

Antioxidant and anti-inflammatory potential of a food produced from irradiated (UV-A LED) sorghum sprouts subjected to in vitro gastrointestinal simulation

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ABSTRACT

Overweight and obesity are global health problems that have been addressed through various methods, such as diet, exercise, and the consumption of functional foods. Among these foods, the use of sorghum grain has been limited. Therefore, to promote the consumption of this cereal, a cookie formulation with potentially functional ingredients was developed using irradiated sorghum sprouts with UV-A LED light. Then, the phenolic content and antioxidant activity of the cookies were evaluated before and after a gastrointestinal simulation process. The anti-inflammatory potential was also determined by measuring the production of nitric oxide in RAW 264.7 cells. Compared to the cookies prepared with sorghum flour, the cookies prepared with irradiated sorghum sprout flour (ISSF) exhibited higher phenolic content and antioxidant activity. Furthermore, the bioaccessibility of phenolic compound was greater and the antioxidant recovery was higher in the ISSF cookies due to the high content of phenolic acids. Finally, the ISSF cookies exhibited better anti-inflammatory potential by reducing nitric oxide production by RAW 264.7 cells at lower concentrations than those of other cookies. Taken together, these results suggest that ISSF cookies are a promising food that could help address overweight, obesity and other related diseases.

1. Introduction

According to the World Health Organization (WHO, 2022), overweight and obesity are pandemics that affect one billion adults worldwide. This condition is associated with an increased risk of chronic noncommunicable diseases, such as diabetes, some types of cancer, and cardiovascular diseases.

To counteract overweight and obesity and their comorbidities, various approaches have been employed, including personalized diets, increased physical activity, bariatric surgeries, and the use of drugs.

This situation can also be prevented through the consumption of functional foods. According to the Food and Agriculture Organization

(FAO, 2022), a functional food contains significant levels of biologically important compounds beyond basic nutrition. Biologically important compounds include polyphenols, which exhibit immunomodulatory/anti-inflammatory effects (Yahfoufi et al., 2018), anticancer effects (Abotaleb et al., 2020), and antidiabetic effects (Savych et al., 2021), among others. These effects are important, as it is widely accepted that function foods contribute to a healthy, balanced, and appropriate diet and reduce health problems (Baker et al., 2022; Karelakis et al., 2020).

Functional foods can be produced through various methods, including removing harmful components such as allergenic proteins, increasing the concentration of bioactive compounds, adding and substituting components, or increasing the bioavailability of bioactive

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compounds (Shaikh, 2022).

The techniques described above have already been used to develop potentially functional foods based on sorghum grains or their derivatives. Examples of these foods are sorghum cookies, which show higher antioxidant activity than that of wheat cookies (Chiremba et al., 2009). This cereal contains high levels of various phytochemicals, such as phenolics, anthocyanins, phytosterols and policosanols, that provide potential benefits for humans (Xu et al., 2021). Various technologies have been applied to increase the bioactive potential of sorghum grains. One technique is germination, as the biological value of germinated sorghum porridges is greater than that of porridges produced from sorghum grains (Ruiz-Hernández et al., 2021). These effects were also observed in wheat, mung beans, soybeans, and alfalfa (Geng et al., 2022). Additionally, the bioaccessibility of total phenols and ferulic acid is greater in fermented sorghum cookies than whole grain sorghum cookies; in addition, the content of phenols and ferulic acid is higher in doughs fermented by backslipping and spontaneous fermentation (Gutiérrez et al., 2022).

In addition to these technologies, which significantly affect the content and bioaccessibility of bioactive compounds, other emerging technologies, such as ultraviolet light, can increase the content of these compounds; this occurs because the technologies can affect the antioxidant defence systems, thermal dissipation and physical quenching of chlorophylls (Emus-Medina et al., 2023) in diverse plants, such as lettuce (He et al., 2021), barley (Bandurska et al., 2012), broccoli (Formica-Oliveira et al., 2017) and sorghum sprouts (Ruiz Hernández et al., 2022). The increase in polyphenol content due to various technologies leads to beneficial effects, and the products are well-accepted. In the food matrix, polyphenols can be bound to macromolecules, organelles, and membranes; confined to cell walls; and trapped between matrices (Parada & Aguilera, 2007). Particularly, sorghum grain, like all cereals, contains a high quantity of ferulic acid; however, most of this compound is linked to structures (bounded or conjugated) of the cell wall, so it is not naturally accessible (De Oliveira et al., 2015; Chandrakanth et al., 2023). Various technologies can release phenolic compounds and thus become available for potential absorption in the gastrointestinal tract. Therefore, the potential beneficial effect of these compounds depend on their bioaccessibility. Therefore, the objective of this research is to determine the biological effect of sorghum sprout cookies irradiated with UV-A light before and after a bioaccessibility process, as well as their potential anti-inflammatory effect in vitro.

2. Materials and methods

2.1. Acquisition of raw materials and food processing

White sorghum (*Sorghum bicolor* (L.) Moench) was germinated for 28 h at 35 °C, 98 % relative humidity, and a light–dark photoperiod of 8/16 h. At the end of germination *sensu stricto*, the sample was left to sprout for 36 h. Subsequently, irradiation was applied to the growing shoots for 11.9 h at 5.1 $\mu\text{W}/\text{cm}^2$ (Ruiz-Hernández et al., 2021; Ruiz-Hernández et al., 2022). The obtained sprouts were frozen at –20 °C, dried at 45 °C and milled until an irradiated sorghum sprout flour (ISSF) was obtained. The formulation of Chiremba et al. (2009) with modifications was used to prepare a food product (cookies). Sodium bicarbonate (1.6 g), margarine (180 g), honey (150 g) and 450 g of flour were used for each type of cookie. The following flours were evaluated in this study: 1) wheat flour (WF), 2) sorghum flour (SF), 3) sorghum sprout flour (SSF) or 4) ISSF. To produce the flours, the growth portions were not removed. Phenol content, antioxidant activity, phenolic profile, bioaccessibility and anti-inflammatory potential were quantified in the cookies obtained.

