

Cassava derivatives in the preparation of unconventional gluten-free snacks

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Abstract

Products derived from cassava are not being sufficiently utilised by the food industry, despite their great potential in the preparation of gluten-free products. In the present work, mixtures of sour starch and leaf flour obtained from cassava were prepared and processed in a single screw extruder under different conditions of temperature, moisture, and screw speed, in order to obtain salty expanded snacks. The products were analysed in terms of expansion index, specific volume, hardness, colour, water-absorption index, water-solubility index, and sensory acceptance. Changes in the percentage of cassava leaf flour significantly affected the physical properties of the snacks, while they were less affected by moisture, temperature and screw speed. Processing with 6% cassava leaf flour blended with sour starch, in the presence of 16% moisture at an extrusion temperature of 100°C and screw speed of 230 rpm produced highly crisp, light-coloured puffed snacks with good overall acceptability scores and the qualities that are desirable in expanded snacks. These results suggest that cassava sour starch and leaf flour can be used as ingredients in the production of extruded gluten-free products.

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Keywords

Manihot Esculenta
Leaf
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Introduction

Cassava (*Manihot esculenta* Crantz), a native Brazilian plant, is prevalent throughout most tropical countries. At present, it is highly important in terms of world food security, and is considered a priority in programs that deal with the trends in the global economy and the challenges imposed by the changing climate of the world, particularly from the viewpoints of its great resistance and better adaptation to droughts. Africa is the largest producer of cassava, producing 53.6% of the total global production, followed by Asia, the Americas, and Oceania, which account for 30.5%, 15.8%, and 0.1% of the global production, respectively. In terms of countries, Nigeria is the major producer, followed by Brazil, Thailand, Indonesia and Congo (FAO, 2017).

Cassava is grown in all states of Brazil, possessing ethnic, social and economic importance. The total production, including derivatives such as flours, starch, and ethanol, is around 24 million tons (FAO, 2017), and is intended for the purpose of both household consumption and industries.

The cassava-producing industry in Brazil is in constant search for technological innovations, and the increasing use of its derivatives in the food industries represents its high demand in the agricultural and industrial sector.

Celiac disease is the permanent intolerance to gluten, characterised by total or subtotal atrophy of the proximal small intestine mucosa that leads to malabsorption of food in genetically predisposed individuals. A permanently gluten-free diet is required for the treatment of celiac disease; that is, foods containing wheat, rye, barley, malt and oats cannot be consumed (Arendt and Nunes, 2010; Stojceska *et al.*, 2010).

Consumers' awareness on the need to have specific diets for the treatment of obesity, diabetes, celiac disease, and other food intolerances has strongly contributed to advances in the specific-foods market. Therefore, the consumption of gluten-free products has been increasing rapidly, even among non-celiac consumers who seek health benefits through the intake of these products (Markets and Markets, 2015). Given this context, the use of cassava

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derivatives in the preparation of high-value products is of great industrial interest. It addresses the demands for products with low cost and fast preparation in the growing market for gluten-free products.

Extrusion is a promising process in the development of gluten-free products because it yields products with different shapes, sizes, flavours and textures (Kaur *et al.*, 2014; Rathod and Annapure, 2017). During extrusion, the raw material is cooked and textured by the combined action of factors such as moisture, pressure, temperature, and shear, thereby leading to the disintegration of molecular structures and the formation of various products that are ready for consumption (Vargas-Solórzano *et al.*, 2014). Extrusion has several beneficial effects such as the gelatinisation of starch, reduction of lipid oxidation, increase of dietary soluble fibres, and reduction of anti-nutritional factors (Singh *et al.*, 2007). However, in the development of extruded products, process parameters such as screw configuration and speed, feed rate, and extruder barrel temperatures as well as raw material characteristics such as moisture and grain size are highly important in controlling the extrusion (Ding *et al.*, 2005; Nikmaram *et al.*, 2017).

Cassava leaf flour (CLF) has high contents of proteins, vitamins, carotenoids and minerals (Ortega-Flores *et al.*, 2003; Wobeto *et al.*, 2006; Latif and Muller, 2015). This composition makes it a potential raw material for use in fortified extruded snacks, considering that extrusion can reduce the effect of anti-nutritional components (Omeire *et al.*, 2012; Salata *et al.*, 2014; Ferrari *et al.*, 2014; Kaur *et al.*, 2015; Trombini *et al.*, 2016). Another product derived from cassava is sour starch. This starch, modified by natural fermentation and dried under the sun, is mainly used in the production of biscuits because of its intrinsic characteristic to expand, and has been studied as a raw material for snack products with soy, quinoa, and flaxseed flours (Leonel *et al.*, 2010; Taverna *et al.*, 2012; Mesquita *et al.*, 2013).

Considering the growing demand for gluten-free products, their commercial appeal, the sustainability of using CLF as a source of protein and fibre in industrial foods, and the differential effects rendered by the use of sour starch and leaf flour blends in extruded products, the present work was therefore aimed to evaluate the quality parameters of cassava gluten-free snacks produced in a single screw extruder under different operating conditions.

