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ORIGINAL ARTICLE

Optimization of nutrient retention in whole flours extruded from cowpea biofortified grain

Otimização da retenção de nutrientes em farinhas integrais extrusadas de grãos biofortificados de feijão-caupi

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Abstract

Whole flour of cowpea grains of the cultivar BRS Tumucumaque biofortified in iron and zinc were processed in a co-rotating twin-screw extruder Clextral HT 25. A central rotational composite design 2^3, combining temperature (from 86.4 to 153.6 °C), screw rotation speed (from 163.6 to 836.4 rpm) and moisture content (from 16.6% to 23.4%), was used to assess the effects on flour constituents. The three variables significantly affected ($p < 0.05$) the levels of protein and copper in the extrudates. The reactions of the extrusion process caused a decrease in the levels of proteins and an increase in the levels of copper, zinc and potassium, and these amounts were accentuated as the values of the process variables approached those of the central point region. Extreme conditions at high rotational screw speeds combined with low moisture content reduced the magnesium content, at the opposite end of the values for these two variables, the intensity of the reduction was lower. In the analysis of global desirability, it was found that the extrusion condition at 112.6 °C, 587.4 rpm and 23.4% moisture provided the highest nutrient retention. The levels of iron and zinc remained above 60 and 40 mg kg^{-1}, respectively, in extruded products.

Keywords: *Vigna unguiculata*; Extrusion; Response surface methodology; Chemical composition; Mineral elements; Instant flour.

Resumo

Farinha integral de grãos de feijão-caupi da cultivar BRS Tumucumaque biofortificados em ferro e zinco foi processada em extrusor de rosca dupla corrotativo Clextral HT 25. Um delineamento composto central rotacional 2^3, combinando as variáveis independentes: temperatura (86,4 a 153,6 °C), velocidade de rotação do parafuso (163,6 a 836,4 rpm) e teor de umidade (16,6% a 23,4%), foi utilizado para avaliar os efeitos sobre os constituintes da farinha. As três variáveis afetaram significativamente ($p < 0,05$) os teores de proteína e cobre dos extrusados. As reações do processo de extrusão provocaram diminuição nos teores de proteínas e aumento nos teores de cobre, zinco e potássio, e essas quantidades se acentuaram à medida que os valores das variáveis de processo se aproximaram aos da região do ponto central. Condições extremas de altas velocidades de rotação das roscas combinadas com baixos teores de umidade reduziram o teor de magnésio, enquanto que, no extremo inverso de valores para essas duas variáveis, a intensidade da redução foi menor. Na análise de desejabilidade global, verificou-se que a condição de extrusão a 112,6 °C, 587,4 rpm e 23,4% de umidade proporcionou a maior retenção de nutrientes. Os teores de ferro e zinco permaneceram acima de 60 e 40 mg kg^{-1}, respectivamente, nos produtos extrusados.

Palavras-chave: *Vigna unguiculata*; Extrusão; Metodologia de superfície de resposta; Composição química; Elementos minerais; Farinha instantânea.

1 Introduction

Cowpea (*Vigna unguiculata* (L.) Walp.) is one of the most important sources of protein in the diet of the semi-arid tropics of the planet, as it is a species of wide adaptability, rusticity, tolerance to conditions of high temperatures and water deficits. It is the 5th most cultivated pulse in the world (Food and Agriculture Organizations of the United Nations, 2020). In 2018, Brazil produced around 738 thousand tons of cowpea, or 25% of the total beans (*V. unguiculata* + *Phaseolus vulgaris*) produced in the country (3 million tons) (Empresa Brasileira de Pesquisa Agropecuária, 2020). On the world stage, Brazil ranks 3rd as the world´s largest producer, behind Nigeria (2.6 million tons) and Niger (2.4 million tons), and ahead of Burkina Faso (631 thousand tons) (Food and Agriculture Organizations of the United Nations, 2020).

The cowpea provides nutritious grains, containing on average 25% of proteins (Food and Agriculture Organizations of the United Nations, 2016) of high digestibility compared to other pulses (Afoakwa et al., 2006), in addition to energy, vitamins, soluble fibers and minerals, which assist in reducing cholesterol and control diabetes, thus improving other aspects related to health (Food and Agriculture Organizations of the United Nations, 2016), as well as presenting low cost of production with sustainability. However, it also contains antinutritional compounds, such as trypsin inhibitors, which can be inactivated by adequate heat treatment, as they are thermolabile (Doblado et al., 2007).

Thermoplastic extrusion is one of the processes that exposes raw materials or formulations prepared to high temperatures for a short time, which significantly reduce or eliminate most antinutritional compounds (Batista et al., 2010; Diouf et al., 2019; Choton et al., 2020), and modifies the format or expands the raw material (Akhtar et al., 2015), allowing to obtain a diversity of products in a short processing time (Choton et al., 2020), and has recently been directed for obtaining versions of products with healthy and nutritious characteristics for consumers of all age groups (Jakkanwar et al., 2018). Ready-to-eat products made exclusively from whole cowpea flour are more nutritious and healthier than those obtained from traditional cereal flours, and can be consumed by individuals with restricted gluten intake, such as: celiac, represented by about 1.4% of the population (Singh et al., 2018); those who are sensitive or who choose not to consume gluten, which represent 7 to 14% of the population (Cabrera-Chávez et al., 2017).

