



# Impact on antioxidant activity of including grape peel flour in a novel sorghum-based extruded food

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## Abstract

The aim of this study was to evaluate the effect of combining grape (*Vitis vinifera* L.) peel flour (GPF) and the colored sorghum BR305 (brown) flour (CSF) by extrusion and the consequent impact on antioxidants in the final product. The physical properties and bioactive compounds of both raw GPF and blended GPF/CSF extruded materials were investigated. The results before extrusion of raw materials, the antioxidants by ORAC the CSF was  $89.111 \pm 0.25$ ; GPF before drying  $321.033 \pm 0.21$ ; GPF flour after drying was  $311.022 \pm 0.30 \mu\text{mol Trolox.g}^{-1}$ , respectively. Considering the different treatments (10, 15 and 20% of GPF), the highest values of the compounds obtained: Antioxidant activity, ORAC, was  $37.889 \pm 0.32 (\mu\text{mol Trolox.g}^{-1})$ ; with 15% of GPF, at 120 °C and 17% moisture processing. ABTS,  $12.222 \pm 0.14 (\mu\text{mol Trolox.g}^{-1})$ ; with 20% GPF at 140 °C and 19% moisture processing as best processing conditions. Anthocyanins  $138.31 \pm 0.11 (\text{mg cyanidin-3-glucoside } 100 \text{ g}^{-1})$ ; Total phenolic  $307.95 \pm 0.11 (\text{mg catechin.}100 \text{ g}^{-1})$ . The water absorption index was  $38.99 \pm 0.19, \text{ g of gel.}(\text{g dry matter})^{-1}$  perhaps are sufficient for the preparation of beverages for breakfast or in porridge in order to contribute to the health of the consumer.

**Keywords:** colored sorghum; grape peel flour; extrusion-cooking; antioxidants; pre-cooked flours.

**Practical Application:** Mixed flour that can be used as an input in the preparation of other foods. Alternative for use in preparing gluten-free foods. Depending on the degree of processing, it can be used in the porridges preparation or drinks in general.

## 1 Introduction

Sorghum is a versatile grain generally consumed in Asian and African countries but which is gaining interest in the United States due to its gluten-free and bioactive compound-enriched health benefits (Davis et al., 2019). In Brazil, the predicted production in 2021 is nearly 25.9454.000 tons (Companhia Nacional de Abastecimento, 2020). Therefore, colored sorghum is utilized in many gluten-free products, as it has good acceptability and an attractive neutral taste and color (Di Cairano et al., 2018).

Mixing ingredients can not only be an advantage but also a unique way to make feasible the consumption of certain powder products such as grape peel flour; each ingredient can contribute to improving the sensory characteristics of the final product, making the product appealing (Di Cairano et al., 2018).

There are several reasons why the use of agro-industrial by-products is interesting. First, due to the conditions of use, it can contribute to a reduction in environmental pollution; second, many by-products contain significant amounts of nutrients, some having a good protein content, or being a good source of fiber, bioactive compounds, other mineral profiles, etc. The third point is that value is added, not only from an economic point of view but also as a health aggregator, which in some cases is greater than the raw material itself (Di Cairano et al., 2018; Matejová et al., 2016).

Grapes (*Vitis* spp.) are one of the most consumed fruits in the world. The winemaking industry generates a lot of waste like peel, seeds, and stems. This waste is called grape pomace and has an economic and environmental impact (Fontana et al., 2013). It contains many bioactive compounds and can be used in added-value products (Sánchez-Tena et al., 2013).

Today, food extrusion technology is one of the most important ways of transforming a wide variety of food types into ready-to-eat foods, whether for human consumption or as animal feed. This is because in the formulation of these foods it is possible to incorporate different raw materials, of animal, vegetable, or mineral origin. All of this is to satisfy the specific needs of a specific consumer. In addition, according to the characteristics imposed in the process, it is possible to incorporate higher levels of dietary fiber or significant percentages of protein, or simply to improve the characteristics of acceptability and sensorial preferences (Román et al., 2017). This technology is also used to improve food by-products (Offiah et al., 2019) and to develop dietary fiber-enriched foods (Leonard et al., 2020).

A large number of products have been developed using food extrusion technology, mainly due to the possibility of using different raw materials, creating texture properties that are highly acceptable to consumers. The technology also has high productivity, low operating costs, and minimal energy costs. From a nutritional point of view, foods produced by this

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type of processing have greater bioavailability and digestibility. For these reasons, it has become one of the most used tools in the formulation of new products derived from agro-industrial by-products (Brennan et al., 2011).

It is very important to note that it is not feasible to consume many inputs derived from agro-industrial by-products due to their own characteristics, for example grape skin powder, shrimp powder, etc. In this sense, there is a need to associate these raw materials with starchy sources, such as flours from rice, corn, wheat, cassava, potatoes, etc., to be processed later by extrusion. Matejová et al. (2016) added grape pomace to gluten-free biscuits. Alves et al. (2018) extruded passion fruit shell and colored sorghum flour. Other combinations explored are extruded colored sorghum flour and apple pomace (Mehraj et al., 2018); cereal breakfast extrudates with colored sorghum and pomelo rind (Shi et al., 2017); sugar replacement using citrus fiber extracted from orange pulp in wheat-corn extrudates (Pitts et al., 2016); cherry pomace and direct expanded corn starch (Wang et al., 2017); and cassava-soy composite with grape pomace (Oladiran & Emmambux, 2018).

Following the tendency for healthier products, sustainable development, and gluten-free foods, the combination of colored sorghum and grape peel by extrusion cooking is a good alternative to formulate new products based on these extruded flours. The aim of this work was to develop mixed colored sorghum and grape peel flours by extrusion cooking, evaluate the corresponding impact on antioxidant content, and consider the usefulness of the mixture for future possibilities.