Materials and methods

Raw materials and preparation

Sour cassava starch (SCS) was commercially

obtained (Ouro Minas, São Pedro do Turvo, São Paulo, Brazil) and CLF. To produce CLF, cassava leaves were obtained from the cassava cultivar IAC 14 harvested in the eighth month after plantation. CLF was obtained from ripe leaves sanitised in chlorinated water by drying them in an oven with air circulation (30°C). Following drying for 72 h, the material was ground in a knife mill and sieved (0.3 mm) to obtain the flour. SCS and CLF were then analysed for moisture, ash, fibre, lipid, protein, carbohydrate and total cyanide content (Cooke and Cruz, 1982).

SCS and CLF mixtures were prepared in a “Y” homogeniser (Tecnal, TE201/5, Brazil). The moisture content was adjusted according to the experimental design (Table 1), and following homogenisation for 5 min, 30 samples of 1,000 g each were set aside. The samples were transferred to closed containers and stored overnight at 4°C for equilibration until extrusion.

Table 1. Variables and their levels employed in the central composite design.

Experiment	Coded value				Uncoded value			
	X ₁	X ₂	X ₃	X ₄	CLF	T	M	SS
1	-1	-1	-1	-1	4	90	14	215
2	-1	-1	-1	1	4	90	14	245
3	-1	-1	1	-1	4	90	18	215
4	-1	-1	1	1	4	90	18	245
5	-1	1	-1	-1	4	110	14	215
6	-1	1	-1	1	4	110	14	245
7	-1	1	1	-1	4	110	18	215
8	-1	1	1	1	4	110	18	245
9	1	-1	-1	-1	8	90	14	215
10	1	-1	-1	1	8	90	14	245
11	1	-1	1	-1	8	90	18	215
12	1	-1	1	1	8	90	18	245
13	1	1	-1	-1	8	110	14	215
14	1	1	-1	1	8	110	14	245
15	1	1	1	-1	8	110	18	215
16	1	1	1	1	8	110	18	245
17	-2	0	0	0	2	100	16	230
18	2	0	0	0	10	100	16	230
19	0	-2	0	0	6	80	16	230
20	0	2	0	0	6	120	16	230
21	0	0	-2	0	6	100	12	230
22	0	0	2	0	6	100	20	230
23	0	0	0	-2	6	100	16	200
24	0	0	0	2	6	100	16	260
25-30	0	0	0	0	6	100	16	230

X₁ = CLF (cassava leaf flour), X₂ = T (temperature in third zone of barrel extruder), X₃ = M (moisture of raw materials), X₄ = SS (screw speed).

Extrusion

Extrusion was conducted using an Inbra RX50 single screw extruder (Inbramaq, São Paulo, Brazil) that has a processing capacity of 50 kg h⁻¹. The constant extrusion parameters were the extrusion temperatures in the first (25°C) and second zones (45°C), screw compression ratio (3:1), screw diameter (32.6 mm), die diameter (4 mm), feed rate (160 g min⁻¹), and cutting speed (90 rpm). During extrusion, a portion of the next test material was used to purge the extruder, and about 500 g extruded sample was collected after a steady-state flow was attained in the extruder. The cylindrical rod-shape products were put into an oven with air circulation at 40°C to obtain the dry extrudates with 5–7% moisture content. The samples were stored in polyethylene bags and used for subsequent analyses.

Experimental design and statistical analysis

The central composite rotatable design was adopted in the present work. The content of CLF ranged from 2% to 10%, and the content of SCS ranged from 90% to 98%. The extrusion temperature ranged from 80 to 120°C, the feed moisture ranged from 12% to 20%, and the screw speed ranged from 200 to 260 rpm. The eight dependent variables were the radial expansion index (REI), specific volume (SV), colour (L^* , a^* , b^*), hardness (H), water-solubility index (WSI), and water-absorption index (WAI). A total of 30 experiments were conducted based on the design presented in Table 1.

Response surface methodology describes the behaviour of a system in which the independent variables (X_i) and the dependent variables or responses (Y_i) are combined. The response (Y_i) is a function of the combination of the parameters (X_i) scaled by certain factors (β_{ij}). Within the range of these parameters, the behaviour of each response can be predicted using a general equation:

$$Y_i = \beta_0 + \beta_1 X_1 + \beta_2 X_2 + \beta_3 X_3 + \beta_4 X_4 + \beta_{11} X_1^2 + \beta_{22} X_2^2 + \beta_{33} X_3^2 + \beta_{44} X_4^2 + \beta_{12} X_1 X_2 + \beta_{13} X_1 X_3 + \beta_{14} X_1 X_4 + \beta_{23} X_2 X_3 + \beta_{24} X_2 X_4 + \beta_{34} X_3 X_4 + e, \quad (\text{Eq. 1})$$

where

Y_i = dependent variable or response function;

X_1, X_2, X_3, X_4 = values of the independent variables;

β_0 = coefficient corresponding to intercept in the response axis;

$\beta_1, \beta_2, \beta_3, \beta_4$ = linear coefficients assessed through least square method;

$\beta_{11}, \beta_{22}, \beta_{33}, \beta_{44}$ = coefficients of quadratic variables;

$\beta_{12}, \beta_{13}, \beta_{14}, \beta_{23}, \beta_{24}, \beta_{34}$ = coefficients of interaction between independent variables;

e = experimental error.

The effects of independent variables on the dependent variables were statistically investigated by the SAS program (SAS Institute, 1990). The obtained model was validated by an F -test, with the pure error mean square as the denominator. Response surface graphs were plotted with Statistica® 6.0.