2.2. Obtaining methanolic and alkaline extracts

To solubilize the free phenolic compounds in the samples,

methanolic extracts were obtained. In conical tubes (15 mL), 1 g of sample was weighed, and 15 mL of 80 % methanol was added and sonicated for 1 h. The sample was then centrifuged at 4000 rpm, and the supernatant was recovered. The process was repeated 2 times, and Whatman filter 1 was used. The supernatants were evaporated under vacuum and resuspended in 50 % methanol (Salazar López et al., 2016).

To obtain the bound compounds from the food matrix, the solid residue resulting from the methanolic extraction was dried in an oven at 45 °C. Subsequently, 100 mg of the residue was obtained and mixed with 5 mL of NaOH (2 N degassed). The air was displaced with nitrogen and sonicated for 3 h. After this time, the pH was adjusted to 1.5–2.0 with 6 N HCl. Then, ethyl acetate (7 mL) was added and shaken vigorously for 2 min. For phase separation, the mixture was centrifuged at 4000 rpm for 15 min. This step was repeated 2 times. Ethyl acetate was recovered, which was evaporated under vacuum in a rotary evaporator, and the sample was resuspended in 7 mL of 50 % methanol (Guo & Beta, 2013). To obtain the conjugated phenolic compounds, 1 mL of the free fraction was obtained and sonicated for 1 h. Next, the methodology for the bound fraction was applied.

2.3. Determination of total phenols

Total phenolic compounds were quantified with the Folin–Ciocalteu reagent. Following the methodology proposed by Singleton and Rossi (1965), the assay was conducted with a FluoStar Omega microplate reader (BMG Labtech Inc., Ortenberg, Germany) with modifications. Thirty microlitres of each extract was mixed with 150 μL of Folin–Ciocalteu's reagent diluted 1:9 with deionized water. Then, 120 μL of a sodium carbonate solution (0.075 mg/mL) was added, and the mixture was homogenized and reacted in the dark for 60 min. Changes in absorbance were monitored at 765 nm against a reagent blank. A concentration curve was generated with gallic acid as a standard to calculate the concentration of total phenols. The results are expressed as mg gallic acid equivalents (mg GAE) per g of sample.

2.4. Antioxidant capacity

2.4.1. Trolox equivalent antioxidant capacity (TEAC)

This assay is based on the ability of antioxidant molecules to scavenge the $\text{ABTS}^{\bullet+}$ cation radical, as a colour changes is produced that can be quantified spectrophotometrically (Blancas-Benitez et al., 2015). Potassium persulfate (17.6 μL of a 37.8 mg/mL solution) was used for activation of the ABTS radical solution (3.86 mg/mL), which was incubated for 16–18 h at room temperature. A working solution was prepared by mixing 1 mL of the ABTS solution with ≈ 88 mL of ethanol and adjusted to an absorbance of 0.7 ± 0.02 . In a microplate well, 280 μL of the working solution was mixed with 20 μL of each of the extracts and incubated at room temperature for 5 min. Changes in absorbance were monitored at 715 nm. The results were expressed as μM Trolox equivalents (TE) per gram of sample, for which a Trolox curve was used.

2.4.2. 1,1-Diphenyl-2-picrylhydrazyl (DPPH)

The DPPH assay was performed according to the technique described by Salazar-López et al. (2017). The assay is based on the scavenging capacity of the DPPH radical, which is manifested by a change in absorbance at 515 nm. Briefly, 280 μL of a methanolic solution of DPPH (0.025 mg/mL) was mixed in microplate wells with 20 μL of each extract. The mixture was incubated for 30 min at room temperature in the dark. Changes in absorbance were monitored at 515 nm in a microplate reader and recorded as DPPH radical scavenging activity. A standard curve was generated, though which the results were expressed as μM TE/g sample.

2.5. Quantification of phenols by ultra high-performance liquid chromatography (UHPLC-DAD)

To evaluate the phenolic profile, the components were separated using an Agilent 1290 Infinity Binary LC system. A Zorbax Eclipse Plus C18 column (2.1 × 50 mm 1.8 µm) at 30 °C was used for this purpose. The mobile phase consisted of a linear gradient of 0.1 % formic acid in water (A) and 0.1 % formic acid in acetonitrile (B) as follows: 97–93 % A, 0–3 min; 93–90 % A, 3–5 min; 90–88 % A, 5–8 min; 88–85 % A, 8–10 min; 85–85 % A, 10–15 min; 85–45 % A, 15–18 min; 45–10 % A, 18–20 min. The flow rate was 0.4 mL/min, and the injection volume was 10 µL. The retention times and spectra of each compound were evaluated for identification (Lee et al., 2012).

2.6. In vitro digestion

To mimic gastrointestinal digestion conditions, the oral, gastric, and intestinal phases were conducted according to a protocol by Salazar-López et al. (2018), through which the bioaccessibility of free phenols, antioxidant recovery and individual phenolic compounds were evaluated for the different samples (cookies). Briefly, for the oral phase, 1 g of sample was chewed by asymptomatic volunteers that had undergone fasting. After this phase, the volunteers expelled the liquid and rinsed their mouth twice with 5 mL of distilled water. The samples used in the oral phase were frozen at −20 °C, and the remaining samples were saved for the following phases. Blanks were prepared following the same protocol but without a sample. Subsequently, in the gastric phase, 0.2 M HCl-KCl buffer was added to the sample submitted to the oral phase at a pH of 1.5. Then, 700 µL of pepsin solution (300 mg/mL) from porcine gastric mucosa (Cat. P7000 powder ≥ 250 units/mg solid, Sigma-Aldrich) was added to reach a final enzyme concentration of 2000 U/mL. Next, the gastric digest was incubated in a water bath at 37 °C and 100 rpm for 1 h. At the end of the incubation time, the samples were frozen, and the remaining samples were saved for the intestinal phase. In this phase, phosphate buffer (0.1 M) at pH 7.5 was first added. Then, 1 mL of pancreatin (17 mg/mL) from porcine pancreas (Cat. P1750 4 × USP specifications, Sigma-Aldrich) was added to reach a final enzyme concentration of 100 U/mL (trypsin activity), and bile salts (80 mg) (Cat. B8756 Sigma-Aldrich) were added. The samples were shaken at 37 °C at 100 rpm for 6 h. After incubation, the oral, gastric, and intestinal samples were frozen at −20 °C, freeze-dried, and resuspended in 50 % methanol for further analysis.

