

Research Article

Bioactive Properties of Sorghum-based Beverages from Whole or Refined Grains

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2023, volume 3, issue 3
doi:10.21926/rpn.2303013**Received:** March 08, 2023
Accepted: August 16, 2023
Published: August 21, 2023

Abstract

Sorghum-based beverages were developed and the effects of decortication on nutritional composition and biofunctional properties after a simulated gastrointestinal digestion were analyzed. For that, white sorghum flours from whole (WS) and decorticated (DS) grains were obtained and used to prepare sorghum beverages. They were analyzed regarding chemical composition, minerals, and phenolic acid contents. Moreover, the bioaccessibility of phenolics, proteins, and the potential bioactivity (ABTS⁺ scavenging, ACE-I inhibition and DPP-IV inhibition) after a simulated gastrointestinal digestion were determined. The grain decortication increased the content of carbohydrates and Fe in the flour, but decreased all the other compounds analyzed. Gallic and ferulic acids were the most abundant free and bound phenolics, respectively. WS-beverage exhibited higher gallic acid bioaccessibility, while DS presented higher p-coumaric acid bioaccessibility, with no differences for ferulic acid. DS-beverage showed higher peptide bioaccessibility than WS, probably because of the higher degree of hydrolysis of proteins from refined flour. Regarding bioactive properties, WS-beverage presented 20% more ABTS scavenging than DS, possibly related to the higher phenolic content. On the other hand, DS-beverage presented higher ACE-I and DPP-IV inhibition than WS (40 and 100% more, respectively) because of the generation of bioactive



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peptides. Whole or refined sorghum could be used to produce beverages analogs to milk, with health potential benefits.

Keywords

Sorghum; phenolic compounds; bioactive peptides; bioaccessibility; plant-based beverages

1. Introduction

In recent years, consumers have tended towards a plant-based diet due to various reasons, such as aversion to the consumption of animal products, desire for a healthy lifestyle, and environmental awareness. Therefore, the emphasis of new research in the development of food products is to meet current consumer demands by creating new nutritious and healthy food and beverage alternatives. In this sense, the need arises to develop animal-based food substitutes using vegetable ingredients, providing vegans, vegetarians and flexitarians with a greater range of products [1]. Plant-based milk substitutes are water extracts of legumes, oil seeds, cereals or pseudocereals that resemble cow's milk in appearance. The most widely consumed and the oldest plant milk substitute is soy milk [2], while almond, rice, coconut and oat milk have increased their presence in the market and are utilized as plain, flavored and / or sweetened [3]. However, Bernardo et al. [4] reported that sorghum beverages prepared with powdered extruded sorghum presented good sensory acceptance, and suggested that sorghum beverage could be a potential health food.

Sorghum (*Sorghum bicolor* (L.) Moench) is one of the most cultivated cereals in Africa, Asia and Latin America [5]. Sorghum grains are mainly used for animal feed. However, there is a growing interest in using sorghum to produce biofuels and develop healthy and functional foods, mainly due to the presence of beneficial compounds in sorghum [6-8].

Sorghum grain composition is similar to maize, regarding carbohydrates, proteins, lipids, dietary fiber and ash contents [9]. Additionally, whole grain is rich in vitamins, minerals and phenolic compounds, with phenolic content in sorghum being one of the highest among cereals [10]. Phenolics have free radical scavenging activity, anti-diabetic, anti-inflammatory, anti-viral, immune-modulatory, and anti-cancer capacities [11]. However, whole grains decrease the organoleptic acceptance of foods and beverages [12]. In this sense, decortication of cereals is an effective alternative to improve the flavor, despite reducing phenolic content and other phytochemicals. It is an important primary process in cereals, which effectively separates the bran to obtain flours with better appearance, texture and cooking qualities, palatability and digestibility [13]. Nevertheless, there are no studies about incorporating whole or decorticated sorghum flours in milk analogs.

This study aimed to prepare sorghum-based beverages using whole and decorticated sorghum extracts, to compare the chemical composition and to evaluate the bioactivity after a simulated gastrointestinal digestion.

