






# Combining germination-extrusion as strategy to improve nutritional and nutraceutical value of whole sorghum grain

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**Abstract:** Sorghum (*Sorghum bicolor* L.) is one of the most important cereals in the world; is an important source of bioactive compounds. The germination is a very useful tool to improve the nutraceutical value of cereals, associated with the reduction of chronic-degenerative diseases; the extrusion has a positive effect on microbiological stability and sensory properties. The response of the combined germination-extrusion processes applied under optimised conditions, on proximal composition, *in vitro* protein digestibility (IVPD), total phenolic compounds (TPC), antioxidant activity (AoxA), hypoglycemic potential and microbiological quality of sorghum grains were studied. Sorghum was processed by germination (37 °C for 69 h) and extrusion [137 °C for 134 rpm (revolutions per minute)]. The germination increased protein content (+21%), insoluble dietary fibre (+50%), IVPD (+10%), TPC (+26%), AoxA (+97%). The extrusion increased soluble dietary fibre (+100%) and IVPD (+13%). The combined germination-extrusion processing reduced the content of total coliforms, total mesophilic aerobics and molds below the maximum limits established by the Mexican Official Standards NOM-147-SSA1-1994. Regarding hypoglycemic potential, germinated sorghum and germinated-extruded sorghum presented the best half maximal inhibitory concentration ( $IC_{50}$ ) value. The combination of germination-extrusion processes is an effective strategy to increase bioactive compounds with antioxidant activity and inhibition of  $\alpha$ -amylase and  $\alpha$ -glucosidase enzymes.

**Keywords:** functional food; sorghum; optimisation; nutritional properties; microbiological quality

Sorghum is among the most important cereals in the world. Being used mainly in animal feed as forage; however, the attention towards this crop by farmers has grown due to the potential of sorghum grains; this is because it is a drought-resistant cereal and adapts very easily to new conditions; taking into account cli-

mate changes and declining water supplies; sorghum can be a great alternative as a commonly consumed cereal in humans (Hossain et al. 2022). The components of sorghum are starch (65–80%), proteins (7–15%), polysaccharides (> 10%) and lipids (1.5–6%) (Frankowski et al. 2022); this cereal has a high content

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of phenolic compounds: phenolic acids, flavonoids and anthocyanins (Šárka et al. 2020); which are linked to multiple health benefits, such as anti-inflammatory, antioxidant, antibacterial, and antiglycemic properties. In addition, sorghum flour is gluten-free, making it an option in the diet of people with celiac disease (Frankowski et al. 2022). Sorghum flours can be successfully used in the food industry due to their multiple health benefits; to carry out the processing of these flours; a technological option is extrusion, a profitable method for the processing of different products, modifying ingredients and operating conditions, with great advantages (improvement in protein digestibility, acceptability and palatability of the product) (Ofiah et al. 2019); however, as it is a thermal process, the phenolic compounds of the flours can be affected; to improve the loss of these during extrusion, it is suggested to carry out a prior process that improves the properties of the grain, such as germination (Gong et al. 2018); a bioprocess that increases nutritional bioavailability, increases the content of phenolic compounds, as well as the antioxidant activity of cereals (Chavarín et al. 2019). Germination-extrusion have been used as combined processes to produce flours with better nutritional, nutraceutical and sensory properties; as well as greater microbiological quality by eliminating the microbial load of the sprouts when applying the extrusion process (Ohtsubo et al. 2005).

There are no reports on the combination of germination-extrusion technologies in sorghum grain for the development of functional flours. The objective of this research was to study the effect of the processes; germination-extrusion, applied in combination, under optimised conditions for each of them; on the chemical composition, nutritional value, antioxidant activity, content of total phenolic compounds and microbiological stability of grain sorghum. In addition, it is planned to obtain a functional extruded germinated sorghum flour with a high nutritional and nutraceutical value that can be used to create functional foods.

## MATERIAL AND METHODS

### Material and chemicals

Sorghum seeds were purchased at the Rafael Buena local market, Culiacán, Sinaloa, Mexico. The ABTS [2,2'-azino-bis(3-ethylbenzothiazoline-6-sulfonic acid)] and DPPH (2,2-diphenyl-1-picrylhydrazyl), Trolox, sodium hydroxide, hydrochloric acid, hexane, methanol, ethanol, and ethyl acetate reagents was obtained from Sigma Chemical Co. (USA).