However, for some bean cultivars, thermoplastic extrusion, in general, has significantly reduced the protein and starch content (Ai et al., 2016). In a mixture of sorghum and bambara (70:30), the increased extrusion temperature (100 to 130 °C) decreased the protein and lipid content, and maintained or decreased the ash content (Jiddere & Filli, 2016). In the production of snacks based on a mixture of rice, pea and carob flour, no change in protein content was observed, however, it could be noted a reduction in lipid content and an increase in carbohydrate content (Arribas et al., 2017). Mineral elements are unlikely to be lost during water evaporation at the outlet of the matrix (Camire, 1998). However, in some studies, a decrease in the content of some mineral elements has been observed (Anuonye et al., 2010) or an increase or decrease, depending on the cultivar and the process conditions (Korus et al., 2006). The content of most mineral elements can vary (Peluola-Adeyemi et al., 2014) or not in the thermoplastic extrusion process (Singh et al., 2007). Increases in the levels of calcium, phosphorus, iron and copper have been attributed to water and wear on the extruder cylinder (Singh et al., 2000).

If, on the one hand, through food biofortification programs, cowpea cultivars that are able to absorb and accumulate more nutrients in the grains than traditional ones are obtained (Freire Filho, 2011), it is necessary to evaluate the possible nutritional losses that may occur during processing. The contents of the nutrients iron, zinc and proteins have been evaluated in cowpea cultivars. Those whose grains have more than 60 and 40 mg kg^{-1}, respectively, and are considered biofortified in iron and zinc. The observed variation has been from 48.8 to 77.4 mg kg^{-1} for iron and from 35.6 to 53.7 mg kg^{-1} for zinc (Freire Filho, 2011). To be considered biofortified in protein, grains must contain about 30% of this nutrient, so the corresponding cooked grain will provide at least 15 g of protein out of the 50 g of the Reference Daily Intake (RDI), but the variation observed in grains has been from 21.5 to 26.8% (Freire Filho, 2011).

Transforming Biofortified Whole Cowpea Flour (BWCF) into crunchy expanded extrudates or instant flours is a convenient option to consume this nutritious and healthy grain, especially important for those who have reduced nutrient absorption capacity due to intestinal injuries (Ribeiro et al., 2017; García-Manzanares & Lucendo, 2011) and for those who choose not to eat foods containing gluten, whose alternatives are less nutritious, since the functionality of gluten is usually replaced by that of starch (Horstmann et al., 2017; Rai et al., 2018).

In this study, it was evaluated how the different conditions of the thermoplastic extrusion process affected the chemical and mineral composition of the raw BWCF of the cultivar BRS Tumucumaque. The information is valuable for optimizing the extrusion process to identify appropriate conditions for obtaining extrudates with greater nutrient retention.

2 Material and methods

2.1 Raw material

The cowpea grains of the biofortified cultivar BRS Tumucumaque produced and cleaned in Sorriso, in the state of Mato Grosso (MT) were crushed in the knife mil (Renard, MFC-180-75-01, São Paulo, SP, Brazil), with a 3 mm diameter opening mesh attached to the outlet, followed by the grinding of a roller mill (Brabender OHG, Quadrumat Senior, Duisburg, North Rhine-Westphalia, Alemanha), resulting in BWCF.

2.2 Thermoplastic extrusion

The raw BWCF was subjected to the thermoplastic extrusion process in the co-rotating twin-screw equipment (Clextral, Evolum HT 25, Firminy, France), each 25 mm diameter screw, 40:1 length:diameter ratio, screw configuration was kept constant (Figure 1c), die containing four circular holes of 3.8 mm in diameter, ten distinct heating zones and a feed rate of 6.79 kg h^{-1}, according to the central rotational composite design 2^3 (Table 1). The extrusion of the treatments was carried out in random order, after obtaining the extrudates, they were dried in an air circulation oven at 60 °C for 4 hours, followed by packing in polyethylene bags for cooling and grinding (Retsch, MM200, Haan, Germany) for analysis.

Table 1. Variables independent of the thermoplastic extrusion process and levels expressed in coded and real values.

Treatments	Encoded values			Real values		
	x_1	x_2	x_3	Temperature (ºC)	Speed (rpm)	Moisture (%)
T01	-1	-1	-1	100	300	18
T02	-1	1	-1	100	700	18
T03	1	-1	-1	140	300	18
T04	1	1	-1	140	700	18
T05	-1	-1	1	100	300	22
T06	-1	1	1	100	700	22
T07	1	-1	1	140	300	22
T08	1	1	1	140	700	22
T09	-α	0	0	86.4	500	20
T10	α	0	0	153.6	500	20
T11	0	-α	0	120	163.6	20
T12	0	α	0	120	836.4	20
T13	0	0	-α	120	500	16.6
T14	0	0	α	120	500	23.4
T15	0	0	0	120	500	20
T16	0	0	0	120	500	20
T17	0	0	0	120	500	20
T18	0	0	0	120	500	20
T19	0	0	0	120	500	20

x_1 = Temperature of the 10th zone of the extruder (ºC). x_2 = Screws rotation speed (rpm). x_3 = Raw WBCF moisture (%). $\alpha = \sqrt[4]{8} = 1.682$