2. Materials and Methods

2.1 Raw Material

White sorghum cultivar (341 × 120) free of condensed tannins was provided by Nuseed S.A. (Santa Fe, Argentina). The material was carefully cleaned using a Labofix Brabender (Duisburg, Germany) to remove foreign particles and dust. According to previous reports, an aliquot of the grains was decorticated [14]. Briefly, sorghum grains were adjusted to 13.2% of moisture content, and debranned in an abrasive mill for 7.5 min (Gallicet, Entre Rios, Argentina). Whole (WS) and decorticated sorghum (DS) grains were ground in a cyclonic mill (UDY Corporation, USA), obtaining flours with particle sizes smaller than 500 µm. Figure 1 shows the grains before and after decortication and the corresponding flours.

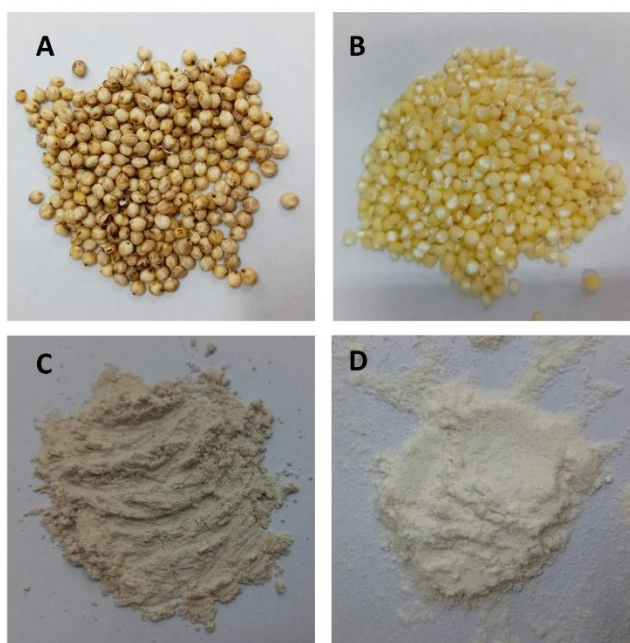


Figure 1 A. Whole sorghum grains, B. Decorticated sorghum grains, C. Whole sorghum flour, D. Decorticated sorghum flour.

2.2 Preparation of Extracts of Sorghum Flours

In order to obtain the water extracts used to formulate sorghum milk-like products, WS or DS flour dispersions were prepared, heated for 10 min at 85°C, cooled at 75°C and added with α -amylase enzyme following manufacturer instructions. After 40 min reaction, the enzyme was inactivated for 10 min at 90°C. Samples were stored at -20°C until analysis.

2.3 Chemical Composition

Protein, ether extract, and ash contents were determined according to the AOAC methods [15]. Total dietary fiber was assessed by Megazyme® commercial kit. The content of carbohydrates was determined by difference. The results were expressed as g/100 g dry base.

Mineral content was determined after dry ashing. Fe, Zn, Ca, Cu and Mg were measured by Flame Atomic Absorption Spectroscopy and Na and K by flame photometry using an Atomic Absorption spectrophotometer Analyst 300 (Perkin Elmer). P content was performed according to AOAC [15].

2.4 Phenolic Acid Analysis

Free and bound phenolic acids from flours and their water extracts were determined by RP-HPLC according to Garzón et al. [8], using a Shimadzu Series LC-20AT pump, with Shimadzu SPD-M20A diode array detector. Compounds were separated on a 250 mm × 4.6 mm, 5 µm particle size, Gemini 110A C-18 Phenomenex column. Data were processed using Shimadzu LC solution software. The sum of free and bound phenolics calculated the total content of phenolic compounds.

2.5 Bioaccessibility of Bioactive Compounds and Potential Bioactive Properties

The *in vitro* method of the simulated gastrointestinal digestion described by Van de Velde et al. [16] was used for estimating the bioaccessibility of the phenolic acids, peptides, and potential bioactive properties from WS and DS extracts. Both samples were prepared at 10 g solids/100 g dispersion. The intestinal phase of the digestive process was performed using dialysis bags. After digestion, the bag contents (dialysates of the *in vitro* intestinal phase) were transferred to flasks, weighted and frozen at -20°C until analysis.

Phenolic acids were determined in dialysate samples according to the 2.4 section. Intestinal bioaccessibility of phenolic compounds was calculated as the dialyzable fraction of phenolic compounds about the total phenolic compound content.

Intestinal bioaccessibility of proteins was calculated as the dialyzable fraction of proteins about the total protein content of the analyzed sample. The protein content in dialysates was determined according to Lowry et al. [17] using bovine serum albumin (A7906, Sigma-Aldrich) as a standard.