### Methods

**Obtention of the optimised germinated sorghum flour (OGSF).** A 0.02% NaClO solution was employed to soak 1.2 kg of sorghum seeds for 23 h; after, the seeds were soaked for 1 h in water. Once the sorghum seeds were hydrated, they were placed in germination trays (100 g per tray). The trays were collocated in the germinator (Percival GR-36L; Perry, USA) with controlled temperature and humidity (80–90%). The optimal germination conditions used were those reported by Salcido (2015): germination temperature (*GT*) and germination time (*Gt*) are 37 °C and 69 h. Seed germination was carried out under periods of light/darkness (50%/50%) during the daily germination time with white light [fluorescent tubes (16 W, 2 700 K); Tecnilite, Mexico]. Trays were watered once a day with a 0.02% NaClO solution throughout the experiment to keep the seed moist. The germinated sorghum seeds were subjected to a drying process at 50 °C for 8 h in an oven (forced circulation; Carbolite, Spain) until reaching the water activity  $a_w = 0.6$ , they were ground (LM 3100; PerkinElmer, USA) (0.177 mm) and stored (4 °C).

**Obtention of the extruded germinated sorghum flours (EGSF).** The methodology of León et al. (2021) was slightly modified for the extrusion of the OGSF. Water was added to the OGSF to obtain a final moisture content of 28% (278 mL per 1 kg). Extrusion was performed in a laboratory extruder (single-screw model 20 DN; CW Brabender Instruments, USA) equipped with a 19 mm diameter screw, length:diameter = 20:1, 1:1 nominal compression ratio, and 3 mm die opening. The operating conditions of the extruder were: extrusion temperature *ET* = 50–160 °C and screw velocity *SV* = 50–240 rpm. The extruder was fed at a rate of 70 g·min<sup>-1</sup>; germinated sorghum extrudates were allowed to stand at 25 °C, ground (LM 3100; PerkinElmer, USA) (0.177 mm) and stored at 4 °C.

**Extraction of phenolic compounds, antioxidant activity (AoxA) and total phenolic content (TPC).** The methodology of Salas et al. (2018) for the extraction of phenolic compounds was used; 10 mL of ethanol-water (80:20, v/v) was added to 0.5 g of sample defatted, shaken, the supernatant was recovered by centrifugation (3 000 × g for 10 min) (Sorvall RC5C; Thermo Fisher Scientific, USA). For bound phenolic compounds, the precipitate was hydrolysed with 2 M NaOH (95 °C for 30 min). HCl was then used for neutralisation (25 °C for 60 min). The extraction was carried out with 10 mL of ethyl acetate (four times). Both extracts were evaporated to dryness (Savant SC250 DDA Speed Vac Plus centrifugal evaporator; Yamato, USA) and reconstituted in 2 mL of metha-

Table 1. Experimental design\* used to obtain different combinations of extrusion temperature and screw velocity for producing extruded germinated sorghum flours

Assay**	Process variables	
	extrusion temperature (°C)	screw velocity (rpm)
1	66	77
2	143	77
3	66	212
4	143	212
5	<b>50</b>	145
6	<b>160</b>	145
7	105	<b>50</b>
8	105	<b>240</b>
9–13 (replicates)	105	145

\*Central composite rotatable design with two factors and five levels; \*\*does not correspond to order of processing; rpm – revolutions per minute; bold – minimum and maximum values used

nol. The extractions were performed in triplicate. Servín de la Mora et al. (2018) for the evaluation of *AoxA* [ABTS and DPPH:  $\mu\text{mol TE}$  (Trolox equivalent) per 100 g sample (DW, dry weight)] and *TPC* [mg GAE (gallic acid equivalents) per 100 g sample (DW)] were used.

***In vitro* protein digestibility (IVPD).** A multienzymatic system was used to determine *IVPD* (Rathod and Annappure 2016).