Analyses of extruded products

The REIs of the snacks was evaluated after extrusion and before drying. Expansion indexes result from the division of radial expansion by the die orifice diameter (4 mm) (Kasprzak *et al.*, 2013).

The SV was determined according to the mass displacement method (millet seed) using a graded cylinder (Faubion and Hosoney, 1982).

The hardness of the samples was determined with a TA-XT2 texture analyser (Stable Micro Systems, Surrey, England), with a 50 kg load cell and HDP/WBV probe (Warner Bratzler set with a “V” slot blade for USDA Standard, pre-test speed: 5 mm s⁻¹, test speed: 5 mm s⁻¹, post-test speed: 10 mm/s).

The colour measurements (CIE L^* , a^* , and b^* colour space) were performed on the raw materials before extrusion and on the ground extruded samples using a Minolta CR-400 model colorimeter (Konica Minolta, Ramsey, NJ, USA) with illuminant D65. A Hunter Lab colour space was used to measure the lightness (L^*), red/green ($+a^*/-a^*$), and yellow/blue ($+b^*/-b^*$) variables.

The WAIs and WSIs were determined following the method described by Anderson *et al.* (1969).

After the extrusion and data analysis were complete, the processing conditions were established that allowed snacks to be obtained with the desired expansion, density, crispness, colour, WAI and WSI. Consumer testing was conducted at the university. The criterion for the recruitment of panellists was the regular consumption of snacks. Each product was coded with a random three-digit number. Panellists were instructed to share their overall liking of the product using a nine-point hedonic scale (Chalermchaiwat *et al.*, 2015). The implementation of sensory analysis was approved by the Ethics Committee of the University.

Results and discussion

SCS is a naturally fermented, sun-dried product used to prepare low-density, gluten-free bakery products. Due to its production which includes the disintegration of cassava roots, starch extraction, purification, natural fermentation and sun-drying, SCS has in low levels of non-starch components (Table 2), which was also observed by Onitilo *et al.* (2007).

Cassava leaves form a major part of the diet in some countries (Latif and Müller, 2015); but its consumption is restricted to only some regions in Brazil. CLF assessed in the present work had high protein and fibre contents (Table 2), as also observed by Ortega-Flores *et al.* (2003), Wobeto *et al.* (2006), and Latif and Müller (2015). Although cassava leaves are a source of valuable nutrients, they also contain cyanogenic glucosides. However, the steps involved during its production in the present work yielded a low content of cyanide in the CLF, as compared with those reported by Latif and Müller (2015) in cassava leaves.

The mixtures had WAI values ranging from 2.72 to 3.06 g g⁻¹ and WSI values ranging from 1.03% to 2.07%. The mixtures exhibited increased indexes with increasing percentage of leaf flour. Colour

analysis of the mixtures showed lower luminosity and higher levels of green ($-a^*$) and yellow ($+b^*$) chromas with increasing amount of leaf flour.

Analyses of extruded products

Extrusion parameters such as temperature, screw speed, feed rate, die diameter, and raw-material composition (moisture, protein, fibre, lipids, salt, sugar), have great influence on the expansion, crispness and colour of puffed snacks (Ding *et al.*, 2005; Tsokolar-Tsikopoulos *et al.*, 2015).

Data analysis showed that CLF was the most important factor and affected all the dependent variables significantly ($p < 0.05$, $p < 0.01$ and $p < 0.001$). Moisture content was the second most important factor and had significant effects on all the dependent variables except the REI (Table 3).

Effect of variable parameters on expansion

The expansion index is an important parameter for assessing the quality of expanded extrudates. This parameter characterises the structure and texture of the extrudates. During extrusion, the viscoelastic material at the exit of the die encounters a sudden drop in pressure, which causes the evaporation of water and leads to the formation of expanded porous products (Salata *et al.*, 2014).

Table 2. Proximate analysis and physicochemical properties of raw materials and mixtures.

Proximate composition	Raw material		Mixture (% leaf flour)				
	Sour starch	Leaf flour	2	4	6	8	10
Moisture (g 100g ⁻¹)	11.2 ± 0.73	8.7 ± 0.69	11.15 ± 0.72	11.1 ± 0.71	11.05 ± 0.70	11.0 ± 0.70	10.95 ± 0.06
Ash (g 100g ⁻¹)	0.17 ± 0.02	5.19 ± 0.97	0.27 ± 0.04	0.37 ± 0.05	0.47 ± 0.07	0.57 ± 0.08	0.67 ± 0.09
Fibre (g 100g ⁻¹)	0.28 ± 0.01	26.52 ± 0.16	0.80 ± 0.01	1.33 ± 0.02	1.86 ± 0.02	2.38 ± 0.02	2.9 ± 0.02
Lipid/Ether extract (g 100g ⁻¹)	0.21 ± 0.04	13.3 ± 0.08	0.47 ± 0.03	0.73 ± 0.04	0.99 ± 0.04	1.26 ± 0.04	1.52 ± 0.04
Protein (g 100g ⁻¹)	0.19 ± 0.02	24.05 ± 0.22	0.67 ± 0.02	1.14 ± 0.03	1.62 ± 0.03	2.1 ± 0.03	2.58 ± 0.03
Carbohydrate (g 100g ⁻¹)	87.52 ± 1.2	21.91 ± 0.71	86.21 ± 1.19	84.9 ± 1.2	83.58 ± 1.2	82.27 ± 1.18	80.96 ± 1.1
Total cyanide (mg 100g ⁻¹)	0.17 ± 0.02	0.33 ± 0.04	0.17 ± 0.01	0.17 ± 0.01	0.18 ± 0.01	0.18 ± 0.01	0.18 ± 0.01
WAI (g g ⁻¹)	2.08 ± 0.02	6.87 ± 0.27	2.72 ± 0.13	2.76 ± 0.12	2.86 ± 0.10	2.92 ± 0.12	3.06 ± 0.18
WSI (%)	0.64 ± 0.02	14.85 ± 0.12	1.03 ± 0.05	1.17 ± 0.04	1.36 ± 0.02	1.76 ± 0.02	2.07 ± 0.06
L^*	94.7 ± 0.01	47.52 ± 0.13	94.28 ± 0.51	90.22 ± 0.44	86.24 ± 0.38	82.29 ± 0.47	77.93 ± 0.56
a^*	1.09 ± 0.01	-0.66 ± 0.02	0.26 ± 0.03	-0.04 ± 0.01	-0.07 ± 0.0	-0.09 ± 0.01	-0.17 ± 0.0
b^*	5.87 ± 0.05	15.97 ± 0.03	6.27 ± 0.11	6.59 ± 0.12	7.10 ± 0.09	7.49 ± 0.13	7.93 ± 0.11

