiphyllum peltatum* against sodium fluoride-induced oxidative stress. *Industrial Crops and Products, 44*, 50–55. https://doi.org/10.1016/j.indcrop.2012.10.024.

Neilson, M., Giddins, R., & Richards, G. N. (1986). Effect of tannins on the palatability of mangrove leaves to the tropical sesarminid crab *Neosarmatium smithi*. *Marine Ecology Progress Series, 34*, 185–186. https://doi.org/10.3354/meps034185.

Pasko, P., Zagrodzki, P., Bartoń, H., Chłopicka, J., & Gorinstein, S. (2010). Effect of quinoa seeds (Chenopodium quinoa) in diet on some biochemical parameters and essential elements in blood of high fructose-fed rats. *Plant Foods for Human Nutrition, 65*(4), 333–338. https://doi.org/10.1007/s11130-010-0197-x.

Price, M. L., Scoyoc, S. Van, & Butler, L. G. (1978). A critical evaluation of the vanillin reaction as an assay for tannin in sorghum grain. *Journal of Agricultural and Food Chemistry, 25*, 1214–1218. https://doi.org/10.1021/jf60219a031.

Ramaiah, S. K. (2007). A toxicologist guide to the diagnostic interpretation of hepatic biochemical parameters. *Food and Chemical Toxicology*. https://doi.org/10.1016/j.fct.2007.06.007.

Ranhotra, G. S., Gelroth, J. A., Glaser, B. K., Lorenz, K. J., Johnson, D. L., Myojin, C., ... Goshev, I. (1993). Composition and protein nutritional quality of quinoa. *Food Chemistry, 70*(3), 303–305. https://doi.org/10.1016/j.foodchem.2015.02.111.

Reeves, P. G., Nielsen, F. H., Fahey, G. C., & Fahey, G. C., Jr. (1993). AIN-93 purified diets for laboratory rodents: Final report of the American Institute of Nutrition ad hoc writing committee on the reformulation of the AIN-76A rodent diet. *Journal of Nutrition, 11*, 1939–1951. https://doi.org/10.1017/CBO9781107415324.004.

Repo-Carrasco, R., Espinoza, C., & Jacobsen, S.-E. (2003). Nutritional value and use of the andean crops quinoa (Chenopodium quinoa) and kañiwa (Chenopodium pallidicaule).pdf. *Food Reviews International, 19*(1–2), 179–189. https://doi.org/10.1081/FRI-120018884.

Rojas, W., Alandia, G., Irigoyen, J., & Blajos, J. (2011). *Quinoa: Millennial cultivation to contribute to the world food security. Oficina Regional para America Latina y el Caribe, Vol. 37*FAOhttps://doi.org/10.1016/j.jaridenv.2009.03.010.

Roncal-Jimenez, C., Lanaspa, M. A., Jensen, T., Sanchez-Lozada, L. G., & Johnson, R. J. (2015). Mechanisms by which dehydration may lead to chronic kidney disease. *Annals of Nutrition and Metabolism, 66*(suppl 3), 10–13. https://doi.org/10.1159/000381239.

Stefoska-Needham, A., Beck, E. J., Johnson, S. K., & Tapsell, L. C. (2015). Sorghum : An underutilized cereal whole grain with the potential to assist in the prevention of chronic disease. *Food Reviews International, 31*(8755–9129), 401–437. https://doi.org/10.1080/87559129.2015.1022832.

Teixeira, N. D. C., Queiroz, V. A. V., Rocha, M. C., Amorim, A. C. P., Soares, T. O., Monteiro, M. A. M., ... Junqueira, R. G. (2016). Resistant starch content among several sorghum (Sorghum bicolor) genotypes and the effect of heat treatment on resistant starch retention in two genotypes. *Food Chemistry, 197*, 291–296. https://doi.org/10.1016/j.foodchem.2015.10.099.

Valcárcel-Yamani, B., & Lannes, S. C. da S. (2012). Applications of quinoa (Chenopodium quinoa willd .) and amaranth (Amaranthus spp .) and their influence in the nutritional value of cereal based foods. *Food and Public Health, 2*(6), 265–275. https://doi.org/10.5923/j.fph.20120206.12.

Watanabe, K., Kawanishi-Asaoka, M., Myojin, C., Awata, S., Ofusa, K., Kodama, K., ... Kawanishi-Asaoka, M. (2014). Amino acid composition, oxidative stability, and consumer acceptance of cookies made with quinoa flour. *Food Science and Technology Research, 20*(3), 687–691. https://doi.org/10.3136/fstr.20.687.