## 2 Material and methods

### 2.1 Raw materials

A sorghum genotype with varied pericarp color, BR305 (brown), was produced by Embrapa Milho e Sorgo (Sete Lagoas, Brazil) at the agricultural experimental field located at 19° 28' south latitude, 44° 15' 08" west longitude and an altitude of 32 m. The grape pomace was supplied by Embrapa Semiárido, Petrolina, PE, -Brazil (in the Municipality of Petrolina, PE, 09° 09' south latitude, 40° 22' west longitude and an altitude of 365.5 m). The pomace was submitted to drying oven with air circulation SL102 (SOLAB, São Paulo, Brazil) at 45 ± 0.5 °C and 1.0 m/s of air velocity until reaching constant weight, using stainless steel mesh trays, containing layers of bagasse 0.6 cm high for

48 consecutive hours, reaching constant weight, with a moisture near of 8,00%.

Colored sorghum and grape peel were ground, separately, in a Lab Mill 3600 disc mill (Perten Instruments, model 3600, Kungens Kurva, Sweden). Colored sorghum flour (CSF) and grape peel flour (GPF) with a particle size of less than 450 µm were used.

### 2.2 Extrusion cooking process

A single-screw Brabender 20DN DSE extruder coupled to a module 330 torque rheometer (Duisburg, Germany) was used. The screw speed and feed rate of 2.5 kg.h<sup>-1</sup> were constant throughout the process at a pressure of 8.5 ± 10 MPa. The screw configuration was L/D 1:3 (compression ratio, Figure 1) and included a circular die 3 mm in diameter. The proportion of grape peel flour, barrel temperature, and moisture content were the independent variables described in the experimental design. After extrusion cooking, the extrudates were dried in a forced-air drier (Fabble-Primar, São Paulo, Brazil) at 60 °C for 4 h to obtain a moisture range of 4-7 g.100 g<sup>-1</sup>. In the extrusion assays, flours were ground in a disc mill with a 0.8 mm sieve size. The extruded flours were maintained under refrigeration (5-8 °C) until further analysis.

### 2.3 Experimental design

The experimental design was based on a central composite rotatable design (CCRD) for two levels and three independent variables. The experiment was conducted with 19 runs with 8 factorial points, 6 axial points, and 5 central points. Independent variables were  $X_1$ : proportion of grape peel flour,  $X_2$ : barrel temperature, and  $X_3$ : moisture content; the coded and decoded levels studied are shown in Tables 1 and 2, respectively. The moisture content of the mixed raw materials was calculated in order to equalize the moisture ( $X_3$ ) according to the real values used in the experimental design (Table 1 and 2) using Equation 1.

$$W = \left( \frac{M_f - M_0}{100 - M_f} \right) \times G \quad (1)$$

where  $W$  is the amount of water to be added,  $M_0$  is the initial moisture (%) of the mixture determined by the method of the Association of Official Analytical Chemistry (2010),  $M_f$  is the

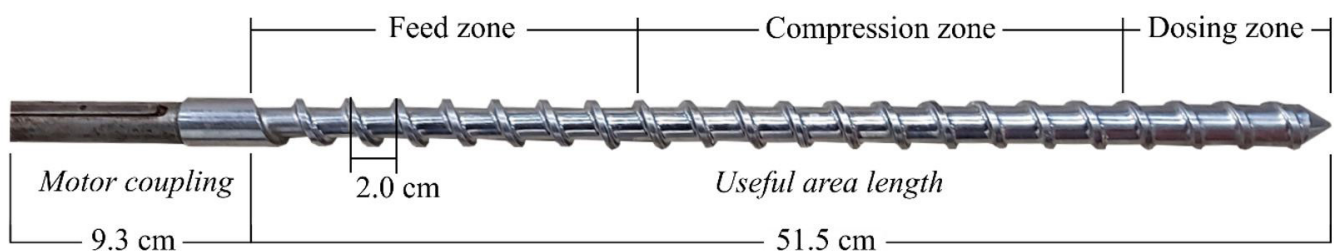


Figure 1. Brabender® extruder screw with 3:1 compression ratio.

**Table 1.** Real values used in experimental design for the production of products extruded from mixtures of colored sorghum and grape peel flours.

Independent variable	Coded level				
	-1.682	-1	0	+1	+1.682
$X_1$ : Proportion of grape peel flour (%) <sup>a</sup>	6.6	10	15	20	26.4
$X_2$ : Barrel temperature (°C)	113.2	120	130	140	146.8
$X_3$ : Moisture content (%) <sup>b</sup>	13.6	15	17	19	20.4

<sup>a</sup>dry basis; <sup>b</sup>wet basis.

**Table 2.** Experimental design and responses for proximate composition, pasting properties, and expansion properties of colored sorghum-grape peel extrudates and raw ingredients.