WAI = water absorption index; WSI = water solubility index

Under the conditions used in the processing of the cassava leaf flour and sour starch mixtures, the REI values were similar to those reported for cereal extrudates (Meng *et al.*, 2010), and to those observed by Leonel *et al.* (2010) after extrusion of mixtures of sour starch and soy flour, and by Mesquita *et al.* (2013) in their extrusion study with blends of sour cassava starch and flaxseed flour.

Regression analyses indicated that the REIs decreased with increasing CLF content in the blend. Data analysis also showed negative quadratic effects of temperature, moisture, and screw speed. There was a significant correlation between CLF content and screw speed (Table 3). Figure 1a illustrates the effects of CLF content and screw speed (SS) on the REIs. A low REI was observed with a combination of high SS and high CLF content while a high REI was observed with a combination of low CLF content and high SS.

The lower REI at a higher percentage of CLF could be attributed to the high content of fibre in this flour. The inclusion of fibrous material in blends in order to produce expanded extruded snacks results in a more compact structure and smaller expansion. The expansion is related to the size, number, and distribution of gas cells, and the higher fibre content reduces the size of the cells causing rupture, thereby reducing the expansion (Taverna *et al.*, 2012; Wang and Ryu, 2013).

In addition to fibres, high amounts of proteins in CLF may also have an adverse effect on the expansion. Protein-starch reactions may interfere in the radial expansion of the material by increasing the susceptibility of elastic recoil through the formation of an amylose-protein complex. In addition, these reactions may decrease the expansion of amylopectin and inhibit the release of water vapour during extrusion, thereby reducing the expansion and increasing the density of the extrudate (Moraru and Kokini, 2003).

A higher screw speed, lower percentage of CLF, higher water vapour pressure, and viscosity would increase the expansion index. The temperature and screw speed have a profound effect on the expansion of the snack, because the dissipation of the mechanical energy of the screw rotation provides the energy required to reach the temperatures in the extruder barrel.

At the extreme opposite conditions of extrusion temperature and moisture, the expansion index was lower (Figure 1b). Moisture and temperature are intrinsically related to starch disruption during extrusion. The expansion ratio of the extrudates

increases with temperature until it reaches a critical level, after which it declines. At a higher temperature, more water is flashed to steam, which causes an increase in the melt viscosity, a decrease in bubble growth, and thus, a decrease in expansion (Meng *et al.*, 2010).

The SV is the sum of the radial and axial expansions, and is associated with measures of crispness, WAIs and WSIs of the extruded products (Ferrari *et al.*, 2014).

The SV of extruded products were higher than those obtained by Salata *et al.* (2014) in their study with extrusion of blends of cassava flour and cassava leaf flour (1.8 to 8.71 mL g⁻¹), as well as the results observed by Ferrari *et al.* (2014) (3.13 to 10.15 mL g⁻¹), who extruded mixtures of cassava leaf flour and cassava starch. These differences may be ascribed to the composition of the raw materials, because of the higher levels of fibre in blends with cassava flour and the intrinsic characteristics of expansion of the SCS used in the present work.

In Brazil, the quality of SCS is measured by the specific volume of the expanded biscuits that are produced by scalding starch with a mixture of water, oil, and salt, followed by moulding and baking in the oven. The biscuits produced are then classified as having low (<5 mL g⁻¹), moderate (5–10 mL g⁻¹), and high (> 10 mL g⁻¹) expansion. Based on this parameter, the extruded products in the present work showed moderate to high expansion that was independent of the experimental conditions.

Data analysis showed a negative linear effect of CLF content, temperature, and moisture on the SV, and a positive quadratic effect on all the variable parameters. Significant correlations between CLF content and temperature as well as temperature and moisture were observed (Table 3). The SV increased at a lower percentage of CLF, moisture, and temperature. The correlation of CLF content and temperature is shown in Figure 1b.

Low moisture content may restrict the material flow inside the extruder barrel and increase the shear rate, which increases starch gelatinization, and thus, expansion.