2.3 Chemical analysis

The contents of proteins, lipids, ash and carbohydrates were determined according to the methodology described by AOAC (2010). To determine the levels of Fe, Zn, Cu, Mn, P and K, an extract was initially prepared as following: 0.2 g of samples were deposited in tubes containing 5 mL of nitric acid: perchloric acid (2:1); digested at 200 °C; and then the volume was made up to 20 ml with distilled water. Aliquots of the extract were used to quantify the levels of minerals in the atomic absorption spectrophotometer (GBC, Model 90C, IL, USA). For P content, a 0.2 mL aliquot of the extract was diluted in 8.4 mL of distilled water, and 1 mL of acid ammonia molybdate solution and 4 mL of 2% vitamin C were added, followed by homogenization in Vortex, rest for 5 minutes, followed by reading at 725 nm on a UV-VIS spectrophotometer (Thermo Spectronic, model Genesis 20 4001/4, CA, USA).

2.4 Statistical analysis

The data obtained were analyzed by multiple linear regression (Barros Neto et al., 2010) and response surface graphs were plotted using the Statistical program (StatSoft, Version 10, OK, USA). The significant experimental models ($p \leq 0.05$) were submitted to analysis of global desirability, in an equity condition, adopting values of “s” and “t” equal to 1, to identify the process condition that favors the retention of these nutrients.

3 Results and discussions

3.1 Effect of the extrusion process on chemical properties

Tables 2 and 4 show the results of the chemical composition of raw and extruded BWCF under different conditions of temperature and rotation speed of the screws and conditioning moisture of the samples. The protein content in the raw BWCF was 24.76% (db), whereas that of the extruded BWCF ranged from 23.54%

to 24.90% (db) (Table 2). Similar reductions have been observed in other extruded leguminous flours (Ai et al., 2016; Cardoso-Santiago & Arêas, 2001), extruded cereal flour (Razzaq et al., 2012) and flour mixes (Anuonye et al., 2010).

Table 2. Effect of extrusion variables on the proximate composition (dry basis (db) of raw and extruded BWCF.

Treatments	Proteins (%)	Lipids (%)	Ashes (%)	Carbohydrates (%)
Raw WBCF	24.76 ± 0.17	1.98 ± 0.18	3.55 ± 0.06	69.73 ± 0.22
T01	24.13 ± 0.06	1.21 ± 0.26	4.38 ± 0.14	70.29 ± 0.34
T02	24.07 ± 0.08	0.96 ± 0.02	3.88 ± 0.07	71.09 ± 0.13
T03	24.10 ± 0.15	1.21 ± 0.26	3.95 ± 0.55	70.75 ± 0.80
T04	24.25 ± 0.05	1.36 ± 0.10	4.11 ± 0.27	70.29 ± 0.27
T05	24.84 ± 0.22	1.15 ± 0.19	3.81 ± 0.10	70.20 ± 0.32
T06	24.90 ± 0.08	1.06 ± 0.14	4.01 ± 0.14	70.03 ± 0.23
T07	24.72 ± 0.03	1.17 ± 0.19	3.74 ± 0.12	70.37 ± 0.23
T08	24.15 ± 0.18	1.09 ± 0.08	3.96 ± 0.50	70.80 ± 0.64
T09	24.11 ± 0.05	1.30 ± 0.34	4.03 ± 0.03	70.57 ± 0.30
T10	24.01 ± 0.12	1.07 ± 0.24	3.89 ± 0.03	71.05 ± 0.27
T11	24.26 ± 0.05	1.57 ± 0.14	3.93 ± 0.07	70.25 ± 0.22
T12	24.35 ± 0.15	1.34 ± 0.25	3.91 ± 0.06	70.40 ± 0.40
T13	24.23 ± 0.03	1.16 ± 0.08	3.99 ± 0.09	70.61 ± 0.11
T14	24.41 ± 0.11	1.30 ± 0.24	3.97 ± 0.12	70.32 ± 0.07
T15	23.69 ± 0.17	1.22 ± 0.15	3.91 ± 0.01	71.17 ± 0.16
T16	23.79 ± 0.23	1.55 ± 0.41	3.98 ± 0.05	70.68 ± 0.38
T17	23.64 ± 0.21	1.09 ± 0.13	3.55 ± 0.02	71.72 ± 0.31
T18	23.89 ± 0.12	1.37 ± 0.14	3.55 ± 0.05	71.19 ± 0.20
T19	23.54 ± 0.12	1.20 ± 0.06	3.57 ± 0.06	71.68 ± 0.01
CV[1] (%)	0.57	16.18	5.03	0.47

All values are presented as mean ± standard deviation of three determinations. [1]Coefficient of variation.

For the protein content of the extruded BWCF, the model obtained was significant ($p = 0.008$) with a good fit to the observed data ($r^2 = 0.85$) (Table 3). The lowest protein contents were quantified close to the central point region and as the values of the extrusion parameters moved away from the process conditions of that region, there was less reduction in the protein content (Figure 1b). The milder conditions of the extrusion process were associated with higher conditioning moisture content, lower temperature and speed of rotation that preserved the BWCF protein content.