The free amino groups were determined according to Nielsen et al. [18]. The total protein degree of hydrolysis (DH) was calculated considering the sum of free amino groups in dialysates and digested samples about the total hydrolyzable peptide bounds in the protein (7.9 mEq/g protein).

Bioaccessibility of bioactive properties was determined measuring dialysates' antioxidant, antihypertensive and antidiabetogenic activities.

2.6 Bioactive Properties

To estimate the antioxidant activity (AOA) of dialysates, the inhibition of 2,2'-azinobis (3-ethylbenzothiazoline-6-sulfonic acid) (ABTS^{•+}) was performed according to Cian et al. [19]. The absorbance reading was taken at 734 nm after 6 min initial mixing using a spectrophotometric plate reader (Asys UVM340). Results were expressed as a scavenging percentage.

Angiotensin-converting enzyme-I (ACE-I) inhibition activity was determined on dialysates according to Hayakari et al. [20], and used to estimate the antihypertensive activity (AHA). Results were expressed as ACE-I inhibition (%).

Antidiabetogenic activity (ADA) of dialysates was evaluated through the inhibition of dipeptidyl peptidase IV (DPP-IV) according to Wang et al. [21]. Results were expressed as inhibition percentages of DPP-IV (%).

2.7 Statistical Analysis

Each experiment was performed at least in triplicate. All results were expressed as mean \pm SD (standard deviation). Statgraphics Centurion XV 15.2.06 software was used to analyze the data by analysis of variance (one-way ANOVA) and Duncan's multiple range tests to determine differences between samples, and paired sample t-test to determine differences between WS and DS samples ($p < 0.05$).

3. Results

3.1 Chemical Composition

The chemical composition of sorghum extracts is shown in Table 1. It was observed that decorticated process increased carbohydrate and Fe content by 16 and 44%, respectively, but decreased all the other evaluated compounds.

Table 1 Chemical composition of sorghum extracts made with whole (WS) or decorticated sorghum flour (DS).

(g/100 g d.b.)	WS	DS
Protein	10.7 \pm 0.2 ^b	8.1 \pm 0.4 ^a
Total dietary fiber	8.38 \pm 0.1 ^b	3.46 \pm 0.1 ^a
Ether extract	3.8 \pm 0.1 ^b	0.6 \pm 0.0 ^a
Carbohydrates	75.7 \pm 0.2 ^a	87.6 \pm 0.5 ^b
Ash	1.5 \pm 0.2 ^b	0.2 \pm 0.0 ^a
Minerals (mg/kg d.b.)		
Fe	33.6 \pm 0.8 ^a	48.6 \pm 0.9 ^b
Zn	10.9 \pm 0.9 ^b	7.7 \pm 0.2 ^a
Ca	83.0 \pm 6.1 ^b	66.4 \pm 5.0 ^a
Na	83.6 \pm 3.2 ^b	43.8 \pm 3.7 ^a
K	4199.7 \pm 342.2 ^b	923.2 \pm 64.5 ^a
Cu	6.3 \pm 0.6 ^b	4.9 \pm 0.4 ^a
Mg	1899.2 \pm 39.7 ^b	540.0 \pm 21.4 ^a
P	2845.5 \pm 9.5 ^b	760.7 \pm 47.4 ^a

d.b.: dry basis. Values with different superscript letter in a row are significantly different ($p < 0.05$).

3.2 Phenolic Acid Profile

The profile of phenolic acids in sorghum flours and extracts is shown in Table 2. Gallic acid was the most abundant free phenolic acid in all the samples, while ferulic acid was the highest-bound phenolic. In addition, 4-hydroxybenzoic acid, caffeic acid and p-coumaric acid were found both, in

free and bound form, and their content depended on decortication. In this regard, decorticated sorghum presented lower phenolic acid content than the whole sorghum.

Table 2 Phenolic acid profile of sorghum flours and extracts made with whole (WS) or decorticated sorghum flours (DS).