***Experimental design and extrusion process modelling and optimisation.*** A rotatable central composite experimental design was used. The analysed factors were *ET* and *SV*, and the optimised response variables were *AoxA*, *TPC*, and *IVPD*. The design consisted of 13 treatments (Table 1), therefore statistics such as the mean and standard deviation were not reported. Second-order polynomial models that establish the mathematical relationship between the process and response variables were adjusted using least squares regression, with the purpose of obtaining a prediction model for each response studied and using them for the optimisation of the extrusion process. The optimisation was done ensuring that *AoxA*, *TPC*, and *IVPD* had the highest possible values. The graphical method was used for optimisation (Design-Expert software, version 7.0.0).

***Characterisation of sorghum flours.*** Unprocessed sorghum flour (USF), optimised germinated sorghum flour (OGSF) and optimised extruded germinated sorghum flour (OEGSF) were produced by triplicate with the purpose of characterising them according to the methodologies described as follows:

- Proximal composition. The chemical composition of sorghum flour was determined using the Association of Official Analytical Chemists (AOAC 2012) chemical methods.
- Hypoglycemic potential [half maximal inhibitory concentration ( $IC_{50}$ ),  $\alpha$ -amylase and  $\alpha$ -glucosidase inhibition activities]. The inhibitory activity of the free and bound phenolic extracts against  $\alpha$ -amylase and  $\alpha$ -glucosidase was determined following the methodology reported by León et al. (2021).
- Microbiological analysis of flours. A sample of each flour was taken and incorporated into a buffer solution of water with peptone at 0.1% w/v. The mixture was stirred and filtered (Whatman No. 1) to obtain 50 mL. Serial dilutions were made with a phosphate buffer solution. 1 mL of each dilution was placed in 3M Petrifilm™ plates (TECHNOPATH, Ireland) for the enumeration and identification of *Staphylococcus aureus* [AFNOR method validated (3M-01/19–04/03)], *Escherichia coli*, total coliforms (official methods AOAC 998.08 and 991.14), and aerobic mesophiles (AOAC 990.12 official method). Molds and yeasts were grown in Petri dishes in Sabour and dextrose agar for quantification according to the Mexican Official Standards NOM-111-SSA1-1994. The identification of *Salmonella* spp. was carried out by incubation in Selenite Cystine broth and Rappaport Vassiliadis broth (Merck, Germany) (37 °C for 24 h), and subsequent isolation in Hektoen enteric agar and Xylose Lysine Deoxycholate agar (Merck, Germany) (37 °C for 24 h) (ISO 6579:2002).
- Statistic analysis. The results of the sorghum flour characterisation were analysed [analysis of variance (ANOVA) and Tukey's multiple range test;  $P \leq 0.05$ ] using an unifactorial experimental design [factor: type of flour, with three levels: USE, OGSF, and OEGSF; these flours (3 treatments) were produced per triplicate and each replicate was evaluated for quality characteristics of the flours (studied responses)]. Normal distribution and homoscedasticity of data were verified through a residual analysis (Kolmogorov-Smirnov and Bartlett's tests;  $\alpha = 0.05$ ). The Minitab (version 21) statistical package was used.

## RESULTS AND DISCUSSION

**Prediction models for antioxidant activity, total phenolic content, and *in vitro* protein digestibility of extruded germinated sorghum flours.** The experimental values of the response variables (*AoxA*, *TPC*, and *IVPD*) of the EGSE; reflect data ranging

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6 412–7 770  $\mu\text{mol TE}$  per 100 g of sample (DW), 276–329 mg GAE per 100 g of sample (DW), and 52–76%, respectively. The relationship between the response variables (*AoxA*, *TPC*, and *IVPD*) and the process variables (*ET* and *SV*) were obtained by regression, which presented a second-order prediction model that includes the linear, quadratic, and interaction terms of *ET* and *SV*, which were significant ( $P \leq 0.05$ ). Whereas *AoxA* and *TPC* included the linear and quadratic terms. We clarify that the results shown below correspond to the estimated values of the regression coefficients ( $\beta$ 's) significant ( $P \leq 0.05$ ) of the mathematical models using uncoded variables, as well as the parameters related to the significance of the models:

$$\begin{aligned} AoxA = & +6.999 + 853.13 \times ET + 174.29 \times SV + \\ & - 68.75 \times ET \times SV - 18.44 \times (ET)^2 + \\ & + 219.56 \times (SV)^2 \end{aligned} \quad (1)$$

where:  $P_{\text{model}} = 0.0001$ ,  $R^2_{\text{adj}} = 0.9707$ ,  $P_{\text{lof}} = 0.3737$ ,  $CV = 0.0104$ ; *AoxA* – antioxidant activity; *ET* – extrusion temperature; *SV* – screw velocity;  $P_{\text{model}}$  – probability of model significance;  $R^2_{\text{adj}}$  – adjusted coefficient of determination;  $P_{\text{lof}}$  – probability of testing for lack of model fit;  $CV$  – coefficient of variance.