In compound flour (amaranth, beans, peanuts and pumpkin), it has been reported that the extrusion temperature and moisture content had a linear and negative interaction effect on protein content (Akande et al., 2017). The reduction in protein content has been attributed to denaturation (Peluola-Adeyemi et al., 2014), loss of some amino acids and nitrogen compounds on heating (Rehman & Shah, 2005), and the degradation of lysine at high temperatures because of its two available reactive amino groups (Singh et al., 2007).

One of the main reactions that occurs during the extrusion process is the Maillard reaction, being dependent on the amount of substrate (reducing sugars and reactive amino acids) and process conditions, differences in discernible shades in the extruded BWCF (Figure 1a). Although sensorially desirable due to the development of color and flavor, it negatively affects protein quality by the loss of essential amino acids in this reaction (Bjorck & Asp, 1983; Singh et al., 2000, 2007).

The lipid content in the BWCF was 1.98% (db). In the extruded samples, the concentrations of lipids ranged from 0.96% to 1.57% (db) (Table 2). The reduction in lipid content was attributed to partial decomposition and volatilization during extrusion processing (Marzo et al., 2002) and formation of amylose-lipid complexes and lipid proteins. In most comparisons between the lipid content of raw and extruded bean flours from different cultivars, there was a decrease or no change in content after thermal processing (Korus et al., 2006). In other comparisons between raw and extruded flours, a reduction in lipid content was also observed (Razzaq et al., 2012; Anuonye et al., 2010; Rampersad et al., 2003; Alonso et al., 2000), being attributed to the formation of complexes between amylose and fatty acids. The model obtained by multivariate regression analysis was not predictive (r^2 = 0.39) or significant (p = 0.739) (Table 3).

The carbohydrate content in raw BWCF was 69.73% (db), i.e., this was the lowest quantified value, however, the levels between the samples of the extruded treatments ranged from 70.03 to 71.72% (db) (Table 2).

Table 3. Coefficient values estimated by multivariate regression for proteins, lipids, ash and carbohydrates.

Coefficients	Proteins	Lipids	Ashes	Carbohydrates
β_0	23.70 *	1.29 *	3.71*	71.29 *
β_1	-0.07 ^{ns}	0.00 ^{ns}	-0.04 ^{ns}	0.10 ^{ns}
β_2	-0.02 ^{ns}	-0.05 ^{ns}	0.00 ^{ns}	0.06 ^{ns}
β_3	0.17 *	-0.00 ^{ns}	-0.06 ^{ns}	-0.11 ^{ns}
β_{11}	0.15 *	-0.07 ^{ns}	0.09 ^{ns}	-0.17 ^{ns}
β_{22}	0.24 *	0.03 ^{ns}	0.07 ^{ns}	-0.34 *
β_{33}	0.25 *	-0.05 ^{ns}	0.10 ^{ns}	-0.29 *
β_{12}	-0.05 ^{ns}	0.05 ^{ns}	0.08 ^{ns}	-0.08 ^{ns}
β_{13}	-0.13 ^{ns}	-0.04 ^{ns}	0.01 ^{ns}	0.16 ^{ns}
β_{23}	-0.08 ^{ns}	0.01 ^{ns}	0.09 ^{ns}	-0.01 ^{ns}
r^2	0.85	0.39	0.62	0.73
F value	5.66 *	0.64 ^{ns}	1.66 ^{ns}	2.67 ^{ns}
p	0.008 *	0.739 ^{ns}	0.231 ^{ns}	0.080 ^{ns}
Lack	0.12 ^{ns}	0.52 ^{ns}	0.86 ^{ns}	0.76 ^{ns}

*Significant ($p < 0.05$). ^{ns} not significant.

As the carbohydrate content was calculated by difference, the extruded BWCF treatments that showed large decreases in the protein, lipid and ash content, presented higher carbohydrate content, even higher than raw BWCF (Table 2). The increase in the carbohydrate content after extrusion has been observed in other studies (Cardoso-Santiago & Arêas, 2001; Anuonye et al., 2010), however, the decrease has also been reported (Korus et al., 2006; Alonso et al., 2000). The regression model for the carbohydrate content in WCF extrudates showed a relatively high coefficient of determination (r^2 = 0.73), but it was not significant (p = 0.080) (Table 3).

The ash content in the raw BWCF was 3.55% (db), and in the extruded BWCF the concentrations were significantly equal to or higher than the raw BWCF, varying from 3.55% to 4.38% (db) (Table 2). The model had a low determination coefficient (r^2 = 0.62) and was not significant (p = 0.231) (Table 3). Some studies have reported an increase in ash content in extruded products (Marzo et al., 2002; Anuonye et al., 2010; Pérez-Navarrete et al., 2007) and others decrease (Korus et al., 2006; Cardoso-Santiago & Arêas, 2001), and there are reports of an increase in one cultivar and a decrease in another after extrusion (Razzaq et al., 2012).