Bioaccessibility was expressed as a percentage of the total free phenol content according to the following equation:

$$B = (\text{Total phenols content of the intestinal fraction} / \text{total phenols (free + bounded + conjugated) non digested samples}) \times 100$$

TEAC values were also analysed by this procedure and expressed as an antioxidant activity recovery (%).

2.7. Anti-inflammatory capacity

2.7.1. Cell culture

American Culture Collection (ATCC) RAW 264.7 mouse macrophages were cultured in Dulbecco's modified DMEM supplemented with 10 % foetal bovine serum, sodium bicarbonate (3.5 g/L), antifungal antibiotic (1 %), and sodium pyruvate (110 mg/L). The culture was placed in a 75 cm² flask at 37 °C and 5 % CO₂ in a humidified atmosphere, as described by Salazar Lopez et al., 2016.

2.7.2. Effect of extracts on cell viability

RAW 264.7 cells were cultured at 37 °C and 5 % CO₂ for 24 h in 96-well COSTAR plates (1 × 10⁴ cells/well). After this time, the medium was replaced with fresh medium containing different concentrations of food extracts and digests from the intestinal fraction resuspended in DMSO < 1 %. These concentrations were 25, 12.5, and 6.25 µg/mL. The culture was incubated for 24 h, and then the medium with the

treatments was removed. Subsequently, 20 µL of MTT dissolved in the medium was added to the adherent cells and incubated again for 2 h. As a result, MTT was transformed to formazan, which was evaluated at 570 nm (Salazar López et al., 2016). Cell viability (CV) was expressed as a percentage and calculated by the following equation:

$$CV (\%) = (\text{sample absorbance} / \text{absorbance control cells}) \times 100.$$

2.7.3. Determination of nitric oxide production

A methodology by Salazar López et al. (2016) was used to study nitric oxide production. RAW 264.7 cells (2.5 × 10⁵) were cultured in 96-well plates at 37 °C and 5 % CO₂ for 24 h. Then, medium with LPS (1 µg/mL) was added to induce nitric oxide production and treatments (food extracts and digests) at concentrations of 25, 12.5, and 6.25 µg/mL. Nitric oxide production in the culture medium was indirectly evaluated as nitrite by the Griess reaction. One hundred microlitres of the supernatant medium was added to 100 µL of a mixture consisting of 1 % sulfanilamide and 0.1 % naphthylethylenediamine dihydrochloride and then incubated for 10 min. The absorbance was measured at 550 nm. The concentration of nitric oxide in the culture medium was determined by a sodium nitrite standard curve (nM/mL).

2.8. Statistical analysis

In this study, a completely randomized design was used with three replications for each cookie prepared with the different flours. Analysis of variance (ANOVA) of the means was performed. Tukey's test was used at a significance level of $p < 0.05$. Pearson's correlation analysis was performed as needed.

3. Results and discussion

3.1. Total phenol content and antioxidant activity

After sorghum sprouts irradiated with UV-A LED light were produced, a flour was generated, which was used to create ISSF cookies through a formulation described by Chiremba et al. (2009). To determine the antioxidant potential of the sorghum cookies, the phenol content, antioxidant activity, and various phenolic compounds were quantified. The results obtained for the quantification of total phenols in the cookies are shown in Table 1.

The highest content of free phenols was observed in ISSF cookies, contributing 48 % of the total phenols found in this sample, followed by SSF (36 %), SF (34 %) and WF (22 %). In the same way, the best DPPH radical scavenging activity on the free fraction was found in the cookies prepared with SSF (7.97 ± 0.83 µMTE/g) and ISSF (7.73 ± 0.84 µMTE/g) ($p > 0.05$), contributing 35 % and 32 %, respectively. Moreover, the best activity evaluated by TEAC was found in the cookies prepared with

Table 1

Total phenol content and proportions of free, bound, and conjugated phenols (%) in cookies prepared with different flours.

Cookie	Total phenols mgGAE/g			
	Free	Bounded	Conjugated	Total
SF	1.11 ± 0.05 ^c (34.6 %)	2.01 ± 0.11 ^c (62.8 %)	0.08 ± 0.004 ^c (2.5 %)	3.20 ± 0.16
WF	0.67 ± 0.03 ^d (22.4 %)	2.27 ± 0.20 ^b (76.7 %)	0.04 ± 0.004 ^c (1.3 %)	2.98 ± 0.23
SSF	1.76 ± 0.04 ^b (36.9 %)	2.85 ± 0.18 ^a (59.8 %)	0.15 ± 0.01 ^b (3.1 %)	4.76 ± 0.23
ISSF	2.08 ± 0.06 ^a (48.8 %)	1.84 ± 0.14 ^c (43.1 %)	0.34 ± 0.04 ^a (7.9 %)	4.26 ± 0.24

Mean values (±SD, n = 3) of total phenolic content for the cookies under study (the results marked with the same letter in the upper index within each column differ significantly). SF: sorghum flour; WF: wheat flour; SSF: sorghum sprout flour; ISSF: irradiated sorghum sprout flour.

ISSF (Table 2; Fig. 1). When evaluating the possible relationship between phenol content and antioxidant activity (free fractions), we found a positive relationship between phenols-DPPH ($r: 0.92$; $p < 0.0001$) and phenols-TEAC ($r: 0.97$; $p < 0.0001$). Thus, increasing the phenol content increases the antioxidant activity. Importantly, in addition to the flours, other ingredients used to form the cookies contributed to the final activities; honey contributed 0.24 mg GAE/g of total free phenols, while DPPH and TEAC contributed 1.7 and 3.2 μ MTE/g to the antioxidant activity, respectively. This activity may result from the presence of phenolic acids (gallic, ferulic and sinapic, among others) (Lawag et al., 2023). However, it has been reported that phenolic compounds in margarine are lost when subjected to thermal processes (Nadeem et al., 2017).