(µg/g d.b.)	Sorghum Flours		Sorghum extracts		
	WS	DS	WS	DS	
Gallic acid	F	272.5 ± 10.3 ^a	279.4 ± 8.4 ^a	1391.3 ± 46.0 ^c	770.0 ± 66.3 ^b
	B	ND	ND	ND	ND
4-Hydroxybenzoic acid	F	9.9 ± 0.6 ^a	ND	14.2 ± 1.0 ^b	ND
	B	1.4 ± 0.0 ^a	0.1 ± 0.0 ^a	8.6 ± 1.0 ^b	0.4 ± 0.0 ^a
Caffeic acid	F	13.7 ± 0.8 ^b	7.4 ± 0.9 ^a	19.6 ± 1.4 ^c	11.9 ± 1.1 ^b
	B	1.1 ± 0.1 ^a	ND	6.6 ± 0.0 ^b	1.2 ± 0.1 ^a
p-coumaric acid	F	2.6 ± 0.2 ^a	ND	3.7 ± 0.3 ^b	ND
	B	3.4 ± 0.2 ^b	0.3 ± 0.0 ^a	20.2 ± 1.9 ^c	1.4 ± 0.0 ^{ab}
Ferulic acid	F	30.6 ± 3.5 ^b	2.1 ± 0.3 ^a	43.8 ± 4.7 ^c	ND
	B	49.0 ± 1.9 ^b	16.4 ± 3.2 ^a	295.1 ± 31.7 ^d	81.3 ± 0.3 ^c
Total Free phenolics		329.3	288.9	1472.6	781.9
Total Bound phenolics		54.6	16.8	330.5	84.3
Total phenolics (F + B)		383.9	305.7	1803.1	866.2

F, free phenolics; B: bound phenolics; ND, non-detected. d.b.: dry basis. Values with different superscript letters in a row are significantly different ($p < 0.05$).

Additionally, it was observed that the content of each phenolic acid in sorghum extracts was higher than the content in raw flours. The increase ranged from 3-4 and 5-6 times for the total free phenolics and bound compounds, respectively.

3.3 Phenolic Acid Bioaccessibility

Figure 2 shows phenolic acid bioaccessibility (%). It was observed that 4-Hydroxybenzoic acid and caffeic acid were not detected in dialyzates, and the bioaccessibility of the other phenolics depended on the compound evaluated and on the decortication. Beverages made with WS exhibited higher gallic acid bioaccessibility, while DS-based exhibited higher p-coumaric acid bioaccessibility. Moreover, ferulic acid did not present differences between samples.

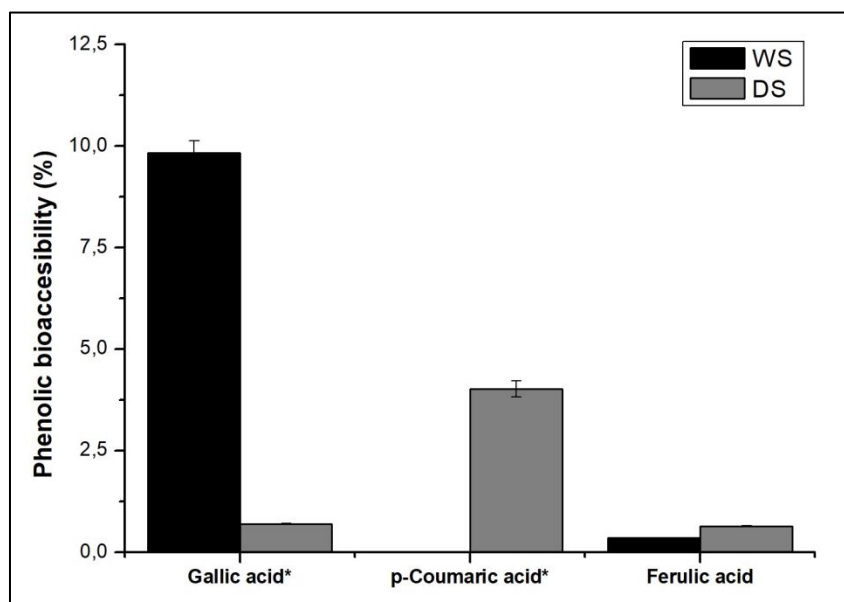


Figure 2 Phenolic acid bioaccessibility (%). * Significant difference ($p < 0.05$) between samples. WS: whole sorghum beverage; DS: decorticated sorghum beverage.

3.4 Protein Bioaccessibility and Degree of Hydrolysis

After digestion, the total protein DH values of WS and DS samples were 43.0 ± 1.2 and 83.4 ± 0.5 %, respectively (p -value < 0.05). The bioaccessibility values for proteins were 9.5 ± 0.3 and 13.4 ± 0.2 %, for WS and DS, respectively (p value < 0.05) indicating a 40% increase when sorghum beverage was produced with refined sorghum flour. Thus, DS proteins can be more easily hydrolyzed by gastrointestinal enzymes, which would favor the generation of peptides and their bioaccessibility.