$$\begin{aligned} TPC = & +44.59 + 2.63 \times ET - 1.32 \times ET \times SV + \\ & + 1.04 \times (ET)^2 \end{aligned} \quad (2)$$

where:  $P_{\text{model}} = 0.0001$ ,  $R^2_{\text{adj}} = 0.9007$ ,  $P_{\text{lof}} = 0.3792$ ,  $CV = 0.0175$ ; *TPC* – total phenolic compounds.

$$\begin{aligned} IVPD = & +74.69 - 1.03 \times SV - 8.46 \times (ET)^2 + \\ & - 10.55 \times (SV)^2 \end{aligned} \quad (3)$$

where:  $P_{\text{model}} = 0.0001$ ,  $R^2_{\text{adj}} = 0.9719$ ,  $P_{\text{lof}} = 0.2404$ ,  $CV = 0.0262$ ; *IVPD* – *in vitro* protein digestibility.

According to Salas et al. (2018), the adequacy tests of a square model are the model is significant ( $P \leq 0.05$ ), a non-significant lack of fit ( $P > 0.05$ ), a high adjusted quadratic coefficient of determination (adjusted  $R^2 \geq 0.8$ ), and a small coefficient of variance ( $CV < 0.1$ ). The results obtained in the presented adequate statistical parameters, as can be seen above. Statistical assumptions (normality, constant variance, independence, randomness) were also verified through residual analysis, obtaining a sat-

isfactory fulfillment. Figure 1A shows that the *AoxA* of EGSF increased with higher *ET* and *SV*, reaching the highest value [8 150  $\mu\text{mol TE}$  per 100 g sample (DW)] at 160 °C and 240 rpm. On the other hand, the *TPC* increased with high *ET* and low *SV*, reaching a maximum value [334 mg GAE per 100 g sample (DW)] at 160 °C for 50 rpm (Figure 1B). In Figure 1C it can be seen that at medium temperatures and speeds, the *IVPD* increases, reaching maximum values of 75% at 105 °C for 145 rpm.

**Optimisation.** A contour plot overlay was used to obtain the optimal combination of process variables (Figure 1A–C) and produce the OEGSF. As shown in Figure 1D, the best process conditions (*ET* = 137 °C and *SV* = 134 rpm) with the highest values of *AoxA*, *TPC*, and *IVPD* are located at the centre point of the optimisation region. With the optimal extrusion conditions and the prediction models, the predicted values for the variables were obtained. The production of OEGSF using the optimal combination of process variables was performed in triplicate, obtaining experimental values of *AoxA*, *TPC*, and *IVPD* similar to those predicted.

**Chemical composition and nutritional properties of sorghum flours.** After optimised germination-extrusion, there was a significant increase in sorghum protein content (12% vs. 14%). This increase can be attributed; to the reduction of nutrients (carbohydrates) through the respiration process in germination (Salas et al. 2018).

Zhu et al. (2017) evaluated the effect of germination-extrusion on the nutritional and physicochemical properties of wheat tortillas. They reported that the germination process caused a significant increase ( $P < 0.05$ ) in protein content; however, extrusion did not show a significant difference compared to germinated wheat flour.

Regarding the lipid content of sorghum, a decrease in germination-extrusion was observed (3% vs. 1%). This decrease can be attributed to the use of lipids as a source of energy for the development of seedlings (germination), which are used for various metabolic activities (Salas et al. 2018); furthermore, the conditions of the extrusion process (high temperatures and speeds, cutting strength and humidity) can cause the formation of lipid complexes (Félix et al. 2021).