Run	Experimental design			Proximate composition <sup>a</sup>				Pasting properties <sup>b</sup>		Expansion properties			
	$X_1$	$X_2$	$X_3$	<i>ASH</i>	<i>PRO</i>	<i>LIP</i>	<i>CHO</i>	<i>BDV</i>	<i>STV</i>	$\frac{BD}{\text{kg.m}^{-3}}$	<i>REI</i>	<i>LEI</i>	<i>VEI</i>
1	10	120	15	1.11	6.91	1.99	89.99	301	355	250.02	7.701	0.766	5.899
2	10	120	19	1.33	6.23	1.84	90.60	358.5	377.5	410.01	4.601	0.688	3.165
3	10	140	15	1.26	6.22	1.88	90.64	611	310.5	205.22	7.675	0.799	6.132
4	10	140	19	1.15	6.25	2.22	90.38	501	471	412.01	4.602	0.688	3.166
5	20	120	15	1.88	6.8	2.11	89.21	330	128.5	236.11	6.306	0.898	5.663
6	20	120	19	1.79	5.64	2.6	89.97	478.5	222.5	426.61	3.501	0.779	2.727
7	20	140	15	1.83	5.68	2.95	89.54	396.5	177	236.02	6.205	0.923	5.727
8	20	140	19	1.71	5.53	2.88	89.88	452	211.5	369.25	3.612	0.944	3.41
9	6.6	130	17	1.04	7.52	1.78	89.66	669	320	251.07	6.25	0.821	5.131
10	23.4	130	17	2.1	4.96	2.97	89.97	338.5	170.5	326.01	4.311	0.877	3.781
11	15	113.2	17	1.65	5.25	2.79	90.31	508.5	277.5	309.27	5.79	0.701	4.059
12	15	146.8	17	1.94	4.89	2.98	90.19	381.5	227	266.55	5.289	0.865	4.575
13	15	130	13.6	1.36	5.22	2.76	90.66	318.5	164.5	180.01	6.876	0.917	6.305
14	15	130	20.4	2.39	5.48	2.85	89.28	578.5	345.5	435.33	3.989	0.806	3.215
15	15	130	17	2.77	5.52	2.79	88.92	481.5	225	288.87	5.499	0.788	4.333
16	15	130	17	2.6	5.45	2.96	88.99	429.5	226	256.1	6.081	0.845	5.138
17	15	130	17	2.59	5.28	2.98	89.15	427.5	255.5	248.07	5.201	0.899	4.676
18	15	130	17	2.56	5.09	2.61	89.74	525.5	271	283.2	5.213	0.811	4.228
19	15	130	17	2.68	5.7	2.86	88.76	514.5	249	271.11	5.391	0.844	4.55
Raw materials			<i>MOIST</i>	<i>ASH</i>	<i>PRO</i>	<i>LIP</i>	<i>CHO</i>						
Grape peel flour			8.12	2.55	11.65	4.76	72.92						
Colored sorghum flour			8.17	1.82	9.59	2.17	78.32						

$X_1$  : proportion of grape peel flour;  $X_2$  : barrel temperature;  $X_3 = MOIST$  : moisture content; *ASH* : ash content; *PRO* : protein content; *LIP* : lipid content; *CHO* : carbohydrate content; *BDV* : breakdown viscosity; *STV* : setback viscosity; *BD* : bulk density; *REI* : radial expansion index; *LEI* : longitudinal expansion index; *VEI* : volumetric expansion index. <sup>a</sup>g.100 g<sup>-1</sup> dry basis; <sup>b</sup>mPa or cP.

feed moisture ( $X_3$ , %), fixed according to the levels of Table 1 and 2,  $G$  is the mass of mixture to be moistened.

#### 2.4 Determination of proximate composition

Analysis of moisture, lipids, and ash was based on Association of Official Analytical Chemistry (2010) methods.

The total carbohydrates were obtained by difference, subtracting from 100 the values obtained for moisture, proteins, lipids, and ash (Instituto Adolfo Lutz, 2008). The results of the proximate composition were expressed in g.100 g<sup>-1</sup>.

The total crude fiber content of pre-cooked mixed colored sorghum and grape peel flour was determined according to the enzymatic-gravimetric method (Association of Official Analytical Chemistry, 2010), using a Sigma enzyme kit. This

method is based on the non-hydrolyzed portion of the food that resists sequential enzymatic digestion with  $\alpha$ -amylase, protease, and amyloglucosidase.

#### 2.5 Determination of physical properties

The melt expansion of extruded product is related to its degree of porosity, which affects mechanical and transport properties such as the shear rate and the diffusion of aromas (Włodarczyk-Stasiak & Jamroz, 2009).

##### Pasting properties

Pasting properties were analyzed using a Rapid Visco Analyser (RVA Super-4 model, Newport Scientific Pvt. Ltd, Australia). The particle sizes of the samples used were between

125 and 250  $\mu\text{m}$ . The samples (3.0 g in 25 g of water) were corrected for moisture (14%), adding water to achieve a total weight of 28 g. The pasting profile was held at 25 °C for 2 min and heated to 95 °C. It was stabilized at this temperature for 3 min and then cooled to 25 °C. The test was performed in 20 min. The initial viscosity, maximum viscosity, final viscosity, and setback viscosity were used to evaluate the cooking degree of each sample and were expressed in Pascal-seconds (Pa.s).

#### Expansion properties

The radial expansion index ( $REI$ ), longitudinal expansion index ( $LEI$ ), and volumetric expansion index ( $VEI$ ) of the extruded snack products were determined for each treatment with the aid of a digital caliper (ZAAS Precision, Curitiba, Brazil). To determine the  $EI$  (Equation 2), the diameter at the beginning, middle, and end of each extrudate was measured, to obtain the average diameter ( $D$ ).

$LEI$  and  $VEI$  were calculated using Equations 3 and 4, respectively.

$$REI = \left( \frac{D}{D_0} \right)^2 \quad (2)$$

$$LEI = \left( \frac{\rho_d}{BD} \right) \left( \frac{1}{REI} \right) \left( \frac{1 - M_d}{1 - M_e} \right) \quad (3)$$

$$VEI = REI \times LEI \quad (4)$$

where  $\rho_d$  is the density of the molten product inside the extruder before it leaves the die, considered to be 1400  $\text{kg}\cdot\text{m}^{-3}$  (density of starch);  $BD$  is the density of the extruded product;  $M_d$  is the moisture content of the wet mass of the molten product inside the extruder; and  $M_e$  is the moisture content of the extruded product, as described by Alvarez-Martinez et al. (1988).

The bulk density ( $BD$ ), in  $\text{kg}\cdot\text{m}^{-3}$ , was calculated according to Fan et al. (1996), using Equation 5.

$$BD = \frac{4M}{\pi D^2 L} \quad (5)$$

where  $M$  is the mass, in g;  $L$  is the length of extrudate, in m; and  $D$  is the extruder diameter, in m. The analysis was performed with 15 replicates of each treatment.