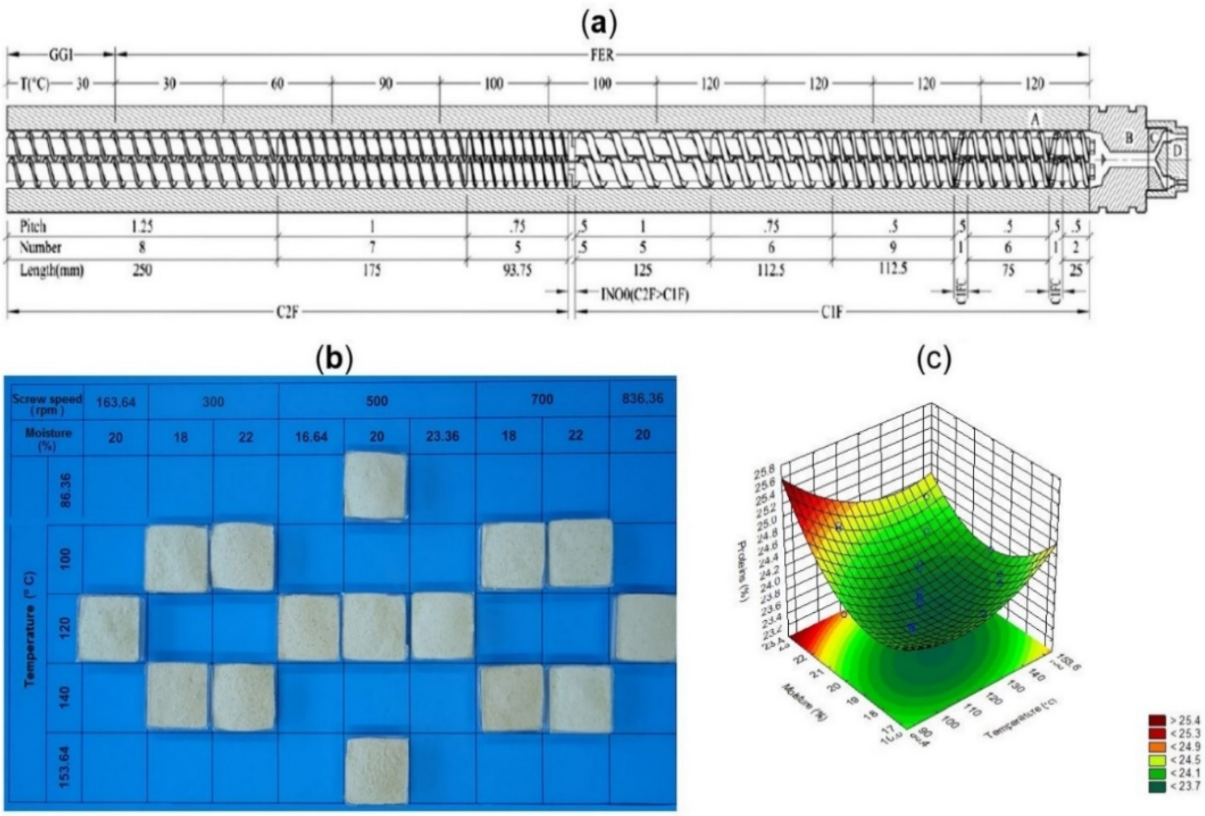

Figure 1. (**a**) Schematic representation of the top view (in mm) of the barrel, screws and front plate assembly (die) of the Clextral Evolum HT25 extruder: A, the barrel; B, the central manifold plate; C, the distributor plate; D, the holder-inserts plate; E, the insert hole; T, module temperature; GG1, feeding module; FER, closed modules; C1F/C2F, conveyor screw of single/double pitch; INO0, interface screw for transition between screws of single and double pitch; and C1FC, reverse screw of single pitch, slotted; (**b**) Differences in the color shade of extruded flours; (**c**) Effect of moisture content and process temperature on protein content (%, db) in whole cowpea flours extruded at 500 rpm screws rotation speed.

All extruded samples continued to be rich in Fe, as they maintained levels above 60 mg kg^{-1}. There was a variation from 72.32 to 103.09 mg kg^{-1} for this element among the extruded samples, the raw BWCF showed highest levels (Table 4). The regression model was neither predictive ($r^2 = 0.65$) nor significant ($p = 0.191$).

The zinc contents ranged from 29.87 to 50.22 mg kg^{-1} (Table 4). The model presented a good fit to the observed data ($r^2 = 0.85$) and significant ($p = 0.009$), the model's lack of adjustment was also significant (lack off it = 0.001) (Table 5). According to Henika (1972) whenever the mean square of the experimental error assumes extremely low values, the significance of the tests due to lack of adjustment should be considered irrelevant. In this case, the value was 5.5 times lower than the mean square of the regression, and the significance of the lack of adjustment was disregarded. Thus, the Zn content was influenced by the linear and quadratic effect of the rotation speed of the screws and by the quadratic effect of the temperature (Table 5). It was observed that as the processing conditions moved away from the central point region, the zinc levels decreased (Figure 2a).

Mg levels ranged from 23.72 to 29.98 mg kg^{-1} (Table 4). The regression model showed a good fit to the observed data ($r^2 = 0.87$) and significant ($p = 0.017$). It was influenced by the linear and quadratic effects of the thread rotation speed, and its interaction with temperature and moisture (Table 5). In general, the Mg content decreased as the rotation speed of the screws became close to the extreme values (Figure 2b).