On the other hand, the application of both processes increased total dietary fibre (6% vs. 9%). Fibre content changes during germination are associated with increased levels of cellulose, hemicellulose, and polysaccharides (Hübner and Arendt 2013). Gong et al. (2018)

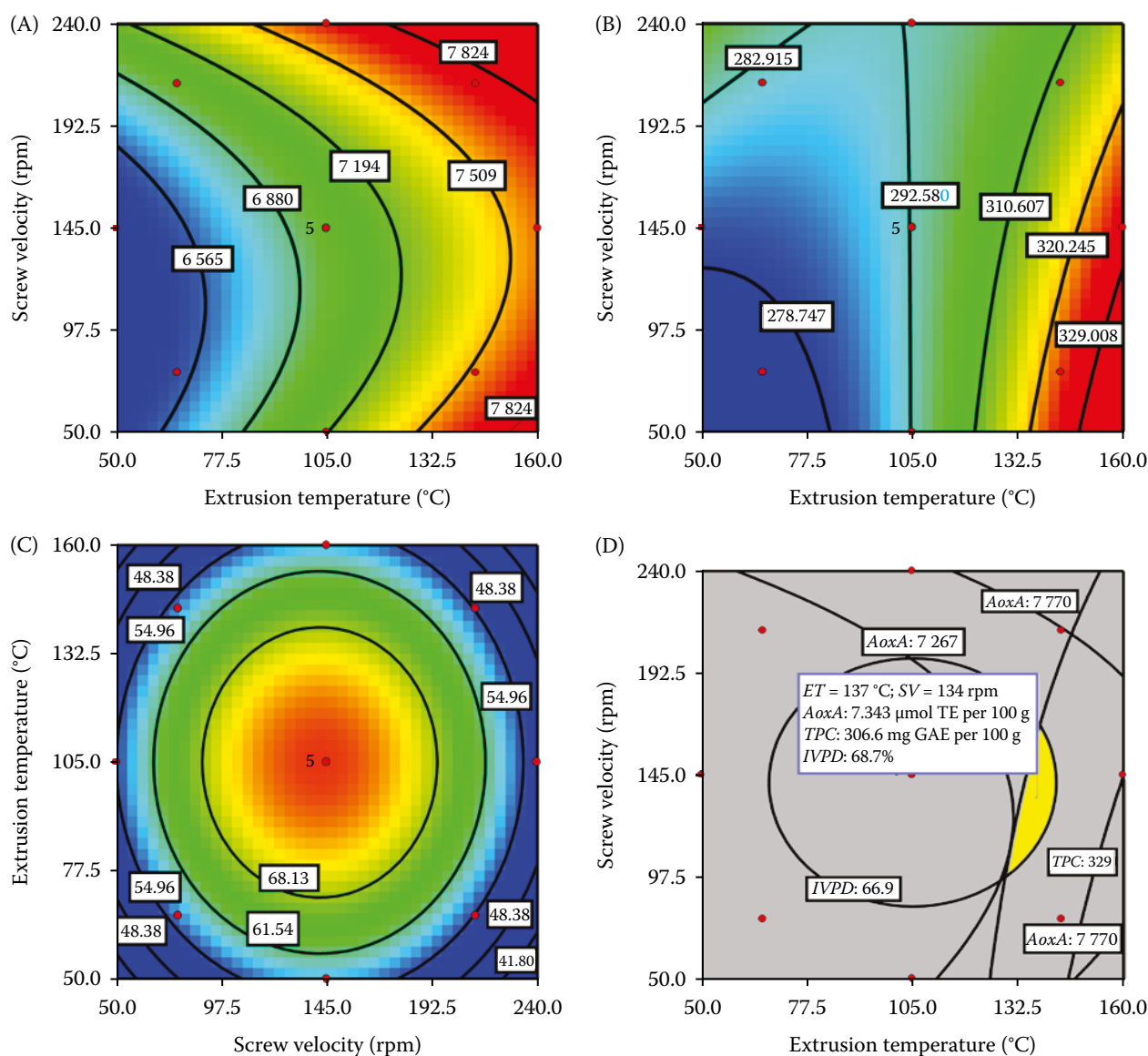


Figure 1. Contour plots showing the effect of extrusion temperature and screw velocity – (A) antioxidant activity, (B) total phenolic compounds, and (C) *in vitro* protein digestibility; (D) region of the optimal combination of process variables

ET – extrusion temperature; SV – screw velocity; AoxA – antioxidant activity; TPC – total phenolic compounds; IVPD – *in vitro* protein digestibility; rpm – revolutions per minute; TE – Trolox equivalents; GAE – gallic acid equivalents

reported similar results when subjecting whole corn grains to germination-extrusion, observing increases in the content of soluble dietary fibre.