#### Water solubility and water absorption indices

The water solubility index ( $WSI$ ) and water absorption index ( $WAI$ ) of the samples were determined in quadruplicate as described by Anderson et al. (1969).  $WSI$  is the sample mass in the supernatant divided by sample mass, and  $WAI$  is the sample mass with absorbed water divided by the sample mass. The  $WAI$  was calculated using Equation 6, while the  $WSI$  was calculated using Equation 7, modified by Doğan & Karwe (2003). The results are expressed in g of gel.(g dry matter)<sup>-1</sup> and percentage, respectively.

$$WAI = \frac{WRC}{W_s - WRE} \quad \text{g of gel. (g dry matter)}^{-1} \quad (6)$$

$$WSI = \frac{WRE}{W_s} \quad (\%) \quad (7)$$

where  $WRC$  is the weight of the centrifuge residue, in g;  $W_s$  is the sample weight, in g; and  $WRE$  is the weight of the evaporation residue, in g.

#### 2.6 Determination of antioxidant capacity and phenolic compounds in grape skin flour and extruded products

Antioxidant capacity was determined using removal of the peroxy radical (ORAC – oxygen radical absorbance capacity) and the ability to remove the organic radical ABTS (2,2'-azino-bis(3-ethylbenzothiazoline-6-sulfonic acid)). The total phenolic compounds were determined using the Folin-Ciocalteu assay, which is the most widely used rapid reaction procedure for the quantification of phenolic compounds in plants. The complete analytical procedure was performed as previously described by Georgé et al. (2011).

**Extract Preparation:** To determine the antioxidant capacity, an extract was used for the ABTS and ORAC analysis. For grape skin flour, 0.1 g was weighed, and for extruded mixed flours, 0.5 g was put, in triplicate, into centrifuge tubes; 10 mL of 50% methanol was added to the tubes, then the mixture was homogenized in a vortex mixer (Genie 2 Scientific Industries, Bohemia, NY, USA) and left to stand for 60 min at room temperature and protected from light. The tubes were then centrifuged in a Universal 320R centrifuge (Hettich, Tuttingen, Germany) at 2000 rpm for 15 min and the supernatant content was transferred to 25 mL amber volumetric flasks; 10 mL of 70% acetone was added to the residues from the first extraction, homogenized, and left to stand for 60 min at room temperature and protected from light. The tubes were centrifuged again (under the same conditions) and the supernatant content was collected and next to the supernatant from the first centrifugation. The balloons were filled with distilled water.

The extracts were then transferred to Eppendorf tubes, frozen at -10 °C, and kept away from light to be used to analyze antioxidant capacity.

#### ABTS method

The antioxidant capacity equivalent to Trolox was estimated according to the procedure proposed by Re et al. (1999), with some modifications. The ABTS•+ radical was prepared from the reaction of 7 mM ABTS aqueous solution with 140 mM potassium persulfate, leaving the mixture at room temperature for 16 h, in the absence of light. Soon the ABTS solution was diluted with ethanol, in order to obtain a level of absorbance of  $0.70 \pm 0.05$  at 734 nm. Aliquots of 30  $\mu\text{L}$  of the samples were added to 3 mL of the diluted ABTS solution, and the absorbance of the mixture was recorded after 6 min. The antioxidant capacity was calculated using a standard Trolox curve (100 to 2000  $\mu\text{M}$ ) and respective percentage inhibition, and the test results were expressed in  $\mu\text{mol}$  of equivalent Trolox per gram of fresh weight ( $\mu\text{mol TE}\cdot\text{g}^{-1}$  PF).

### ORAC method

ORAC was analyzed as proposed by Dávalos et al. (2004). In microplates, aliquots of 25  $\mu\text{L}$  of the extracts were mixed with 150  $\mu\text{L}$  of the fluorescein solution (40 nM) and incubated at 37  $^{\circ}\text{C}$  for 30 min, before adding 25  $\mu\text{L}$  of the AAPH solution (153 nM). All reagents were prepared in phosphate buffer (75 mM, pH 7.1). The fluorescence intensity (excitation at 485 nm and emission at 525 nm) was monitored every minute, for 60 min, in a Sinergy Mx microplate reader (BioTeK, Winooski, VT, USA). The standard curve was prepared with Trolox solution (6.25 to 100 mM), and the results were expressed in  $\mu\text{mol}$  equivalent of Trolox per gram of fresh weight ( $\mu\text{mol}$  Trolox. $\text{g}^{-1}$  PF).

### Determination of total phenolic compounds

Quantification of the total phenolic content of the extracts and products was carried out as recommended by Georé et al. (2005). The reading was performed at 720 nm, after reduction of the reagent by the phenolic compounds. The results were expressed in mg of catechin per 100 g of grape skin flour and in the extruded product, in order to evaluate the effect of extrusion on the content of total phenolic compounds.

### Determination of total anthocyanins

pH difference methodology was used to determine total anthocyanins in the extracts, according to Lee et al. (2005). Two buffer solutions were made, one of potassium chloride/hydrochloric acid of pH 1.0 (0.025 M), another of sodium acetate/hydrochloric acid of pH 4.5 (0.4 M). The samples were diluted in these buffer solutions, and the concentration of the sample at pH 1.0 showed a reading between 0.2 and 1.4 AU, as it is the linearity range of the spectrophotometer. Readings were taken at 520 nm and 700 nm, in both pH 1.0 and pH 4.5 buffer. The 700 nm reading was performed to discount the sample turbidity. The final absorbance ( $A$ ) value was calculated using Equation 8.

$$A = (A_{520\text{nm}} - A_{700\text{nm}})_{\text{pH} 1.0} - (A_{520\text{nm}} - A_{700\text{nm}})_{\text{pH} 4.5} \quad (8)$$

The total concentration of monomeric anthocyanins was expressed in terms of cyanidin-3-glucoside, according to Equation 9.

$$MA = A \times MW \times DF \times \frac{100}{\epsilon - 1} \quad (9)$$

where  $MA$  corresponds to monomeric anthocyanins, in  $\text{mg} \cdot 100 \text{g}^{-1}$ ;  $A$  corresponds to absorbance;  $MW$  is the molecular weight;  $DF$  is the dilution factor; and  $\epsilon$  corresponds to molar absorptivity.