The fibre and protein content of CLF can interfere in the extrudate matrix. The fibres weaken the extrudate by destroying the continuity of the aligned mass. Moreover, at a given temperature, higher moisture contents result in softer and less texturized extruded products as a result of the reduced protein-protein interactions and lower viscosity (Santillán-Moreno *et al.*, 2011).

Effect of variable parameters on colour and hardness

Colour in foods plays a vital role in determining the consumers' acceptance. Extruded snacks showed L^* , a^* and b^* values similar to the ranges observed by Trombini *et al.* (2016) for extruded products from cassava leaf flour. Among the variables of the process, the percentage of CLF had the highest effect on the colour parameter, followed by moisture and screw speed (Table 3).

The browning of the products observed with the conditions of high percentage of leaf flour and high moisture (Figures 1c, 1d, and 1e) may be due to caramelisation, the Maillard reaction, cooking degree, and degradation of pigments present in CLF (Altan *et al.*, 2008). If browning is too intense, undesirable colours and tastes may occur. Thus, the colour changes during the process can be considered as an indicator of chemical and nutritional changes.

The hardness is the maximum force required by a probe to penetrate the extrudate. The hardness of the extrudates ranged from 5.33 to 17.31 N, and were significantly affected by the CLF content, moisture, and extrusion temperature (Table 3). Products with higher hardness were obtained with higher amounts of leaf flour, higher moisture, and higher temperature (Figure 1f and 1g).

Increased hardness is not a desirable attribute for extruded snacks. Addition of high-fibre protein flour affects the texture and expansion of the extruded

snacks. Interestingly, at lower temperature and moisture, the hardness of the extrudate was lower. If the extrusion is carried out at high feed moisture, the vapour pressure will be low, which will result in a reduced expansion ratio and increased hardness (Chalermchaiwat *et al.*, 2015).

Effect of variable parameters on WSI and WAI

During extrusion, the biopolymers undergo transformations such as loss in crystallinity, starch depolymerisation, amylose-lipid reactions, denaturation, and protein cross-linking (Moisio *et al.*, 2015). The WSIs and WAIs of the extruded products are altered because of these transformations.

The WAI represents the ability of a product to associate with water. Moreover, water absorption in high-protein materials is a qualitative test to analyse the cross-linking of the protein.

Regression analysis showed a linear positive effect of leaf flour content on the WAIs, while moisture and screw speed had negative linear effects on the WAIs. Furthermore, temperature had a negative quadratic effect on the WAIs. Significant correlation was found between leaf flour content and moisture (Table 3). The response surface methodology results showed higher WAI values for a higher percentage of leaf flour and low moisture and temperature (Figure 1).

The increase in WAI with increasing CLF content can be attributed to the added fibre that simultaneously has a high water absorption capacity

Table 3. Regression coefficients of the coded models and p-value levels of significant terms.

	REI	SV	L	a	b	H	WAI	WSI	OA
β_0	3.84	11.31	48.54	0.948	16.20	9.02	4.82	33.13	5.76
β_1	-0.258***	-0.640*	-2.641***	0.154**	0.474***	1.395**	0.054*	-4.399***	-0.402**
β_2	ns	-0.677*	ns	ns	ns	0.790*	ns	ns	ns
β_3	ns	-1,479***	-1,116**	0,096*	0,203**	1,127**	-0,183*	-5,387***	-0.305**
β_4	ns	ns	-0,001*	0,005*	ns	ns	ns	2.240*	ns
β_{11}	ns	0,959**	ns	ns	0,145*	ns	ns	ns	ns
β_{22}	-0,145**	1,051**	ns	0,124*	ns	ns	-0.234*	1.202*	ns
β_{33}	-0,203***	0.675*	ns	0,153**	ns	1,011**	ns	ns	ns
β_{44}	-0,133**	0.786*	ns	ns	ns	ns	ns	ns	ns
β_{12}	ns	1.598**	ns	ns	ns	-1,43**	ns	ns	ns
β_{13}	ns	ns	ns	ns	ns	ns	-0,080*	1.107*	0.031*
β_{14}	-0,163*	ns	-0,873*	0,015*	0,140*	ns	ns	ns	ns
β_{23}	0.016*	-0.871*	ns	ns	ns	0,315*	ns	ns	ns
β_{24}	ns	ns	ns	ns	ns	ns	ns	ns	ns
β_{34}	ns	ns	ns	ns	ns	ns	ns	2.147*	ns
R^2	0.8306	0.8358	0.8726	0.7172	0.8576	0.7619	0.6979	0.8379	0.7074
R^2_{adj}	0.7824	0.7960	0.7939	0.6454	0.7939	0.6807	0.5684	0.6994	0.5785

$\beta_1, \beta_2, \beta_3, \beta_4$ = linear coefficients; $\beta_{11}, \beta_{22}, \beta_{33}, \beta_{44}$ = coefficients of quadratic variables; $\beta_{12}, \beta_{13}, \beta_{14}, \beta_{23}, \beta_{24}, \beta_{34}$ = coefficients of interaction between independent variables; REI = expansion index; SV = specific volume; L^*, a^* and b^* = colour parameters; H = hardness; WAI = water absorption index; WSI = water solubility index; OA = overall approval; ns=non-significant; *significant ($p < 0.05$); **significant ($p < 0.01$); ***significant ($p < 0.001$).

and reduced starch. Moreover, denaturation rendered the polar amino acids of the protein more accessible, which enhanced their affinity for water.