3.5 Bioactive Properties

The bioactive properties of dialysates after a simulated gastrointestinal digestion process are shown in Figure 3. Regarding antioxidant potential, WS-beverage presented 20% more ABTS scavenging than DS, probably related to the higher phenolic content. However, DS-beverage presented higher ACE-I and DPP-IV inhibition than WS (40 and 100% more, respectively), probably related to DS-extract's higher content of bioactive peptides.

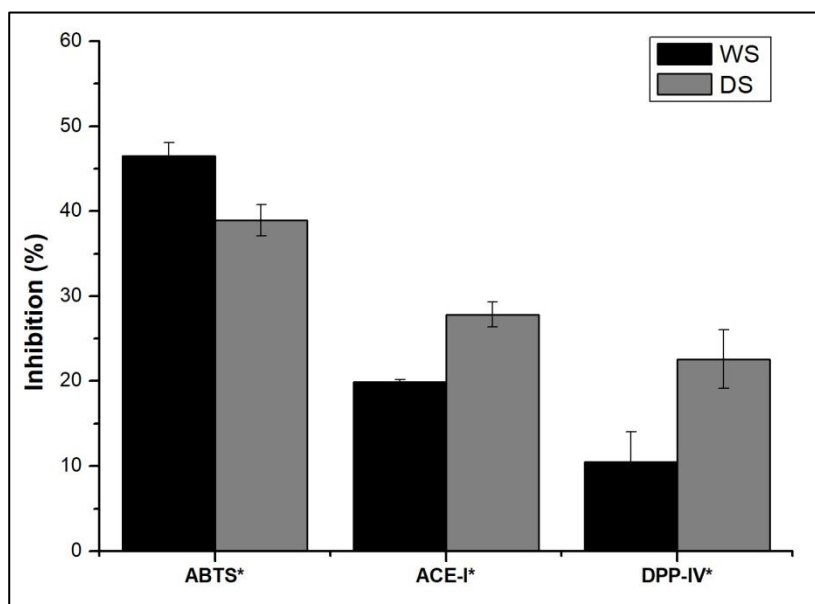


Figure 3 Bioactive potential after a simulated gastrointestinal digestion. *Significant difference ($p < 0.05$) between samples. WS: whole sorghum-based beverage; DS: decorticated sorghum-based beverage; ABTS: scavenging of ABTS⁺ radical; ACE-I: Inhibition of angiotensin converting enzyme I; DPP-IV: Inhibition of dipeptidyl peptidase IV.

4. Discussion

4.1 Chemical Composition

Bran and germ are removed by sorghum decortication [14]. Taking into account that the contribution of lipids, ashes or fiber from the pericarp and the germ of the sorghum grains are around 82, 69, and 71%, respectively [22], and that the losses by decortication obtained in this work were 84, 86 and 59%, for lipids, ashes and fiber, respectively, it is possible to estimate that 90-100% of the germ and pericarp were lost by the pearling process.

The results obtained about the Fe increase and protein decrease in refined flours are similar to those obtained by Sruthi et al. [23], who analyzed loss of nutrients after the decortication of sorghum at different moisture and milling time. They found that the Fe was the only mineral that increased in decorticated sorghum compared with whole sorghum when 12% moisture grains and 15 min of milling were used. Also, a decrease in protein content at all milling conditions was observed. They concluded that proteins are present in higher concentrations in the peripheral layers of the grain than in the endosperm.

Additionally, the composition in the dry base obtained for WS extract is similar to that obtained for sorghum flours [10, 24]. In this regard, the enzymatic process of obtaining sorghum extracts did not generate wet residue. Thus, the enzymatic process can improve the industry's yields and reduce the content of by-products causing environmental problems.

Moreover, preparing the milk analog at 10 g solids/100 mL, the protein content of the beverage is 1 g/100 mL, similar to that obtained in commercial rice milk, which has protein contents ranging from 0.28-1.26 g/100 g [3]. On the other hand, vitamin and mineral supplementation could be used to improve nutritional properties of milk analogs[2].

4.2 Phenolic Acids

The content of phenolic acids was similar to that reported for sorghum grains by other authors [6-11]. Most indicated that ferulic acid had the highest content among the bound phenols, similar to our results. Moreover, since phenolic compounds are in greater quantity located in the pericarp of the sorghum grains [25], the refining process decreases the phenolic content.