The determination was carried out on the grape skin flour sample and on the extruded flours, in order to evaluate the effect of extrusion on the total anthocyanin content.

### 2.7 Statistical analysis

The responses of the extrudates (Table 2) obtained as results of the rotating central composite design  $2^3$  were subjected to a second order polynomial regression analysis shown in the Equation 10:

$$\hat{Y} = \beta_0 + \sum_{i=1} \beta_i X_i + \sum_{i=1} \beta_i X_i^2 + \sum_{i=1} \sum_{j=i} \beta_{ij} X_i X_j \quad (10)$$

where  $\hat{Y}$  is the predict response (proximal composition, paste properties, and expansion properties);  $X_i$ ,  $X_i^2$ , and  $X_i X_j$  are the linear, quadratic, and interaction effects, respectively, of the factors that influence the response ( $\hat{Y}$ ); and  $\beta_0$ ,  $\beta_i$ , and  $\beta_{ij}$  are the coefficients of the model to be determined. Analysis of variance (ANOVA) test was carried out using the Statistica software version 12.0 (StatSoft, Tulsa, USA) with 5% of significance.

## 3 Results and discussion

### 3.1 Proximate composition

The proximate composition (in  $\text{g} \cdot 100 \text{g}^{-1}$  on a dry matter basis) of colored sorghum flour (CSF) and grape peel flour (GPF) and the different tests provided for in the experimental design are shown in Table 2. GPF showed a high fiber content and ash content although the physical and chemical properties of grapes vary according to the climate, soil, variety, and cultivar (Osorio & Silveira, 2013). As an example, the mineral composition may vary according to edaphological conditions, climatic factors, and the use of fertilizers and herbicides among other factors (Bampi et al., 2010). The protein content of the grape depends on the cultivar and its proteins are present mainly in the grape pulp. Crushing of grapes by applying pressure, depending on the intensity can lead to a decrease in the content of soluble proteins in the GPF. At the end of the fermentation process, many proteins precipitate with tannins, mainly in the making of red wine (Jackson, 2020). The data according to the experimental design, with regard to mixed colored sorghum extrudates containing 15%, 20%, and 25% GPF, are presented in Table 2.

The protein content varied between the different treatments from 4.89 to 7.52  $\text{g} \cdot 100 \text{g}^{-1}$  of sample (d.b). These values are reasonable considering the composition of the mixtures. The lipid content varied from 1.43 to 2.59  $\text{g} \cdot 100 \text{g}^{-1}$ . This component is mainly associated with the seeds and, as the flour is made from the grape skin, there may be some remaining seed, presenting a value closer to that for the skin fraction. The lipid content of the grape skin fraction in this study is included among the values found by Romero et al. (2013), which was 4.76  $\text{g} \cdot 100 \text{g}^{-1}$ .

Carbohydrates were the most abundant components in pre-cooked mixed CSF and GPF flour. Although the protein and lipid content is close to that of cereals in general, the mixture has an interesting contribution of nutrients in its consumption.

### 3.2 Physical properties

The pasting properties determined in the RVA indicate significant degrees of conversion after extrusion. Table 3 shows the results of the different treatments for pasting properties, bulk density,  $REI$ ,  $LEI$ , and  $VEI$  for the different tests described in the experimental design. In general, the addition of material containing a significant amount of fiber to the formulations causes lower expansion values. Significant number of train jobs demonstrated this effect; consequently, as GPF is added, lower expansion values are observed. This is because the links are

**Table 3.** Regression coefficients (in coded levels) of adjusted models for proximate composition, pasting properties, and expansion properties of colored sorghum-grape peel extrudates using independent variables: proportion of grape peel flour ( $X_1$ ), barrel temperature ( $X_2$ ), and moisture content ( $X_3$ ).

Coeff.	Proximate composition				Pasting properties		Expansion properties			
	<i>ASH</i>	<i>PRO</i>	<i>LIP</i>	<i>CHO</i>	<i>BDV</i>	<i>STV</i>	<i>BD</i>	<i>REI</i>	<i>LEI</i>	<i>VEI</i>
$\beta_0$	2.65**	5.39**	2.85**	89.11**	452.71**	262.37**	286.95**	5.48**	0.84**	4.52**
$\beta_1$	0.3**	-0.46**	0.34**	-	-49.08*	-75.12**	-	-0.6**	0.05*	-0.23 <sup>ns</sup>
$\beta_{11}$	-0.41**	0.42**	-0.24**	-	-	-	-	-	-	-
$\beta_2$	-	-0.18*	0.13*	-	20.08 <sup>ns</sup>	-	-12.6 <sup>ns</sup>	-	0.04*	-
$\beta_{22}$	-0.33**	-	-	0.39*	-	-	-	-	-0.02 <sup>ns</sup>	-
$\beta_3$	0.09*	-0.11 <sup>ns</sup>	-	-	43.11*	45.1**	82**	-1.2**	-0.03*	-1.18**
$\beta_{33}$	-0.34**	0.11 <sup>ns</sup>	-	0.29*	-	-	15.28*	-	-	-
$\beta_{12}$	-	-	-	-	-51.56*	-	-	-	-	-
$\beta_{13}$	-	-	-	-	-	-	-	-	-	-
$\beta_{23}$	-	0.22 <sup>ns</sup>	-	-	-	-	-	-	-	-
<i>LoF</i>	0.052 <sup>ns</sup>	0.053 <sup>ns</sup>	0.108 <sup>ns</sup>	0.174 <sup>ns</sup>	0.077 <sup>ns</sup>	0.067 <sup>ns</sup>	0.163 <sup>ns</sup>	0.581 <sup>ns</sup>	0.415 <sup>ns</sup>	0.525
$R^2$	0.931	0.7	0.711	0.614	0.446	0.808	0.902	0.925	0.718	0.903