Similar to the observations in the present work, Aguilar-Palazuelos *et al.* (2007) observed in their study of the extrusion of corn starch and coconut fibre that an increase in the fibre content at low barrel temperature resulted in an increase in the WAIs of the extruded blends. However, the effect was reversed at high barrel temperature.

The WSI measures the rate of dissolution of the materials in water, and is an indicator of the degradation of the polymers (Mezreb *et al.*, 2003; Filli *et al.*, 2010; Awolu *et al.*, 2015). Regression

analysis showed significant negative linear effects of the leaf flour content and moisture and a positive effect of the screw speed on the WSIs. There was a significant correlation between the moisture content and screw speed on the WSIs (Table 3). The surface plot showed that the WSI was higher in the presence of low moisture, low amounts of leaf flour, and high screw speed, i.e., there was a greater degradation of the components of the raw material, which increased the amounts of soluble components. The increase in the screw speed and decrease in the moisture introduced high shear, which promoted the degradation of the macromolecules, thereby reducing the molecular weight and increasing the WSI (Figure 1k).

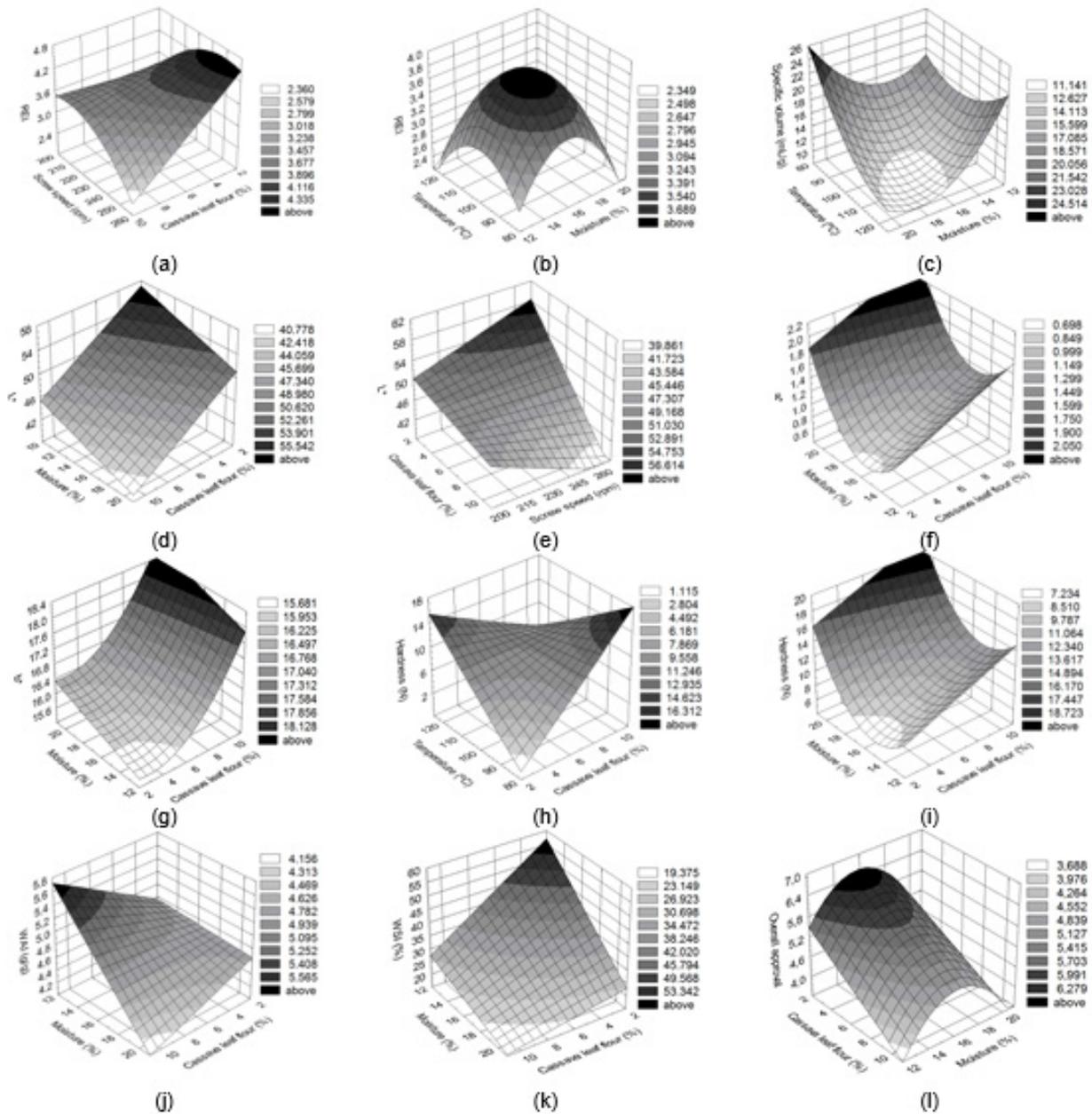


Figure 1. Effect of variable parameters of extrusion on the dependent variables. (a, b) radial expansion index (REI); (c) specific volume (SV); (d, e) luminosity (L^*); (f) a^* value; (g) b^* value; (h, i) hardness; (j) water absorption index (WAI); (k) water solubility index (WSI); and (l) overall approval.