On the other hand, the increase of phenolic compounds in sorghum extracts about flours could be due to the process used to obtain the extracts. The increase in extraction of phenolic compounds after heat treatments was also informed by other authors. Depending on the cooking process, the content of free and bound phenolic acids increased, due to the breaking of the cell walls by heat treatment, which favored the extraction [26]. Moreover, it was reported that the baking temperature and short time (150°C, 5 min) used to make sorghum cookies improved the extraction of phenolic acids. Also, boiling or heating at higher water content of different foods increased total phenolic compound extraction, such as banana pulp boiled for 30 min [27] and garlic boiled for 15-60 min [28].

Moreover, polysaccharides could interact via non-covalent bonds with phenolic compounds. The hydroxyl groups of phenolics can form strong hydrogen bonds with starch molecules [29]. In this sense, hydrolysis with α -amylase used to produce sorghum-extracts could reduce the matrix interactions and favor the extraction of phenolic compounds.

Cereal-base milk could have some favorable effects on human health due to its phenolic compounds [1]. Considering that sorghum contains the highest content of phenolics among cereals [6], it is a really interesting raw material to produce functional plant-based beverages.

4.3 Bioactive Compounds Bioaccessibility

The biological properties of bioactive compounds depend not only on the quantity, but also on the fraction of them which is released from the food matrix in the gastrointestinal tract and becomes available for absorption [30] or for exerting a local activity.

Despite that in vivo digestion phenolic acids are metabolized by xenobiotic enzymes in the gastrointestinal tract [31], the in vitro digestion process is suited for the analysis of phytochemical bioaccessibility.

Intestinal polyphenol bioaccessibility is reported to be low and dependent on the type of phenolic or cereal matrix involved [31], which agrees with our results.

Garzón et al. [8] evaluated the intestinal bioaccessibility of phenolic acids from whole sorghum fermented products and also found that gallic acid was the phenolic acid with higher bioaccessibility (40%). It is important to note that gallic acid was found in free form, which makes dialysis easier during simulated digestion. The higher bioaccessibility of this free phenolic acid from WS could be related to its higher content than in DS-beverage.

Regarding p-coumaric and ferulic acids, they exhibited a low percentage of bioaccessibility (1.4 and 0.9%, respectively). Also, Anson et al. [32] reported low bioaccessibility for ferulic acid from cereal matrix. This could be related to the ferulic acid condensation that generates high molecular weight compounds that could not pass through the dialysis bag. The low absorption of phenolics observed at the intestinal level is mainly due to a combination of several factors including the degradation through gastrointestinal digestion of some of them [33], the isomerization (cis-trans) before they can cross the intestinal mucosa barrier [34], and also their interaction with other

compounds in the cereal matrix, which causes them to accumulate in the colon where they can be metabolized and exert some bioactive functions [31]. Regarding this, dietary fibers have many hydroxyl groups in their structure, which form hydrogen bonds and interact via Van der Waals forces with polyphenols, reducing their bioaccessibility at the gastrointestinal level [29]. In this sense, the greater bioaccessibility found for p-coumaric acid for the DS could be because it contains a lower fiber level than the WS-beverage (Table 1).

On the other hand, food proteins are hydrolyzed by enzymes located throughout the gastrointestinal tract. This protein digestion could generate peptides with beneficial health properties [35]. The bioactive peptides' biological activities depend on their amino acid composition and sequence. Usually, they possess 2-20 amino acid residues and sometimes can exert multiple functions, like antioxidant, anti-inflammatory, and antihypertensive [36]. Moreover, the analysis of the bioaccessibility of the bioactive peptides is important since these compounds must be released from the food matrix to target the systemic site of action.

The lower protein bioaccessibility, related to the WS sample's lower protein DH than the DS sample, might be due to the different food compositions causing different matrix effects. In this sense, several reports found interactions of proteins and peptides with dietary fiber, carbohydrates, and phenolic compounds. For example, phenolic acids could interact with proteins or peptides, and depending on the interaction force between them, it could be negative for producing new bioactive peptides to promote bioaccessibility, or both [35]. Regarding this, Seczyk et al. [30] evaluated the effect of a simulated gastrointestinal digestion on white bean paste added with phenolics, and found that gallic acid decreased the protein digestibility. In this sense, higher gallic acid content found in WS-beverage could negatively influence the generation of peptides.