*ASH* : ash content; *PRO* : protein content; *LIP* : lipid content; *CHO* : carbohydrate content; *BDV* : breakdown viscosity; *STV* : setback viscosity; *BD* : bulk density; *REI* : radial expansion index; *LEI* : longitudinal expansion index; *VEI* : volumetric expansion index; \*\*significant at  $p < 0.01$ . \*significant at  $p < 0.05$ ; <sup>ns</sup>: not significant.

increasingly smaller, due to the blocking of cellulose structures in the formation of hydrogen bridges. Figure 2 shows the response surface plots for expansion properties: (a) *BD* ( $\text{kg}\cdot\text{m}^{-3}$ ); (b) *REI*; (c) *LEI*; and (d) *VEI*. All three of the independent variables had a significant effect on the expansion properties at  $p < 0.01$  (Table 3); this can be seen in Figure 2a in which the apparent density is plotted with the process temperature and moisture content. It is evident that as the moisture content increases, there is an increase in pellet density. The reverse phenomenon occurs with a decrease in temperature, but with less impact.

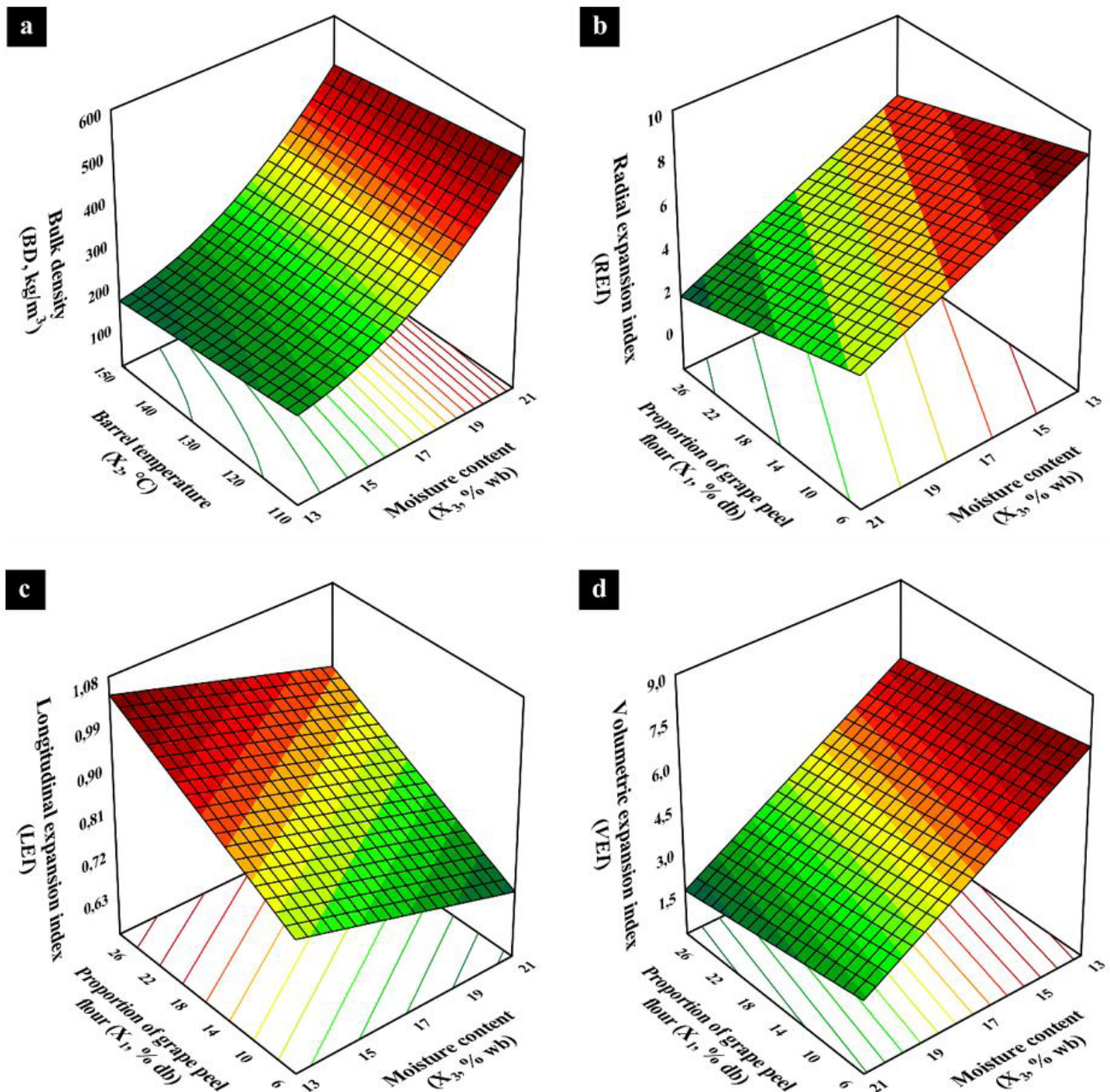
On the other hand, as shown in Figure 2b, the plot of *REI* plot versus the proportion of GPF and moisture content, both variables have a significant effect on radial expansion. Figure 2c, the plot of *LEI* versus the proportion of GPF and moisture content, shows a linear inverse behavior, that is, the lower the moisture content, the higher the value of *LEI* and the lower the content of GPF more than *LEI*. An equivalent phenomenon is shown in Figure 2d, with *VEI* plotted against the proportion of GPF and moisture content. The starch content of sorghum flour is sufficient to cause high expansion values when extruded without any other ingredient. Thus, the levels of GPF substitution used (10%, 15%, and 20%) were sufficient to observe how, as the levels of expansion increase, they also decrease significantly.