Effect of variable parameters on sensory acceptance

The greater demand from consumers for snacks with different nutritional values has led to increasing research in flours with higher contents of functional compounds in the raw materials. Thus, the introduction of CLF into the extrudate formulation meets this demand.

The sensory acceptability of the extrudates was significantly affected by CLF percentage and feed moisture content (Table 3). Higher scores for overall acceptance were observed for products from extrusions with lower CLF percentages (2–4%) and moisture levels between 14% and 18% (Figure 1). Similar overall acceptance was observed by Chalermchaiwat *et al.* (2015) in germinated brown rice extruded at 18% feed moisture, regardless of the screw speed.

Conclusion

The parameters that had the most pronounced effects on extrusion were CLF content and moisture. Higher concentrations of leaf flour reduced the expansion, increased the hardness and browning of the products, increased the water absorption, and decreased the solubility. The results indicated that, aiming to obtain cassava gluten-free snacks with higher expansion and specific volume, lower hardness and darkening, low solubility index, and a good acceptance, all commercially desirable parameters for puffed snacks, the amount of leaf flour mixed with the sour starch should be up to 6%, and the moisture, temperature, and screw speed should be up to 16%, 100°C, and 230 rpm, respectively.

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References

- Aguilar-Palazuelos, E., Zazueta-Morales, J. J., Jiménez-Arevalo, O. A. and Martínez-Bustos, F. 2007. Mechanical and structural properties of expanded extrudates produced from blends of native starches and natural fibres of henequen and coconut. *Starch/Stärke* 59(11): 533-542.
- Altan, A., McCathy, K. L. and Maskan, M. 2008. Twin-screw extrusion of barley-grape pomace blends: extrudate characteristics and determination of optimum processing conditions. *Journal of Food Engineering* 89(1): 24-32.
- Anderson, R. A., Conway, H. F., Pfeifer, V. F. and Griffin, E. L. 1969. Gelatinization of corn grits by roll and extrusion cooking. *Cereal Science Today* 14(1): 4-12.
- Arendt, E. K. and Nunes, M. H. B. 2010. Processing gluten-free foods. In: Boye, J. I. and Godefroy, S. B. (Eds). *Allergen Management in the Food Industry*. New Jersey: John Wiley and Sons.
- Awolu, O. O., Oluwaferanmi, P. M., Fafowora, O. I. and Oseyemi, G. F. 2015. Optimization of the extrusion process for the production of ready-to-eat snack from rice, cassava and kersting's groundnut composite flours. *LWT-Food Science and Technology* 64(1): 18-24.
- Chalermchaiwat, P., Jangchud, K., Jangchud, A., Charunuch, C. and Prinyawiwatkul, W. 2015. Antioxidant activity, free gamma-aminobutyric acid content, selected physical properties and consumer acceptance of germinated brown rice extrudates as affected by extrusion process. *LWT-Food Science and Technology* 64(1): 490-496.
- Cooke, R. D. and Cruz, E. M. 1982. The changes in cyanide content of cassava (*Manihot esculenta* Crantz) tissues during plant development. *Journal of the Science of Food and Agriculture* 33(3): 269-275.
- Ding, Q-B., Ainsworth, P., Tucker, G. and Marson, H. 2005. The effect of extrusion conditions on the physicochemical properties and sensory characteristics of rice-based expanded snacks. *Journal of Food Engineering* 66(3): 283-289.
- Faubion, J. M. and Hosoney, R. C. 1982. High temperature and short time. Extrusion-cooking of wheat starch and flour. I- Effect of moisture and flour type on extrudate properties. *Cereal Chemistry* 59(6): 529-533.
- Ferrari, A. C., Leonel, M. and Mischan, M. M. 2014. Physical properties of snacks made from cassava leaf flour. *Semina Ciências Agrárias* 35(1): 317-326.
- Filli, K. B., Nkama, I., Abubakar, U. M. and Jideani, V. A. 2010. Influence of extrusion variables on some functional properties of extruded millet-soybean for the manufacture of 'fura': a Nigerian traditional food. *African Journal of Food Science* 4(6): 342-352.
- Food and Agriculture Organization (FAO). 2017. FAOSTAT: Production-Crops. Retrieved from FAO website: <http://faostat3.fao.org/home/E>.
- Kasprzak, M., Rzedzicki, Z., Wirkijowska, A., Zarzycki, P., Sobota, A., Sykut-Domańska, E. and Błaszczak, W. 2013. Effect of fibre-protein additions and process parameters on microstructure of corn extrudates. *Journal of Cereal Science* 58(3): 488-494.
- Kaur, G. J., Rehal, J., Singh, A. K., Singh, B. and Kaur, A. 2014. Optimization of extrusion parameters for development of ready-to-eat breakfast cereal using RSM. *Asian Journal of Dairy and Food Research* 33(2): 77-86.
- Kaur, S., Sharma, S., Singh, B. and Dar, B. N. 2015. Effect of extrusion variables (temperature, moisture) on the antinutrient components of cereal brans. *Journal of Food Science and Technology* 52(3): 1670-1676.
- Latif, S. and Muller, J. 2015. Potential of cassava leaves in human nutrition: a review. *Trends in Food Science and Technology* 44(2): 147-158