IVPD of sorghum flour increased (58% vs. 72%) after germination-extrusion; this is attributed to the reduction of antinutritional factors due to both processes (Albarracín et al. 2015) and the denaturalisation of proteins due to extrusion (Félix et al. 2021). Albarracín et al. (2019) studied the effect of germination-extrusion processes on protein digestibility in rice grains; observed an increase when applying the germination

bioprocess and a decrease when applying the extrusion process.

**Phenolic content and antioxidant activity (AoxA) of sorghum flours.** The germination increased the TPC contents in sorghum (Table 2). Xu et al. (2020) reported that germination begins with the hydration of seeds, increasing respiratory activity and the novo synthesis of gibberellic acids in the germ; functioning as a molecular signal that causes the production and secretion of enzymes in the endosperm that degrade macromolecules, which participate in the respira-

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Table 2. Antioxidant activity, total phenolic content and hypoglycemic potentials of sorghum flours

Property	USF	OGSF	OEGSF
<b>Antioxidant activity ABTS<sup>c</sup></b>			
Free phytochemicals	2 070 ± 46 <sup>C</sup>	5 330 ± 108 <sup>A</sup>	4 196 ± 117 <sup>B</sup>
Bound phytochemicals	2 202 ± 175 <sup>B</sup>	3 080 ± 160 <sup>A</sup>	3 320 ± 219 <sup>A</sup>
Total phytochemicals	4 273 ± 129 <sup>C</sup>	8 410 ± 256 <sup>A</sup>	7 517 ± 274 <sup>B</sup>
<b>Antioxidant activity DPPH<sup>c</sup></b>			
Free phytochemicals	355.03 ± 19 <sup>C</sup>	710 ± 71 <sup>A</sup>	604 ± 24 <sup>B</sup>
Bound phytochemicals	403.28 ± 6 <sup>C</sup>	668 ± 54 <sup>A</sup>	601 ± 21 <sup>B</sup>
Total phytochemicals	758.31 ± 15 <sup>C</sup>	1 378 ± 108 <sup>A</sup>	1 204 ± 26 <sup>B</sup>
<b>Phenolic content<sup>d</sup></b>			
Free phenolics	109.56 ± 2 <sup>B</sup>	136 ± 3 <sup>A</sup>	140 ± 1 <sup>A</sup>
Bound phenolics	130.85 ± 3 <sup>B</sup>	168 ± 2 <sup>A</sup>	169 ± 7 <sup>A</sup>
Total phenolics	240.42 ± 2 <sup>B</sup>	304 ± 4 <sup>A</sup>	310 ± 2 <sup>A</sup>
<b>Hypoglycemic potential (IC<sub>50</sub>)<sup>e</sup></b>			
α-amylase inhibition	free phenolics	223.10 ± 1 <sup>A</sup>	84 ± 1 <sup>C</sup>
	bound phenolics	ND	47 ± 1 <sup>A</sup>
α-glucosidase inhibition	free phenolics	ND	138 ± 1 <sup>B</sup>
	bound phenolics	ND	262 ± 1 <sup>A</sup>

<sup>A–C</sup>Means with different superscripts in the same row are different (Tukey,  $P \leq 0.05$ ); <sup>c</sup>μmol Trolox equivalents (TE) per 100 g sample (DW, dry weight); <sup>d</sup>mg gallic acid equivalents (GAE) per 100 g sample (DW); <sup>e</sup>mg extract per mL; data are expressed as means ± standard deviation; ABTS – 2,2'-azino-bis(3-ethylbenzothiazoline-6-sulfonic acid);  $n = 3$  replicates; DPPH – 2,2-diphenyl-1-picrylhydrazyl; IC<sub>50</sub> – half maximal inhibitory concentration; USF – unprocessed sorghum flour; OGSF – optimised germinated sorghum flour; OEGSF – optimised extruded germinated sorghum flour; ND – not detected

tion and synthesis of phenolic compounds. However, extrusion did not significantly modify the *TPC* content in germinated sorghum. Albarracín et al. (2019) reported a significant improvement in the content of *TPC* during the germination of brown rice; when applying the extrusion process, they did not obtain significant changes in the content of bound phenolic compounds. Different trends have been observed in the content of phenolic compounds due to the effect of the extrusion process: decreases, increases and, sometimes, without significant changes (Albarracín et al. 2015; Gong et al. 2018).