Due to the interaction of GPF, mainly with sorghum starch carbohydrates, in the melting and expanded formation of a single product, it is understandable that there is a decrease in the level of expansion, considering the high levels of fiber that

grape peel contains. A similar work by Diez-Sánchez et al. (2019), used blackcurrant pomace rich in polyphenols and dietary fiber, the extrusion treatment of the flour caused the disruption of the starch granules, and affected starch characteristics such as water binding capacity and swelling, this causes the expansion properties in general to be affected due to the fiber content contained in the study material.

In extruded mixed flours, there was a considerable reduction in viscosity values throughout the profile. In the first phase of the viscosity test, a certain water absorption capacity was observed, being characteristic of starch processed by thermoplastic extrusion and which has undergone shearing. The mixture with the highest moisture content, 20.4%, showed a sharp viscosity peak in the temperature increase phase, which may indicate the presence of starch with a certain molecular integrity capable of swelling with the temperature increase, typical of crude starch. According to Khanal et al. (2009), extrusion processing can be used to increase the procyanidin monomer and dimer content in grape seeds and pomace. Table 3 shows the regression coefficients of adjusted models for the proximate composition, pasting properties, and expansion properties of colored sorghum-grape peel extrudates using the independent variables proportion of GPF ( $X_1$ ), barrel temperature ( $X_2$ ), and moisture content ( $X_3$ ), showing that in most events they had a significant effect on the physical properties (pasting and expansion properties).

It is important to emphasize that, the data recorded in Table 3, referring to expansion and density values, pasting properties



**Figure 2.** Response surface plots for expansion properties: (a) bulk density ( $BD$ ,  $\text{kg}\cdot\text{m}^{-3}$ ); (b) radial expansion index ( $REI$ ); (c) longitudinal expansion index ( $LEI$ ); (d) volumetric expansion index ( $VEI$ ).

allow the possibility of use for a given product, for example, tests with a high degree of expansion, can be used for beverage formulation, that of low expansion, can be used to compose porridge formulations, or use them in pastes or bakery products.

### 3.3 Antioxidant capacity and phenolics in grape skin flour and extruded products

Table 4 shows the results for antioxidant capacity determined by the ORAC and ABTS methods, anthocyanin content, and total phenolic content of pre-cooked mixtures of CSF and GFP,

water absorption, and solubility index. The antioxidant values for GFP are considered high so that when fused by the extrusion process with CSF, the product obtained has sufficient quality of antioxidants compared to the available food supplement flours (Shi et al., 2017). Procyanidins in grape by-products have many health benefits, but most are present as large molecular weight compounds, which are poorly absorbed. Extrusion processing appears to be a promising technology to increase the levels of bioactive low molecular weight procyanidins (Khanal et al., 2009). According to this premise, it is considered that during the

**Table 4.** Antioxidant capacity determined by ORAC and ABTS methods, anthocyanin content, total phenolic content, water absorption, and solubility indices of pre-cooked blended colored sorghum and grape peel flours.

Run	Antioxidant activity		Phenolics		Water absorption and solubility indices	
	ORAC	ABTS	Anthocyanins	Total phenolics	WAI	WSI
1	16.040 ± 0.10	4.501 ± 0.11	108.23 ± 0.07	287.66 ± 0.11	18.94 ± 0.12	13.58 ± 0.11
2	28.633 ± 0.12	2.005 ± 0.10	112.43 ± 0.17	297.16 ± 0.16	10.49 ± 0.22	10.41 ± 0.16
3	16.312 ± 0.11	6.991 ± 0.13	109.13 ± 0.03	285.22 ± 0.26	18.45 ± 0.31	12.78 ± 0.21
4	10.715 ± 0.13	5.005 ± 0.10	111.03 ± 0.44	288.19 ± 0.33	15.22 ± 0.05	8.77 ± 0.31
5	29.351 ± 0.21	6.109 ± 0.14	118.53 ± 0.06	298.17 ± 0.13	12.13 ± 0.19	15.16 ± 0.24
6	37.627 ± 0.11	8.990 ± 0.10	122.01 ± 0.12	295.28 ± 0.91	12.15 ± 0.32	10.11 ± 0.21
7	34.570 ± 0.15	11.661 ± 0.11	126.21 ± 0.21	288.19 ± 0.33	20.12 ± 0.51	11.77 ± 0.33
8	32.263 ± 0.10	12.222 ± 0.14	125.90 ± 0.05	281.08 ± 0.72	17.05 ± 0.32	10.05 ± 0.19
9	09.324 ± 0.11	10.433 ± 0.12	95.05 ± 0.03	101.18 ± 0.12	21.56 ± 0.14	13.11 ± 0.25
10	37.122 ± 0.12	10.987 ± 0.10	138.31 ± 0.11	307.95 ± 0.11	19.41 ± 0.24	11.76 ± 0.28
11	24.311 ± 0.15	4.669 ± 0.10	101.27 ± 0.08	277.61 ± 0.22	14.55 ± 0.16	11.94 ± 0.41
12	25.541 ± 0.30	4.644 ± 0.12	112.23 ± 0.11	267.26 ± 0.07	39.89 ± 0.22	11.15 ± 0.42
13	32.335 ± 0.35	6.298 ± 0.10	100.32 ± 0.22	259.61 ± 0.22	38.99 ± 0.19	15.11 ± 0.33
14	18.777 ± 0.32	6.666 ± 0.14	128.21 ± 0.44	285.11 ± 0.04	38.72 ± 0.13	10.14 ± 0.21
15	31.776 ± 0.31	9.276 ± 0.12	110.11 ± 0.22	280.17 ± 0.04	17.88 ± 0.28	11.09 ± 0.41
16	37.889 ± 0.32	10.601 ± 0.16	109.93 ± 0.06	283.99 ± 0.55	16.91 ± 0.13	12.54 ± 0.51
17	33.679 ± 0.28	10.006 ± 0.12	111.88 ± 0.11	281.16 ± 0.07	19.89 ± 0.08	11.33 ± 0.13
18	33.666 ± 0.30	11.127 ± 0.11	112.25 ± 0.05	284.22 ± 0.66	12.93 ± 0.09	10.17 ± 0.41
19	33.176 ± 0.19	9.267 ± 0.16	111.66 ± 0.27	281.99 ± 0.19	16.77 ± 0.14	11.59 ± 0.14
Raw materials	ORAC	ABTS	Anthocyanins	Total phenolics		
Colored sorghum flour	89.111 ± 0.25	32.147 ± 0.19	71.66 ± 0.27	4.09 ± 0.19	-	-
Grape peel flour <sup>a</sup>	321.033 ± 0.21	49.567 ± 0.15	2098.7 ± 0.12	1821.3 ± 0.13	-	-
Grape peel flour <sup>b</sup>	311.022 ± 0.30	44.666 ± 0.10	1822.2 ± 0.02	1669.3 ± 0.03	-	-