Additionally, metal-peptide interactions can lower the bioaccessibility of bioactive peptides [37], and dietary fiber can block the access of digestive enzymes to the protein substrate and also decrease the activity of proteolytic enzymes, which translates into decreased protein and peptide digestibility [38]. Thus, the higher amount of minerals and fiber of WS than in DS-beverage could explain the lower protein hydrolysis of the WS matrix.

Furthermore, some authors showed that refined cereals including sorghum increased protein digestibility due to reduced phenolic compounds and phytic acid that could interact with proteins blocking enzymatic hydrolysis [39, 40].

4.4 Bioactive Properties

The main source of antioxidants in cereal grains is phenolic compounds mainly located in the pericarp [41]. In this sense, it is expected that WS-beverage exhibited a higher AOA since that sample contains higher levels of phenolic acids.

Fadly et al. [42] studied the antioxidant potential of soy milk added with sorghum. They found that the higher content of sorghum added to the formulation, the higher the antioxidant potential of the plant-based milk was, probably due to the higher phenolics content. The antioxidant potential of phenolic compounds is related to eliminating free radicals, which prevents chronic diseases. Moreover, phenolic compounds from sorghum hybrids have been reported to possess antioxidant activities by different in vitro methodologies like DPPH, ABTS, FRAP, and ORAC [11, 24]. Furthermore, Garzón et al. [43] found antioxidant peptides encrypted in sorghum proteins. These peptides generated after in vitro hydrolysis had hydrophobic residues on their peptide sequence.

Thus, the antioxidant potential from WS and DS-beverages could be related to both, bioaccessible phenolic compounds and bioactive peptides generated after gastrointestinal digestion.

Regarding AHA and ADA, the ACE-I and DPP-IV inhibitions were higher for DS than WS, which suggests that the greater bioaccessibility of bioactive peptides from DS samples is related to these activities. Regarding this, Cian et al. [44] analyzed the ACE-I and DPP-IV inhibition potential from sorghum peptides. They found potential ACE-I inhibitory peptides having a MW range of 1000 to 3000 Da and P and Y as probably the main amino acids residues. Also, they found potential DPP-IV inhibitory peptides of 800 Da. Furthermore, Garzón et al. [43] reported DPP-IV inhibitory peptides after an *in vitro* hydrolysis of sorghum proteins using MS/MS analysis. They found six peptides that could interact with the DPP-IV enzyme using *in silico* tools, two in a competitive inhibition mode (PPPGSKSYGT and QADPKTFYGLM).

Sorghum beverages formulated with WS or DS could be functional milk analogs with multiple bioactivities. Plant-based milk analogs mainly of soy, rice, almond, and coconut are produced and commercially available [1-3]. However, as far as we know there are no commercial sorghum milk analogs. Sorghum has a great potential, not only because of its agronomic characteristics, but also because of the evidence about the beneficial effects of its phytochemicals on health. In this sense, it is necessary to develop more innovative ways of incorporating sorghum into the diet, to promote the consumption of this cereal and take advantage of all its benefits.

5. Conclusions

Developing new plant-based milk analogs depends on many criteria: a good or familiar taste, nutritional information, health benefits, and the environmental aspects of the food and its production. In this sense, whole or refined sorghum flours could be used to produce beverages analogs to milk, with health potential benefits, and without generating byproducts. Moreover, despite decorticating process reduced significantly the content of phenolic acids and dietary fiber that have been shown in many studies to have beneficial properties for health, the beverage made with decorticated sorghum presented a higher amount of bioactive peptides with antihypertensive and antidiabetogenic activity than that based in whole sorghum.

Further studies are needed to assess the general consumer acceptability of these new products and the inclusion of vitamins and minerals in these beverages to improve nutritional quality.

Author Contributions

Conceptualization, A.G.G. and S.R.D.; methodology, A.G.G., M.A. and S.R.D.; investigation, A.G.G. and M.A.; data curation, A.G.G.; formal analysis A.G.G., M.A. and S.R.D.; resources, S.R.D.; writing-original draft preparation, A.G.G.; writing-review and editing, A.G.G. and S.R.D.; supervision, S.R.D.; project administration, S.R.D.; funding acquisition, S.R.D. All authors have read and agreed to the published version of the manuscript.

Funding

This research was funded by Universidad Nacional del Litoral [Grant CAI+D PI Tipo II 2020 50620190100064LI] and FONCyT [Grant PICT-2020-3116].

Competing Interests

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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