The antioxidant activity results may vary based on the presence or absence of compounds with different hydroxyl and methoxyl groups, as well as the mechanisms and radicals utilized by the compounds in the assays, including hydrogen atom donation (HAT), one electron followed by proton transfer (SET-PT), and sequential electron transfer with loss of protons (SPLET) (Chen et al., 2020).

3.2. Phenolic compound profile

The phenolic profile of the cookies in the free fraction was evaluated, and the results revealed the presence of gallic acid, protocatechuic acid, coumaric acid, ferulic acid, sinapic acid and catechin (Table 3). The observed results could result from diverse interactions between phenolic compounds and other compounds (Rocchetti, 2022), carbohydrates (Jakobek, 2015), fibre (González-Aguilar et al., 2017), proteins (Ozdal et al., 2013) and other structures in the food matrix. These interactions occur immediately after the food components are mixed, as well as during handling and processing (Guan et al., 2021). On the other hand, cooking by heat treatment causes cell walls to rupture, favouring extraction (Lou et al., 2014).

The highest amounts of gallic acid were found in SSF and ISSF cookies. Similar results were described by Garzón et al. (2020) with sprout cookies from different sorghum cultivars, in which the highest amounts of gallic acid were found in the free fraction. Higher values were found in ferulic acid in sorghum cookies (SF, SSF and ISSF) than in WF cookies because sprouted grain was used for the flour and cookie formulation, while refined flour was used for the WF cookie. Ferulic acid was found in higher concentrations in the outermost fraction of the grain (Hemery et al., 2011). Due to this anatomical arrangement of ferulic acid, the compound can structurally bind to polysaccharides in the cell wall through ester-type bonds; as a result, more than 80 % of the ferulic acid is not free or available for absorption. The presence of these phenolic compounds has already been described by other authors (Dykes & Rooney, 2006; Luthria & Liu, 2013). Likewise, other phenolic compounds, such as syringic acid, apigenin, luteonin and naringenin, can be found in different sorghum cultivars (Xiong et al., 2019).

There is evidence that the growth and development of the new plant increase the content of phenolic acids and flavonoids during the grain sprouting process (Kolton et al., 2022). According to Barnes & Anderson (2018), the release, recycling and rebuilding of cell walls can cause

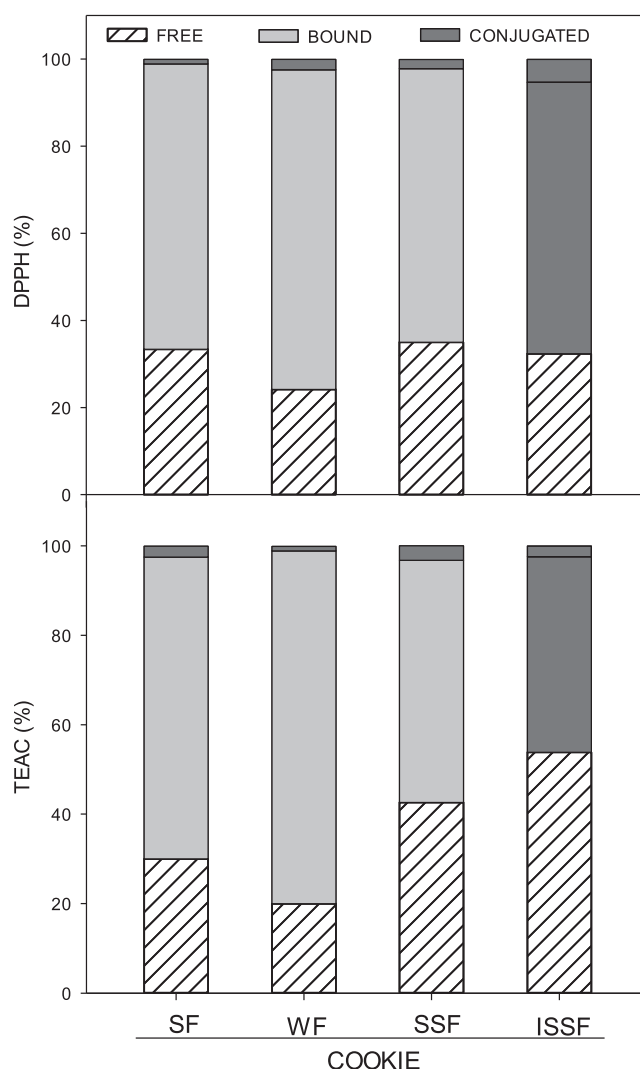


Fig. 1. Antioxidant activity and proportions of free, bound, and conjugated fractions (%) in cookies prepared with different flours.

trapped compounds to be released or increase their production of phenolic compounds needed to defend the new plant (Lone et al., 2023). Therefore, when UV-A light is applied to sorghum shoots, the content of phenolic compounds can increase or decrease according to the conditions and intensity of ultraviolet irradiation. In this case, phenols, flavonoids, anthocyanins, carotenoids and ferulic acid esters can be found in the cuticle, epidermis and mesophyll of plants, participating in heat dissipation, antioxidation, physical quenching of excited states of chlorophyll and singlet oxygen, nucleic acid repair, and protein and lipid synthesis (Emus-Medina et al., 2023).

Table 2

Antioxidant activities (DPPH and TEAC) of free, bound, and conjugated fractions in cookies prepared with different flours.

Cookie	DPPH (μ M TE/g)				TEAC			
	Free	Bounded	Conjugated	Total	Free	Bounded	Conjugated	Total
SF	4.78 $\pm$ 0.46 ^b	9.36 $\pm$ 0.32 ^c	0.15 $\pm$ 0.01 ^d	14.28 $\pm$ 0.79	8.93 $\pm$ 0.45 ^c	20.06 $\pm$ 0.94 ^b	0.72 $\pm$ 0.02 ^b	29.71 $\pm$ 1.41
WF	3.67 $\pm$ 0.05 ^c	11.16 $\pm$ 0.64 ^b	0.36 $\pm$ 0.03 ^c	15.19 $\pm$ 0.72	5.62 $\pm$ 0.19 ^d	22.23 $\pm$ 2.01 ^a	0.29 $\pm$ 0.04 ^c	28.14 $\pm$ 2.24
SSF	7.97 $\pm$ 0.83 ^a	14.28 $\pm$ 0.18 ^a	0.48 $\pm$ 0.01 ^b	22.73 $\pm$ 1.02	11.41 $\pm$ 0.26 ^b	14.52 $\pm$ 0.55 ^c	0.86 $\pm$ 0.09 ^a	26.79 $\pm$ 0.9
ISSF	7.73 $\pm$ 0.84 ^a	14.88 $\pm$ 0.51 ^a	1.26 $\pm$ 0.09 ^a	23.87 $\pm$ 1.44	15.11 $\pm$ 0.49 ^a	12.25 $\pm$ 0.28 ^d	0.68 $\pm$ 0.05 ^b	28.04 $\pm$ 0.82