ORAC: oxygen radical absorbance capacity ( $\mu\text{mol Trolox}\cdot\text{g}^{-1}$ ); ABTS: 2,2'-azino-bis (3-ethylbenzothiazoline-6-sulfonic acid) ( $\mu\text{mol Trolox}\cdot\text{g}^{-1}$ ); anthocyanins (mg cyanidin-3-glucoside  $100\text{ g}^{-1}$ ); total phenolic compounds (mg catechin. $100\text{ g}^{-1}$ ). <sup>a</sup>before drying; <sup>b</sup>after drying.

extrusion process, mainly during melt rather formation in the fusion of the two ingredients, CSF and GPF, part of the molecular structure could be exposed, so that in the determinations appear. On the other hand, considering that extrusion is an HTSH-type thermal process, its effect on the content of antioxidants is not so drastic, mainly because it takes a very short period, that is, approximately 20 to 30 s, in this sense, Pokorný & Schmidt (2010) commented that extrusion usually occurs at temperatures above 100 °C, but the residence time is very short, therefore the decomposition of antioxidants is relatively small, these authors cited an experiment with wheat flour Saracen, where they were extruded at 120 ± 200 °C. in which they observed that the content of phenolic acids increased by the release of their binding to proteins and the sum of polyphenols decreased. Considering GPF as a raw material before the mixing procedure for the extrusion procedure, at the end of Table 4, the values of antioxidants (ORAC, ABTS, total anthocyanins, and phenolic) were added, to verify the influence of the exposed time the drying of the grape skin at a temperature of 45°C before and after drying. In which there was a decrease in the values of antioxidants, ORAC, and ABTS, of 3.2%, and 10%, respectively, in the case of Total anthocyanins and phenolics, 13.18%, and 8.35%, respectively. As an example of losses in cooking activities, during steaming cabbage cooking losses are about 21 ± 23% in ascorbic acid and total phenols about 10%. The antioxidant activity of Trolox decreased by 5 ± 20% and the phenolic compounds increased the pea antioxidant capacity,

due to the phenol ± protein interaction. Hydroxycinnamic acids (such as ferulic, coumaric, and caffeic acids) were the most active ingredients (Pokorný & Schmidt, 2010). Considering the work of Nayak et al. (2011) who used the extrusion process for a mixture of purple potato and yellow pea flour the losses in the total phenolic (TP) content of the formulations under extrusion are expected to occur, due to breaking down of complex polyphenols to other phenolic or non-phenolic compounds, because of high-temperature conditions. However, the effect of the extrusion die at temperatures, 130 and 140 °C, was not significant ( $p > 0.05$ ) on the TP content of the extrudates. On the other hand, Moreno et al. (2017) corroborate what was described above, in which several studies have shown that extrusion and process conditions affect the phytochemical content and antioxidant activity of cereal grains, showing that important losses can occur in the Bioactive compounds due to thermal effect and chemical changes can occur during extrusion since phenolic compounds are highly dependent on the parameters, moisture content, temperature and residence time in the extrusion system.

In another work, researchers (Neder-Suárez et al., 2021) used a blend of blue corn, black beans and sweet chard by extrusion in the production of a type of third-generation snacks, considering that these ingredients contain phytochemical and polyphenols compounds. They concluded that despite thermal processes, anthocyanin retention was high (29.08 mg of cyanidin-3-glucoside equivalents/100 g) under the optimal process



conditions of 122 °C, 133 rpm, and 25% of moisture content. Screw speed and moisture content had the largest effects on the physical responses, while moisture content had the largest effects on total anthocyanin. The highest expansion index, water absorption and water solubility indexes, and hardness were obtained at high screw speed and low extrusion temperature. At low extrusion temperature and moisture content, the highest total anthocyanin was generated.

Among the possibilities of using GPF, Baldán et al. (2021) used this flour; processed at 75 °C (15 and 25%) improved the nutritional composition of the muffins as their content increases, highlighting protein and crude fiber content. Likewise, these had a good level of acceptability by consumers. Taking into account grape pomace is a by-product discarded by wineries, it has a potential benefit and is feasible to use as an ingredient for gluten-free muffins. It should be considered that these authors used GPF without mixing, and not processed by extrusion. In the case of the present study, however, we have the mixture, in the percentages established in the experimental design (10, 15 and 20% of GPF) with the difference of colored sorghum flour. This condition, with the starch material fused to GPF, can contribute to better performance in the elaboration of products, as the functional characteristics like absorption and water solubility can be modified only with variations in the extruder process parameters and meet specific functional properties.

#### 4 Conclusion

These results indicated that extrusion of sorghum colored and grape peel flours produces acceptable extrudates. Changing process conditions affected the physical and functional properties of produced expanded products. However, this impact is not very drastic, making the products resulting from the CSF/GPF mixture have significant values of antioxidants and phenolic compounds. Under these conditions, this resulting mixture with considerable amounts of antioxidants and phenolic compounds can be used as porridge, food formulations among other alternatives. In any case, the resulting products can contribute to the health of the consumer.

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