- Leonel, M., Martins, J. C. and Mischan, M. M. 2010. Production of functional snacks from soybean flour and sour cassava starch. *Ciência Rural* 40(6): 1418-1423.
- Markets and Markets. 2015. Gluten-Free Products Market worth 7.59 Billion USD by 2020. Retrieved from Markets and Markets website: <http://www.marketsandmarkets.com/PressReleases/gluten-free-products.asp>.
- Meng, X., Threinen, D., Hansen, M. and Driedger, D. 2010. Effects of extrusion conditions on system parameters and physical properties of a chickpea flour-based snack. *Food Research International* 43(2): 650-658.
- Mesquita, C. B., Leonel, M. and Mischan, M. M. 2013. Effects of processing on physical properties of extruded snacks with blends of sour cassava starch and flaxseed flour. *Food Science and Technology* 33(3): 404-410.
- Mezreb, K., Goullieux, A., Ralainirina, R. and Queneudec, M. 2003. Application of image analysis to measure screw speed influence on physical properties of corn and wheat extrudates. *Journal of Food Engineering* 57(2): 145-152.
- Moisio, T., Forssell, P., Partanen, R., Damerou, A. and Hill, S. E. 2015. Reorganisation of starch, proteins and lipids in extrusion of oats. *Journal of Cereal Science* 64: 48-55.
- Moraru, C. I. and Kokini, J. L. 2003. Nucleation and expansion during extrusion and microwave heating of cereal foods. *Comprehensive Reviews in Food Science and Food Safety* 2(4): 147-165.
- Nikmaram, N., Leong, S. Y., Koubaa, M., Zhu, Z., Barba, F. J., Greiner, R., ... and Roohinejad, S. 2017. Effect of extrusion on the anti-nutritional factors of food products: an overview. *Food Control* 79: 62-73.
- Omeire, G. C., Iwe, M. O. and Uchechukwu, N. 2012. Effect of extrusion variables on the hydrogen cyanide and haemagglutinin content of extruded blends of cassava products and African yam bean. *Food Science and Quality Management* 6: 23-31.
- Onitilo, M. O., Sanni, L. O., Oyewole, O. B. and Maziya-Dixon, B. 2007. Physicochemical and functional properties of sour starches from different cassava varieties. *International Journal of Food Properties* 10(3): 607-620.
- Ortega-Flores, C. I., Costa, M. A. L., Cereda, M. P. and Penteado, M. V. C. 2003. Avaliação da qualidade protéica da folha desidratada de mandioca (*Manihot esculenta* Crantz). *Nutrire: Revista da Sociedade Brasileira de Alimentação e Nutrição* 25: 47-59.
- Rathod, R. P. and Annapure, U. S. 2017. Physicochemical properties, protein and starch digestibility of lentil based noodle prepared by using extrusion processing. *LWT - Food Science and Technology* 80: 121-130.
- Salata, C. C., Leonel, M., Trombini, F. R. M. and Mischan, M. M. 2014. Extrusion of blends of cassava leaf and flour: physical characteristics of extrudates. *Food Science and Technology* 34(3): 501-506.
- Santillán-Moreno, A., Martínez-Bustos, F., Castaño-Tostado, E. and Amaya-Llano, S. L. 2011. Physicochemical characterization of extruded blends of corn starch-whey protein concentrate-Agave tequilana fibre. *Food Bioprocess and Technology* 4(5): 797-808.
- SAS Institute. 1990. SAS/STAT User's Guide: Version 6, Volume 2, p. 1351-1456. United States: SAS Institute Incorporated.
- Singh, S., Gamlath, S. and Wakeling, L. 2007. Nutritional aspects of food extrusion: a review. *International Journal of Food Science and Technology* 42(8): 916-929.
- Stojceska, V., Ainsworth, P., Plunkett, A. and İbanoğlu, S. 2010. The advantage of using extrusion processing for increasing dietary fibre level in gluten-free products. *Food Chemistry* 121(1): 156-164.
- Taverna, L. G., Leonel, M. and Mischan, M. M. 2012. Changes in physical properties of extruded sour cassava starch and quinoa flour blend snacks. *Food Science and Technology* 32(4): 826-834.
- Trombini, F. R. M., Mischan, M. M. and Leonel, M. 2016. Instant blend from cassava derivatives produced by extrusion. *Ciência Rural* 46(3): 573-579.
- Tsokolar-Tsikopoulos, K. C., Katsavou, I. D. and Krokida, M. K. 2015. The effect of inulin addition on structural and textural properties of extruded products under several extrusion conditions. *Journal of Food Science and Technology* 52(10): 6170-6181.
- Vargas-Solórzano, J. W., Carvalho, C. W. P., Takeiti, C. Y., Ascheri, J. L. R. and Queiroz, V. A. V. 2014. Physicochemical properties of expanded extrudates from colored sorghum genotypes. *Food Research International* 55: 37-44.
- Wang, Y.-Y. and Ryu, G.-H. 2013. Physical properties of extruded corn grits with corn fibre by CO₂ injection extrusion. *Journal of Food Engineering* 116(1): 14-20.
- Wobeto, C., Corrêa, A. D., Abreu, C. M. P., Santos, C. D. and Abreu, J. R. 2006. Nutrients in the cassava (*Manihot esculenta* Crantz) leaf meal at three ages of the plant. *Food Science and Technology* 26(4): 865-869.