In general, the *AoxA* of sorghum seeds increased after germination for phytochemicals (Table 2). When the OGSF was extruded, a decrease in *AoxA* values was observed. However, the *AoxA* values of extruded germinated grain are higher than those of unprocessed grain. Albarracín et al. (2015) reported that brown rice germination increased its *AoxA*, evaluated by ABTS, by 30% and that extrusion, applied immediately, caused another increase in *AoxA*. This increase has been related with other compounds that are formed during thermal processes, such as the Maillard reaction prod-

ucts (Chavarín et al. 2019). However, in this research, colourimetric methods were used to quantify the content of *TPC* and *AoxA*; therefore, there are compounds and reactions between reagents that generate colour and interfere with the measurement.

**Hypoglycemic potential (IC<sub>50</sub>) of sorghum flour.** Table 2 shows the hypoglycemic potential values evaluated in phenolic extracts of sorghum flour. Phenolic extracts from OGSF had better hypoglycemic potential (lower IC<sub>50</sub>) than those from OEGSF for free phenols; in the bound phenolic extracts the IC<sub>50</sub> values for α-amylase were similar for OGSF and OEGSF, meanwhile for α-glucosidase the IC<sub>50</sub> of the phenolic extracts of OEGSF was better than OGSF.

The improvement in the hypoglycemic potential when applying the germination process may be due to the synthesis of new compounds, as well as the transformation of already existing compounds, presenting a correlation between antioxidant activity and inhibitors of α-amylase and α-glucosidase (Gong et al. 2018). Regarding the extrusion process, an improvement in the hypoglycemic potential can be attributed to the release of phenolic compounds and

the formation of Maillard reaction products with hypoglycemic potential due to the conditions of the extrusion process. Phenolic compounds can modify the enzymatic activity of  $\alpha$ -amylase by binding to its reactive site. Phenolic compounds can bind to the enzyme  $\alpha$ -amylase through hydrogen bonds through the galloyl and hydroxyl groups and the polar groups of the enzyme. In this way, phenolic compounds can modify the rate of reaction of the enzyme  $\alpha$ -amylase and affect its hypoglycemic effect (León et al. 2021).

Synthetic inhibitors such as acarbose and miglitol are currently used to suppress the enzyme  $\alpha$ -glucosidase; however, the consumption of these drugs generates side effects such as diarrhea, abdominal pain; for this reason, there is an interest in natural sources of enzyme inhibitors of  $\alpha$ -amylase and  $\alpha$ -glucosidase enzymes (Oyedemi et al. 2017).

**Microbiological stability of sorghum flour.** The germination bioprocess increased ( $P < 0.05$ ) the microbial load compared to unprocessed sorghum. All the samples registered values outside the sanitary specification (NOM 147-SSA1-1994) (Figure 2). This increase may be due to the conditions of the germination process (humidity, temperature, pH) that favour the growth of bacteria inside or outside the seed (Keller et al. 2018).

*Staphylococcus aureus*, *Escherichia coli*, and *Salmonella* were not detected in the unprocessed and opti-

mised germinated sorghum flours (data not shown). Cava et al. (2009) obtained similar results when counting aerobic bacteria, total coliforms, *E. coli*, yeasts, and molds in germinated and non-germinated seeds of two varieties of beans; they also investigated the presence of *Salmonella*, *Listeria monocytogenes*, and *E. coli*; concluding that germination significantly increased the population of aerobic mesophiles, total coliforms, molds, and yeasts of white and black varieties of beans concerning non-germinated seeds. However, the presence of *Salmonella*, *L. monocytogenes*, or *E. coli* was not detected in the germinated seeds of beans.