Table 4. Iron (Fe), zinc (Zn), magnesium (Mg), copper (Cu), manganese (Mn), phosphorus (P) and potassium (K) in raw and extruded BWCF.

Trial	Fe (mg kg^{-1})	Zn (mg kg^{-1})	Mg (mg kg^{-1})	Cu (mg kg^{-1})	Mn (mg kg^{-1})	P (g kg^{-1})	K (g kg^{-1})
Crude WBCF	80.85 ± 2.81	41.64 ± 1.35	25.80 ± 2.48	5.78 ± 0.31	9.90 ± 0.40	5.17 ± 0.29	15.69 ± 0.82
T01	76.35 ± 1.45	32.04 ± 0.25	27.63 ± 0.28	4.59 ± 0.22	9.11 ± 0.41	5.21 ± 0.09	14.83 ± 0.44
T02	72.32 ± 1.48	31.23 ± 0.11	26.59 ± 0.24	4.88 ± 0.14	9.30 ± 0.72	5.02 ± 0.04	14.41 ± 0.11
T03	99.01 ± 3.40	31.41 ± 0.39	29.14 ± 0.10	5.33 ± 0.16	9.84 ± 0.22	5.08 ± 0.03	14.51 ± 0.12
T04	82.84 ± 0.75	30.51 ± 0.81	24.71 ± 5.27	6.18 ± 1.76	10.02 ± 0.39	4.69 ± 0.47	14.96 ± 1.03
T05	76.04 ± 2.55	30.29 ± 0.41	25.95 ± 1.60	5.25 ± 0.04	9.28 ± 0.46	4.99 ± 0.07	13.77 ± 0.21
T06	75.92 ± 0.63	30.43 ± 0.20	27.39 ± 0.85	5.32 ± 0.04	9.77 ± 0.09	5.23 ± 0.19	13.86 ± 0.08
T07	85.96 ± 0.72	29.95 ± 0.32	27.52 ± 0.50	5.41 ± 0.05	9.60 ± 0.44	5.18 ± 0.42	13.77 ± 0.36
T08	75.27 ± 1.11	29.87 ± 0.35	27.65 ± 2.05	5.38 ± 0.08	9.10 ± 0.03	4.85 ± 0.14	14.33 ± 0.29
T09	79.67 ± 0.91	30.10 ± 0.16	27.98 ± 0.59	5.28 ± 0.08	9.38 ± 0.45	5.05 ± 0.03	14.05 ± 0.45
T10	74.76 ± 1.01	30.75 ± 0.74	28.00 ± 0.54	5.44 ± 0.01	9.74 ± 0.71	5.11 ± 0.07	14.29 ± 0.20
T11	73.85 ± 0.26	30.14 ± 0.42	27.34 ± 0.62	5.37 ± 0.05	9.63 ± 0.31	4.87 ± 0.14	14.13 ± 0.21
T12	92.90 ± 0.27	40.40 ± 9.42	23.72 ± 7.80	5.70 ± 0.40	9.71 ± 0.35	5.08 ± 0.13	13.65 ± 0.24
T13	75.88 ± 1.90	49.71 ± 1.00	27.42 ± 0.36	6.05 ± 0.07	9.75 ± 0.50	4.93 ± 0.09	14.10 ± 0.44
T14	88.34 ± 10.74	50.21 ± 2.09	29.98 ± 3.03	6.09 ± 0.25	10.06 ± 0.67	4.86 ± 0.28	13.12 ± 2.05
T15	103.09 ± 1.20	48.56 ± 1.26	27.69 ± 1.40	6.20 ± 0.17	9.96 ± 0.20	4.95 ± 0.10	15.71 ± 0.24
T16	95.53 ± 3.75	48.45 ± 0.37	28.70 ± 0.07	6.04 ± 0.15	10.14 ± 0.18	4.96 ± 0.07	16.26 ± 0.30
T17	99.75 ± 5.37	48.07 ± 0.90	28.01 ± 1.68	6.07 ± 0.14	10.22 ± 0.59	5.07 ± 0.27	14.73 ± 0.76
T18	90.38 ± 1.64	47.53 ± 1.27	27.46 ± 0.37	6.07 ± 0.06	9.55 ± 0.20	4.90 ± 0.24	15.72 ± 0.40
T19	94.39 ± 3.11	46.06 ± 0.44	27.68 ± 1.20	6.08 ± 0.08	9.83 ± 0.34	4.85 ± 0.15	15.95 ± 0.34
Average	84.66	37.87	27.32	5.63	9.70	5.00	14.59
CV (%)	3.80	6.02	8.93	7.47	4.48	3.92	4.16

All values are presented as mean ± standard deviation of three determinations.

The Cu contents ranged from 4.59 to 6.20 mg kg^{-1}, with the raw BWCF showing high levels, in line with the treatments of the central point (Table 4). The model presented a good fit to the observed data (r^2 = 0.85) and significant (p = 0.009). The significance of the lack of adjustment was disregarded, following recommendations of Henika (1972). As conditions in the central point moved away, Cu levels decreased (Figure 2b).