Mean values ($\pm$ SD, $n = 3$) of antioxidant activity (DPPH and TEAC) for the cookies under study (results marked with the same letter in the upper index within each column differ significantly). SF: sorghum flour; WF: wheat flour; SSF: sorghum sprout flour; ISSF: irradiated sorghum sprout flour.

Table 3

Major phenolics in cookies formulated with different flours.

Phenolic ($\mu\text{g/g}$)	SF	WF	SSF	ISSF
Gallic acid	244.8 $\pm$ 6.4 ^d	269.3 $\pm$ 2.4 ^c	2107.6 $\pm$ 60.6 ^a	1121.4 $\pm$ 9.7 ^b
Protocatechuic acid	27.7 $\pm$ 1.2 ^c	81.2 $\pm$ 3.6 ^b	94.23 $\pm$ 0.9 ^a	–
p-Coumaric acid	192.7 $\pm$ 5.7 ^a	43.3 $\pm$ 2.3 ^c	35.3 $\pm$ 1.0 ^d	65.1 $\pm$ 1.2 ^b
Ferulic acid	19.3 $\pm$ 1.0 ^b	9.4 $\pm$ 0.6 ^d	24.4 $\pm$ 1.0 ^a	12.8 $\pm$ 0.7 ^c
Sinapic acid	0.24 $\pm$ 0.0 ^a	–	0.15 $\pm$ 0.0 ^b	0.15 $\pm$ 0.0 ^b
Catechin	58.98 $\pm$ 1.6 ^b	297.9 $\pm$ 3.3 ^a	2.0 $\pm$ 0.1 ^c	1.9 $\pm$ 0.1 ^c

Mean values ($\pm$ SD, $n = 3$) of individual phenolics for the cookies studied (the results marked with the same letter in the upper index within each row differ significantly). SF: sorghum flour; WF: wheat flour; SSF: sorghum sprout flour; ISSF: irradiated sorghum sprout flour. -: not detected.

3.3. Bioaccessibility of phenolic compounds and recovery of antioxidant activity of cookies

To determine the possible antioxidant activity and effects caused by digestion after the phenolic compounds were consumed, *in vitro* gastrointestinal simulation was performed with the cookies. Fig. 2a shows the dynamic change that occurs during the different phases of gastrointestinal simulation.

During the oral phase, the food matrix begins to release phenols, and the released compounds encounter saliva; some compounds remain unchanged, such as catechins, but some phenolic compounds that are affected by deglycosylation, such as flavanol gallate esters, are affected by the saliva (Corona et al., 2014).

On the other hand, during the oral phase, proline-rich acidic proteins can bind to phenolic compounds through hydrophobic interactions or hydrogen bridges, among which interactions can occur between proanthocyanidins and flavanols (Corona et al., 2014; Velderrain-Rodríguez et al., 2014). However, the modifications that occur in the oral phase tend to be insignificant (Grgić et al., 2020).

Once the oral phase has ended and the digestion process enters the gastric phase, the particle size of the food is reduced, which helps release phenols (Grgić et al., 2020). Most of these compounds tend to be released during this phase, and some can even be absorbed in their free form, while others must be hydrolysed and deconjugated (Corona et al., 2014; Bohn, 2014). Once the gastric phase is over, phenolic compounds are absorbed by the small intestine, while others reach the large intestine (Laparra & Sanz, 2010; Velderrain-Rodríguez et al., 2014). During this phase, the components of the food matrix and enzymes are greatly mixed due to changes in pH and effects caused by bile salts and lipases. In addition to releasing phenolic compounds, apolar components (such as lipids and phytochemicals, including flavonoids and aglycones) can micellize (Bohn, 2014) (Chitchumroonchokchai et al., 2012). Additionally, phenolic compounds can interact with proteins (Frazie et al., 2010) and carbohydrates (Saura-Calixto, 2011) due to hydrophobic noncovalent (Yuksel et al., 2010) and covalent interactions (Kroll et al., 2003). On the other hand, phenolic compounds can also interact with enzymes, such as α -amylase, due to hydrogen bridges between phenols and tryptophan residues of the enzyme (Giuberti et al., 2020). These interactions can lead to low bioaccessibility but can also protect nutrients, such as vitamins, lipids, and proteins, from oxidation (Jakobek, 2015). Finally, due to the digestion process, approximately 5 to 10 % of the total content of ingested phenols reaches absorption in the small intestine (Grgić et al., 2020). This finding explains the results obtained for total free phenols during gastrointestinal simulation, in which an increase and decrease was observed during the gastric and intestinal phases, respectively, for all cookies under study. The free phenol content in the intestinal phase was higher in SSF and ISSF cookies than SF and WF cookies.