The extrusion process, applied in combination, caused a reduction of microorganisms ( $P > 0.05$ ) compared to the OGsf. These values comply with the maximum limit allowed by the NOM-147-SSA1-1996. This reduction of microorganisms is related to their inactivation or elimination due to the thermal treatment used in the extrusion process (Anderson et al. 2017). Likewise, in OEGsf the presence of *S. aureus*, *E. coli*, and *Salmonella* was not detected. Ohtsubo et al. (2005) observed similar results when studying the effect of applying processes (germination-extrusion) in rice on *E. coli*; obtaining values of  $3.8 \times 10^3$  CFU·g<sup>-1</sup> for unprocessed rice,  $9.6 \times 10^5$  CFU·g<sup>-1</sup> corresponding to germinated rice and absence of colonies ( $< 10$  CFU·g<sup>-1</sup>) in extruded germinated rice (CFU – colony-forming unit).

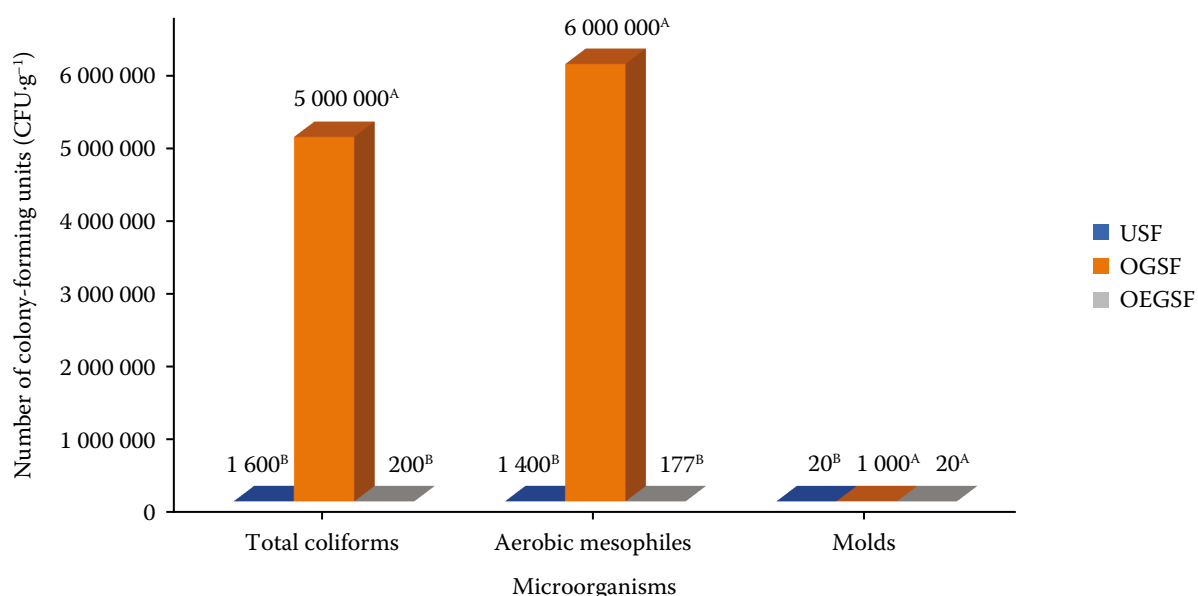


Figure 2. Total coliforms, aerobic mesophiles, and molds in sorghum flours

<sup>A,B</sup> means with different letters for the same type of microorganism present statistical differences (Tukey,  $P \leq 0.05$ ); data are expressed as means;  $n = 4$  replicates; USF – unprocessed sorghum flour; OGsf – optimised germinated sorghum flour; OEGsf – optimised extruded germinated sorghum flour



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## CONCLUSION

The best combination of extrusion process variables, *ET/SV* for producing OEGSF with maximum values of antioxidant activity, total phenolic content, and *in vitro* protein digestibility was *ET* = 137 °C and *SV* = 134 rpm. The combination of optimised processes (germination-extrusion) is an effective strategy to improve protein digestibility, phenolic compound content, and hypoglycemic potential. In addition, it increased the antioxidant activity and decreased the total microbial count. OEGSF could be used as a natural source of dietary fibre, protein, and antioxidants in the development of new functional foods and beverages. The results of this research can serve to promote the use of sorghum for human consumption, given that currently its mayor use is as fodder for livestock.

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