Microstructure of an Extruded Third-Generation Snack Made from a Whole Blue Corn and Corn Starch Mixture

R.O. Navarro-Cortez[1], E. Aguilar-Palazuelos[1], J.J. Zazueta-Morales[1], J. Castro- Rosas[2], J. Hernández-Ávila[3] and C.A. Gómez-Aldapa[2,*], F.S. Aguirre-Tostado [4],

[1]*Universidad Autónoma de Sinaloa, Facultad de Ciencias Químico-Biológicas, Maestría en Ciencia y Tecnología de Alimentos, Boulevard Américas, Culiacán, Sin, C.P. 82000. México*

[2]*Universidad Autónoma del Estado de Hidalgo, Área Académica de Química,* [3]*Área Académica de Ciencias de la Tierra y Materiales, Ciudad del Conocimiento, Carretera Pachuca-Tulancingo Km. 4.5, Col. Carboneras, Mineral de la Reforma, Hgo, C.P. 42184. México*

[4]*Centro de Investigación en Materiales Avanzados, S.C., Alianza Norte 202,Parque de Investigación e Innovación Tecnológica,Apodaca, NL, C.P. 66600, México*

Abstract: Blue corn is a potential material for expanded snack production. Whole blue corn meal was mixed with corn starch and processed by extrusion to produce a third-generation snack. Optimum extrusion conditions were calculated with the response surface methodology using expansion index (EI), penetration force (PF), specific mechanical energy (SME) and total anthocyanins content (TAC). Optimum conditions (zone 1, 67°C; cooking zone, 123°C; zone 3, 75°C; feed moisture, 24.6%) were used to extrude the mixture in a single-screw extruder, and EI,PF,SME and TAC of the expanded pellet were compared against predicted optimum values. Starch structural changes in pellets and expanded were analyzed with DSC, viscosity profiles, x-ray diffraction and SEM. Extruded pellet did not differ ($p>0.05$) from the predicted. However, TAC was lower ($p<0.05$) in the expanded pellet. Structural analyses showed damage starch granular structure during extrusion and pellet expansion. Blue corn is a promising material for production of third-generation snacks.

Keywords: Extrusion, Expansion, Starch microstructure, Differential scanning calorimetry, Anthocyanins.

INTRODUCTION

Over the last thirty years, worldwide obesity incidence has increased substantially, making it one of the fastest growing diseases in the world. Data of World Health Organization (WHO) in the year 2012, 44 millions of child in the world below 5 years had overweight or obese, one in six adult in the world was obese, one in ten was diabetic, and one in three had raised blood pressure. The American continent has the highest obesity rates (26% of adults) [1]. In Mexico, obesity is the principal factor contributing to development of chronic, non-communicable diseases such as diabetes mellitus and cardiovascular diseases, the two main causes of death in the country [2,3]. In 2008, the costs attributable to obesity in Mexico totaled 42 billion pesos, but these could increase to 101 billion pesos by 2017 [4]. Recent data place obesity and overweight prevalence in Mexico at 71.28%, which represents 48.6 million people [5]. In response to this public health challenge, many snack manufacturers have begun modifying production processes to create low-fat products. Extrusion is very useful in producing low-fat improving snack foods and has the added advantages of protein and starch digestibility, solubilizing insoluble fiber, and inactivating toxins, anti-nutritional factors and undesirable enzymes such as lipo-oxygenases and peroxidases [6]. Extrusion of meals and other products with starch in their structure (*e.g*. cereals) is widely used in the food industry to produce snack foods [7].

White corn and yellow corn exhibit good expansion characteristics. Blue corn may also therefore have good physical and technological characteristics for snack food production. When used in the manufacture of a second-generation snack with added calcium hydroxide, it exhibited good technological characteristics for extruded snack production [8]. Blue corn contains anthocyanins, the most frequent being cyanidin 3-glucoside [9,10]. Anthocyanins are natural pigments safe for human consumption that can be used as food additives. Interest in anthocyanins as a promising alternative food coloring has increased in response to their coloring properties and potential health benefits [11]. They are exceptionally potent oxygen radical scavengers with known beneficial effects in diseases outside the cardiovascular system, but may also provide some benefits in heart disease [12].

The present study objective was to determine optimum processing conditions for third-generation snacks using a whole blue corn and corn starch mixture, and to

*Address correspondence to this author at the Universidad Autónoma del Estado de Hidalgo, Área Académica de Química, Ciudad del Conocimiento, Carretera Pachuca-Tulancingo Km. 4.5, Col. Carboneras, Mineral de la Reforma, Hgo, C.P. 42184. México ; Tel: +52 (771) 7172000 Ext. 6501;
Fax: +52 (771) 7172000 ext. 6502.E-mail: cgomeza@uaeh.edu.mx

evaluate anthocyanin content and starch structural damage after extrusion and microwave expansion.