The levels of Mn varied from 9.11 to 10.22 mg kg^{-1} in the extruded samples (Table 4). The regression model was neither predictive (r^2 = 0.69) nor significant (p = 0.125) (Table 5). For P, the variation was from 4.85 to 5.22 g kg^{-1} (Table 4), and the model was not predictive (r^2 = 0.54) nor significant (p = 0.405) (Table 5). The K levels among the extruded samples ranged from 13.12 to 16.26 g kg^{-1}, and the raw BWCF presented one of the highest levels, close to the treatments of the central point (Table 4). The model presented a good fit to the observed data (r^2 = 0.82) and significant (p = 0.017), being significantly affected by the quadratic effects of the rotation speed of the screws and the moisture content of the flours (Table 5). As the conditions of the extrusion process moved away from the central point, there was a reduction in K levels (Figure 2d).

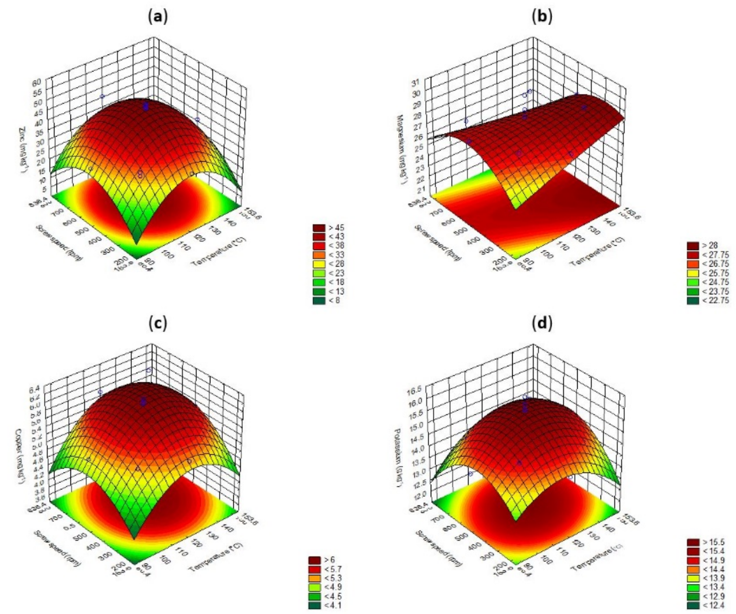

Figure 2. Effect of screws rotation speed (rpm) and temperature of the extrusion process (°C) on the content of (**a**) zinc (mg 100 g^{-1}), (**b**) magnesium (mg 100 g^{-1}), (**c**) copper and (**d**) potassium (g 100 g^{-1}) in BWCF extrudates conditioning at 20% moisture.

Table 5. Coefficient values estimated by multivariate regression for iron (Fe), zinc (Zn), copper (Cu), manganese (Mn), phosphorus (P), magnesium (Mg) and potassium (K).

Coefficients	Fe	Zn	Mg	Cu	Mn	P	K
β_0	96.61*	48.01*	27.92*	6.11*	9.95*	49.43*	15.65*
β_1	2.50 ^{ns}	-0.09 ^{ns}	0.11 ^{ns}	0.19*	0.12 ^{ns}	-0.04 ^{ns}	0.08 ^{ns}
β_2	0.07 ^{ns}	1.14*	-0.73*	0.13*	0.04 ^{ns}	-0.02 ^{ns}	-0.01 ^{ns}
β_3	0.26 ^{ns}	-0.28 ^{ns}	0.35 ^{ns}	0.03 ^{ns}	0.00 ^{ns}	0.01 ^{ns}	-0.34 ^{ns}
β_{11}	-6.75*	-7.67*	-0.03 ^{ns}	-0.33*	-0.17 ^{ns}	0.06 ^{ns}	-0.42 ^{ns}
β_{22}	-4.57*	-5.96*	-0.90*	-0.27*	-0.13 ^{ns}	0.02 ^{ns}	-0.52*
β_{33}	-5.02*	-0.76 ^{ns}	-0.22 ^{ns}	-0.08*	-0.05 ^{ns}	-0.01 ^{ns}	-0.62*
β_{12}	-2.84 ^{ns}	-0.04 ^{ns}	-0.59*	0.06 ^{ns}	-0.13 ^{ns}	-0.09*	0.17 ^{ns}
β_{13}	-2.98 ^{ns}	0.05 ^{ns}	-0.28 ^{ns}	-0.23*	-0.22 ^{ns}	0.03 ^{ns}	0.03 ^{ns}
β_{23}	1.17 ^{ns}	0.22 ^{ns}	0.88*	-0.13*	-0.05 ^{ns}	0.06 ^{ns}	0.08 ^{ns}
r^2	0.65	0.85	0.87	0.85	0.69	0.54	0.82
F value	1.83 ^{ns}	5.51 *	6.77*	5.50 *	2.22 ^{ns}	1.18 ^{ns}	4.51*
p	0.191 ^{ns}	0.009 *	0.004*	0.009 *	0.125 ^{ns}	0.405 ^{ns}	0.017*
Lack of fit	0.080 ^{ns}	0.001 *	0.143 ^{ns}	0.0002 *	0.552 ^{ns}	0.089 ^{ns}	0.669 ^{ns}

*Significant ($p < 0.05$). ^{ns} not significant.