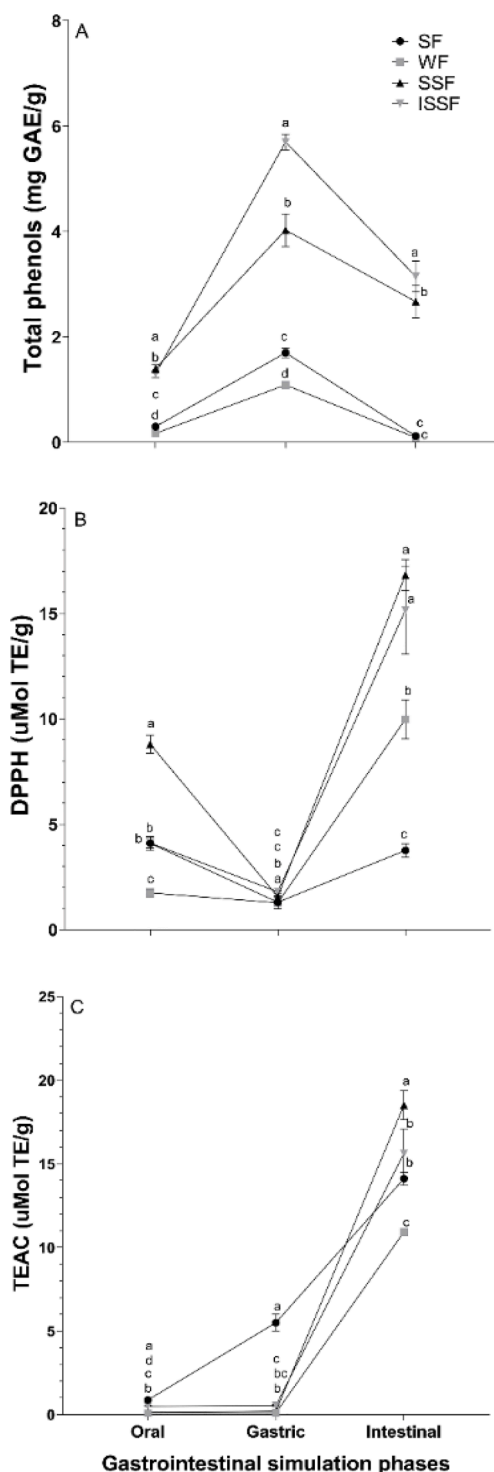


Fig. 2a. Phenol content and antioxidant activity of the free fraction during *in vitro* gastrointestinal simulation of cookies prepared with different flours. A: Free total phenols; B: Antioxidant activity by free DPPH; C: Antioxidant activity by free TEAC. Mean $\pm$ standard deviation of three replicates for each food. Different letters correspond to a significant difference ($p < 0.05$) in each intestinal fraction. Grouped letters follow the order of legends. SF: sorghum flour; WF: wheat flour; SSF: sorghum sprout flour; ISSF: irradiated sorghum sprout flour (ISSF).

Regarding the antioxidant activity exhibited by DPPH, the highest antioxidant capacity with SSF and ISSF cookies was found in the intestinal phase, while for TEAC, the cookie with SSF was significantly higher than the other cookies. On the other hand, the kinetics of gastrointestinal simulation decreased and increased in the gastric and intestinal phases, respectively, for both techniques (DPPH and TEAC), which does not follow the trend observed for phenolic compound contents. Based on the Folin–Ciocalteu technique, it has been demonstrated that compounds such as reducing sugars, amino acids and ascorbic acid tend to react with this technique, and results are usually overestimated (Muñoz-Bernal et al., 2017; Everette et al., 2010). On the other hand, the number and position of hydroxyl groups (particularly those of the A and B rings), as well as the ortho and meta positions, depend on the phenol content (Platzer et al., 2021).

The bioaccessibility of free phenols and antioxidant activity is defined as the proportion (in terms of percentage) of phenols that are potentially available for absorption in the small intestine; this value is based on the total fractions (free, bound and conjugated) of phenols, and the antioxidant activity of cookie samples before digestion is considered as 100 % (Table 1). As shown in Table 4, the bioaccessibility percentages of total phenols obtained in this study were 2.8 %, 3.8 %, 56 % and 74 % for WF, SF, SSF and ISSF cookies, respectively. The above results suggest that intestinal digestion conditions failed to release more phenolic compounds than those found before digestion. Similar behaviour was found for the recovery of antioxidant activity, in which values greater than 100 % were not reached, but greater antioxidant activity was observed in SSF and ISSF cookies.

3.4. Phenolic profile after gastrointestinal simulation

To further elucidate the results obtained for phenol content and antioxidant activity, we evaluated the free phenolic profile of digests in the intestinal fraction, since these profiles represent phenols available for absorption and their possible beneficial effects. The results are shown in Table 5.

Relevantly, gastrointestinal digestion conditions favoured the release of most evaluated phenolic compounds present in cookies with ISSF. The results show that the phenolic acid with the highest content in all cookies (except the cookie with WF) subjected to gastrointestinal simulation was gallic acid; in addition, the highest quantity of gallic acid was found in the cookie with SSF, followed by ISSF. These results agree with the findings of Miafo et al. (2020), in which free but not bound gallic acid was found. Additionally, increases in other phenolic acids, such as chlorogenic acid (which increases after digestion), have been found in other foods (Kashyap et al., 2022). The decrease in phenol content may result from the interactions these compounds have with the food matrix during digestion, as mentioned above. On the other hand, extraction methods are diverse, and emerging technologies improve the extraction ratio (Lampakis et al., 2021); therefore, using enzymes and other conditions during gastrointestinal simulation may improve the content vs. the cookie subjected to chemical extraction.

As previously demonstrated, the bioaccessibility of individual

Table 4

Bioaccessibility (%) of free phenolics and antioxidant recovery of cookies produced with different flours.

Cookie	Phenolics	Bioaccessibility (%)	
		DPPH	TEAC
SF	3.82	26.29	47.74
WF	2.8	65.71	38.79
SSF	56.18	73.98	69.12
ISSF	74.05	64.58	55.70

Bioaccessibility of free total phenols and antioxidant recovery. SF: sorghum flour; WF: wheat flour; SSF: sorghum sprout flour; ISSF: irradiated sorghum sprout flour.

Table 5

Phenolic profiles of the intestinal fraction of cookies prepared with different flours.