There is little information about the effect of the extrusion process on the levels of minerals in food. A greater emphasis has been directed to the element Fe, relating the increases in the contents of the extrudates due to the wear of the metal parts during processing, in general, severe processing conditions can transfer the iron element from the extruder to the food due to the wear of the parts (Camire, 1998). The non-uniform hydration in flour particles and abrasive particles can also cause wear of the metal parts (Rokey et al., 2010) or due to deflection of the screws and high rotation speeds (Martin, 2016). The addition of mineral elements can also be attributed to the water used to moisten the raw material (Singh et al., 2000), which was not the case, as distilled water was used.

For the same model of mono-screw extruder, the following were published: 1) in extrudates of the mixture of dehulled pigeon pea flour with green banana flour (3:1), conditioned at 25% of moisture, and extruded at 100 °C and 120 rpm, the levels of Fe, Zn, Cu, Mg, Ca, K and Na were higher in relation to the raw mixture (Anuonye et al., 2012); 2) In extrudates made up of dehulled soy flour and green banana pulp flour (3:1), 25% of moisture, 150 °C and 150 rpm, an increase in the content of Fe (69.44%), Ni (28.57%), Se (6.25%) and Na (38.89%), and reductions in the content of Zn (93.57%), Ca (28.29%), Mg (34.57%), Cr (70.56%) and K (51.11%), were observed (Anuonye et al., 2010); 3) in whole flours of 5 bean cultivars under 4 different conditions (moisture/temperature: 14%/120 °C; 20%/120 °C; 14%/180 °C; 20%/180 °C), it was demonstrated that in relation to the 20 extruded combinations, the raw flours presented Fe contents always higher; for Zn of the 20 combinations, six presented contents equal to or less than the raw BWCF (6/20); for Mg, this ratio was 12/20; Cu 11/20; Mn 2/20; P 7/20; and K 8/20 (Korus et al., 2006).

In a double-screw extruder, 25% of moisture, 100 rpm and at 150 °C, the Fe content in pea flours extrudates was significantly higher than raw flour, but without differences in the levels of Ca, Mg, Cu, Mn, and P (Alonso et al., 2001). This same behavior was observed between extruded bean flour and its respective raw flour, processed at 155 °C. This increase in the Fe content in the extrudates was probably related to the wear and tear of metallic parts, mainly of the extruder thread (Alonso et al., 2001).

The variations observed in the chemical composition of BWCF should occur similarly in whole flours from non-biofortified cultivars.

3.2 Optimization of process conditions using desirability analysis

Using the significant experimental models, the process conditions that resulted in lower nutrient losses or overall desirability (D) were determined, obtaining a value equal to 0.75 (Figure 3), a relatively high value, being considered acceptable and good, according to the Lazic (2004) scale, and the process condition that provided this greater preservation of nutrients was the combination of 112.6 °C, 587.4 rpm and 23.4% of moisture (Figure 3).

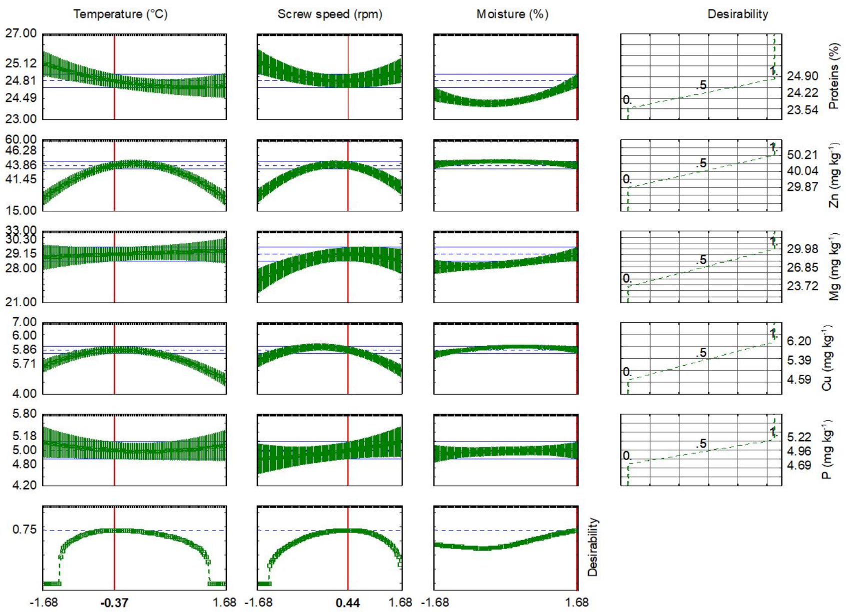

Figure 3. Graph of the desirability function for nutrient preservation responses (proteins, zinc, magnesium, copper and phosphorus).

4 Conclusions

The extrusion temperature, screw rotation speed and conditioning moisture significantly affected the protein and copper contents of the Transforming Biofortified Whole Cowpea Flour (BWCF) extrudates. Zinc levels were not influenced by moisture. The magnesium and potassium contents were not affected by the temperature. It was possible to obtain an acceptable and good global desirability for nutrient retention in BWCF extrudates, but it is necessary to reconcile this information with the sensory characteristics of the extrudates.

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