Phenolic (µg/g)	SF	WF	SSF	ISSF
Gallic acid	813.59 ± 6.4 ^c	–	2388.20 ± 5.7 ^a	1807.31 ± 6.0 ^b
Protocatechuic acid	392.81 ± 2.2 ^b	179.42 ± 2.8 ^c	20.57 ± 0.6 ^d	503.00 ± 4.8 ^a
p-Coumaric acid	26.10 ± 0.8 ^c	11.63 ± 0.2 ^d	37.26 ± 1.3 ^b	41.83 ± 1.1 ^a
Ferulic acid	13.61 ± 0.3 ^b	4.7 ± 0.19 ^c	15.29 ± 0.23 ^a	13.67 ± 0.3 ^b
Sinapic acid	67.21 ± 0.4 ^b	–	–	144.51 ± 0.3 ^a
Catechin	72.11 ± 1.6 ^b	58.80 ± 0.4 ^c	93.13 ± 0.4 ^a	–

–: not detected; The values represent the mean ± standard deviation of three replicates for each food. The differences correspond to each compound among the different cookies. SF: sorghum flour; WF: wheat flour; SSF: sorghum sprout flour; ISSF: irradiated sorghum sprout flour (ISSF).

phenolic compounds depend on their molecular weight, shape, polarity, and structure (Tarko et al., 2020). For example, polyphenols undergo metabolic transformations and absorption in the small intestine (Fernandes et al., 2014) or become methylated with hydroxyl groups in the ortho position (Kroon et al., 2004).

3.5. Nitric oxide production

Once it was determined that the extracts did not negatively influence the viability of RAW264.7 cells (Fig. 2b), nitric oxide production was quantified at various concentrations of cookie extracts and digests. The results are shown in Fig. 3.

Regarding the concentrations observed in food (A), all cookies formulated with different flours at a concentration of 25 µg/mL were statistically equal. For concentrations of 12.5 µg/mL, cookies with SF and ISSF showed the greatest decrease in nitric oxide production. Significantly similar results were observed at a concentration of 6.25 µg/mL. On the other hand, for some wheat and sorghum sprout samples, no differences were observed compared with the positive control. On the other hand, in digests (B), cookies prepared with SSF and ISSF showed lower nitric oxide content at all three concentrations. Among these cookies, no significant differences were found under the different concentrations. However, with ISSF cookies, the same results can be obtained with low and high concentrations.

These results may be due to the presence of various phenolic compounds in the sample. Based on correlation analyses and the Pearson correlation, there is a negative relationship between the nitric oxide produced and the presence of phenolic compounds ($r: -0.82p < 0.0255$); therefore, the higher the content of these compounds is, the lower the production of nitric oxide. There was no correlation between the rest of the cookies and nitric oxide production.

Although a reduction in nitric oxide production in food and its digestion has been observed, these findings do not necessarily reflect what happens in the human body, since the amount and structure of polyphenols in the body can vary significantly.

For example, these polyphenols are metabolized in vivo and circulate in the body as sulfated, glucuronidated and methylated metabolites, which may exhibit higher or lower bioactivity (Heleno et al., 2015). These effects have already been observed in phenolic acids present in plasma from coffee (Koriet & Gad, 2022) and results from the interactions between the compounds and components of the food matrix during digestion (Rocchetti et al., 2022).

The decrease in nitric oxide may result from several factors. According to Taira et al. (2009), some flavonoids can directly eliminate nitric oxide in RAW 264.7 cells. Another mechanism involves

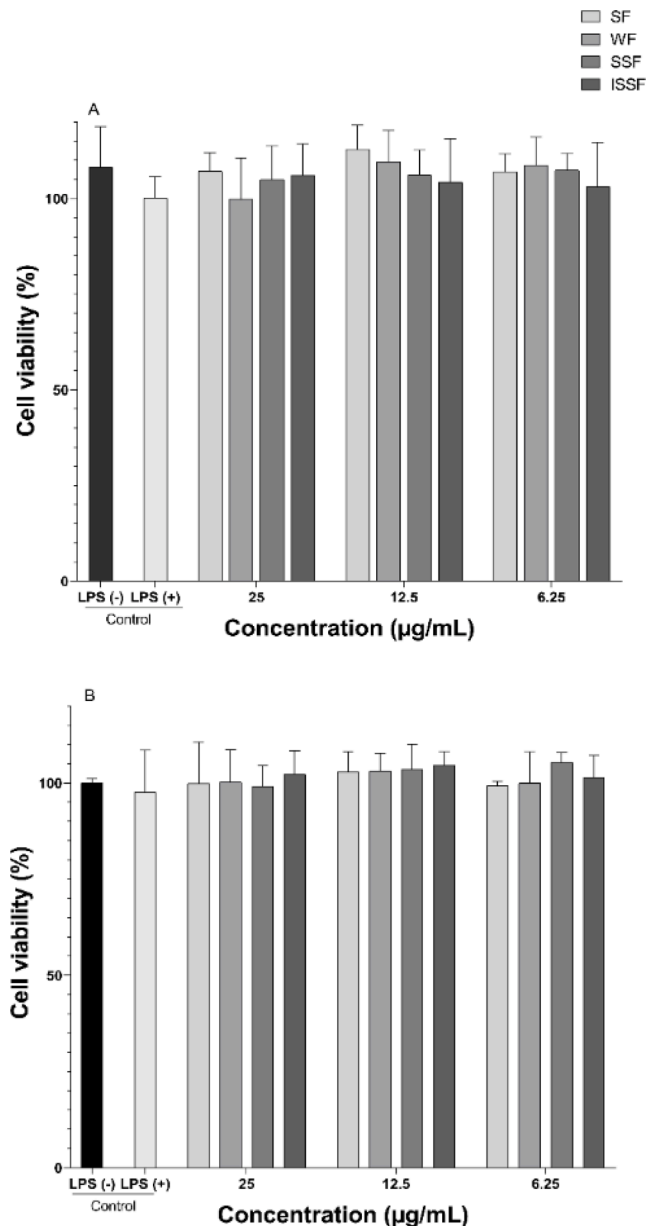


Fig. 2b. Viability (%) of RAW 264.7 cells treated with cookie extracts and digests of SF: sorghum flour; WF: wheat flour; SSF: sorghum sprout flour; ISSF: irradiated sorghum sprout flour. A: Food; B Digest. Control (-) represents untreated cells, and control (+) represents cells treated only with LPS. Each bar represents the mean of three replicates $\pm$ standard deviation.

controlling nitric oxide production through transcription of nuclear factor kappa beta (NF KB) (Yahfoufi et al., 2018). One of these compounds is caffeic acid ester (Onori et al., 2009; Karaboğa, 2019). In addition, researchers have found that phenolic compounds can help inhibit the enzyme nitric oxide synthase, an enzyme that catalyses the formation of nitric oxide (Serreli and Deiana, 2023), and NADPH oxidase (NOX), a multiprotein complex responsible for producing reactive oxygen species; these enzymes are precursors of nitric oxide (Petrônio et al., 2013). Among other known mechanisms, chlorogenic acid can inhibit cyclooxygenase (COX 2) and proinflammatory cytokines (IL-1 β and TNF- α), possibly through competing with the enzymatic active site (related enzyme for activation of proinflammatory genes) (Hwang et al., 2014; Zhang et al., 2018), as well as suppressing the activation of inflammasomes, such as NLRP3, a multiprotein complex related to inflammation (Spano et al., 2022).

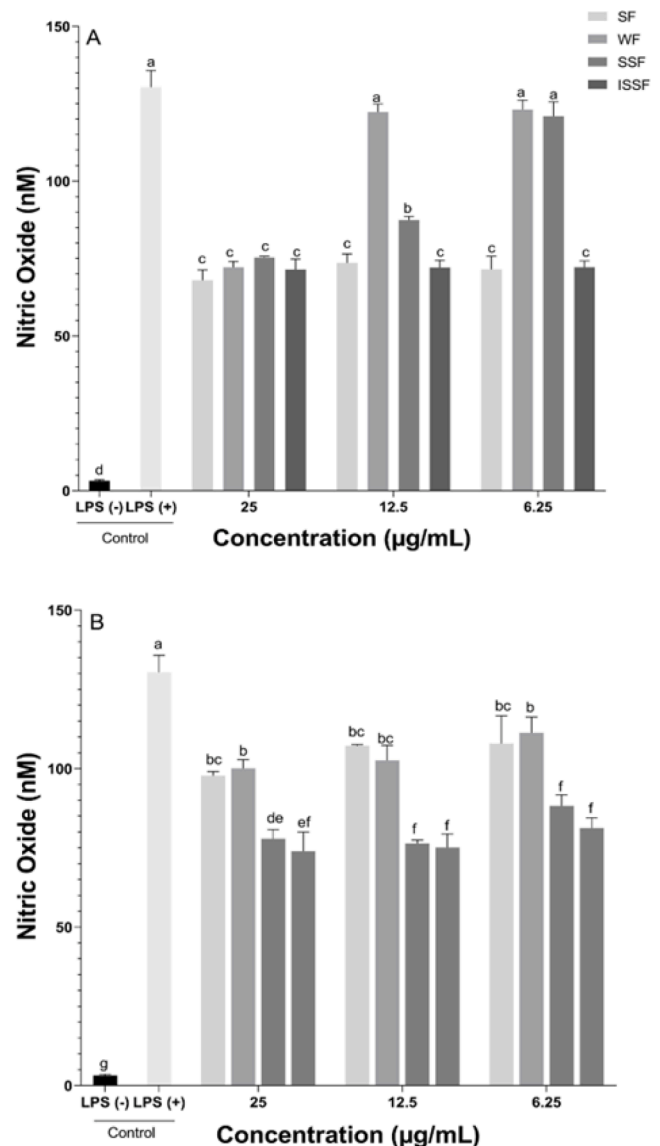


Fig. 3. Nitric oxide production of RAW 264.7 cells treated with various concentrations of extracts from cookies prepared with different flours. A: Food; B Digest. Control: LPS (-) cells cultured without lipopolysaccharide, LPS (+) cells cultured with lipopolysaccharide. SF: sorghum flour; WF: wheat flour; SSF: sorghum sprout flour; ISSF: irradiated sorghum sprout flour (ISSF). The cookies were cultured at different concentrations with LPS. The bars represent the mean $\pm$ standard deviation of three replicates at each concentration of each food. Different letters correspond to a significant difference at $p < 0.05$.

On the other hand, the observed findings may result from the synergistic effects exhibited by the observed and unobserved phenolic compounds; for example, synergistic effects can be found in caffeic + sinapic acid, ferulic + p-coumaric acid, and gallic + ferulic acid, among others (Olszowy et al., 2019). In addition, adverse effects, such as antagonism of some bioactive compounds (gallic + vinylic) (Rúa et al., 2017) or negative effects by acrylamides obtained during cookie baking (Koohi et al., 2023; Wang et al., 2023), may interfere with the results. These findings indicate that a potentially functional food resulting from ultraviolet irradiation, with potential antioxidant and anti-inflammatory effects, can be used to counteract chronic non-communicable diseases related to overweight and obesity. These benefits of sorghum are consistent with those previously described by Xu et al. (2021).

4. Conclusion

Irradiation with ultraviolet light increased the phenolic content and antioxidant activity of compounds in ISSF cookies. Gallic acid was the most abundant phenolic acid in ISSF cookies. Due to gastrointestinal simulation effects, phenol content and antioxidant activity varied during the different phases of the simulation. ISSF cookies showed better bioaccessibility results than those of some control cookies. The phenolic acid with the highest content in the intestinal fraction was gallic acid. The anti-inflammatory activity shows that ISSF cookies present an equal or greater decrease in nitric oxide at low concentrations than some control cookies. ISSF cookies show promising results as a potential functional food and could help counteract overweight/obesity and related chronic degenerative diseases.

Ethical statement

There is no formal documentation process available on the gastrointestinal simulation assessment conducted in this study. We agree to provide this statement to confirm that adequate protocols were used to protect the rights and privacy of all participants during the research.

CRediT authorship contribution statement

Alan A. Ruiz-Hernández: Writing – original draft, Investigation. **Ofelia Rouzaud-Sánchez:** Juana Frias: Supervision, Methodology, Conceptualization. **Fernando Ayala-Zavala:** Writing – review & editing, Formal analysis, Conceptualization. **Humberto Astiazarán-García:** Norma J. Salazar-López: Resources, Methodology, Investigation. **Carmen M. López-Saiz:** Sandra C. De la Reé-Rodríguez: Investigation. **Maribel Robles Sánchez:** Writing – review & editing, Project administration, Conceptualization.

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.

Data availability

Data will be made available on request.

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