MATERIALS AND METHODS

Raw Materials

Commercially available blue corn (*Zea mays* L. var. chalqueño) was purchased in a local market in the State of Mexico. Normal corn starch was purchased from Grupo Emsiana S.A. de C.V. (Puebla, Mexico).

Meal Production

Blue corn grain was ground in a hammer mill with a 1 mm mesh. The resulting meal was mixed with normal corn starch at a 65:35 ratio and 0.1% monoglycerides added to improve final product appearance.

Process Optimization

Optimization of the manufacturing process for a third-generation snack from whole blue corn and corn starch was done using the response surface superposition methodology. The response variables used in the optimization of the extrusion process were the expansion index (EI), penetration force (PF), specific mechanical energy (SME) and total content anthocyanins (TAC). These responses were selected based on the most important traits required in a third-generation snack. The SME was used to evaluate process energy expenditure and indirectly determine starch granule damage. Total anthocyanins content (TAC) was quantified due to their ability to neutralize damaging reactive species in the human organism. Once the optimum processing area was calculated, the central point was taken and these optimum extrusion conditions were tested experimentally. The design experimental used was a central composite rotatable where was evaluated the effect of extrusion temperature (98.79-141.21) and moisture feed (19.93-34.07) (Table **1**), on the responses variables EI, SME, FP and TAC. A linear regression analysis was conducted to relate each response with extrusion temperature and moisture feed. The response surface model used for each response was with main, interactions, and quadratic terms:

$$y = \beta_0 + \beta_1 x_1 + \beta_2 x_2 + \beta_{12} x_1 x_2 + \beta_{11} x_1^2 + \beta_{22} x_2^2$$

Table 1: **Rotatable Central Composite Design used to Evaluate Responses for EI, PF, SME and TAC**

	Levels				
Independent Variables	-α	-1	0	+1	+α
Temperature (°C)	98.79	105	120	135	141.21
Moisture feed (%)	19.93	22	27	32	34.07

Extrusion Process

A single-screw laboratory extruder (Brabender Instruments Inc., model 20DN/8-235-00, CW, Duisburg, Germany) was used with a 19 mm internal barrel diameter, 20:1 length/diameter ratio, a 2:1 screw compression ratio and a rectangular matrix with a 20 x 1.5 mm exit aperture area. The extruder barrel was divided into two independent heating zones (feed and cooking), using air circulation to maintain an accurate temperature. Feed zone temperature was 67±3°C, cooking zone temperature was 122±3°C and exit matrix temperature was 75±3°C. The blue corn/corn starch mixture was fed into the extruder at a 1.56 kg/h feed rate with an 80 rpm screw speed, under these conditions the mixture of blue corn and starch flowed continuously into the extruder and the product had good appearance. Cooking zone temperature (123°C) and mixture feed moisture content (24.6%) were selected from the optimization graph (Figure **1**). The resulting products were analyzed to quantify their physical and chemical characteristics.

Characterization of Extruded Products

The strips produced in the extrusion process were cut into 3 cm-long pellets and expanded in a microwave oven (LG® brand, model R-501CW, 900 W/2450 Hz power, Beijing, China) for 26s; were tested different times of expansion in the microwave oven 20, 23, 26, 29 and 32s, shorter times to 26s the pellet not expanded complete and times higher to 26s the pellet was burning. Randomly were selected lots of 30 pellets and were expanded. Expansion index was measured for the expanded pellets using the seed displacement method [13], with millet as the seed.

The force required to penetrate the expanded product was measured with a penetrometer (Chatillón TCD 200, Viennacourt, Lammas Road, Godalming, Surrey, UK). Expanded samples were placed horizontally on a 1 cm thick plastic platform and penetrated with a 2 mm flat tip cylindrical probe. Probe descent rate was 0.83 mm.s^{-1}, and maximum penetration distance was 3 mm. Thirty measurements were taken per treatment and values reported as Newton's (N).

Specific mechanical energy is a numerical value expressed as energy per weight unit (J/g). It is calculated from the torque (T) (N*m), screw speed (Ss, rpm) and feed rate (F, g/min) values:

$$SME = 2\pi TSs/F$$

Specific mechanical energy is defined as the net mechanical energy per mass unit required by the extruder to turn the screw, and provides data on extruder operation [14].

Total anthocyanins content was measured according to Abdel-Aal & Hucl, 1999 [15]. Samples containing 9-13% moisture were ground into meals and passed through No. 60 mesh. From these meals, a 1 g sample was placed in a 50 mL centrifuge tube with 8 mL acidified ethanol (85 mL 95% EtOH + 15 mL 1.0 N HCl), the tube was manually agitated for 2 min and pH adjusted to 1.0 with a water distilled:HCl (1:1) solution. The tube was agitated for 30 min and pH readjusted if necessary. It was then centrifuged at 3220 x g for 30 min at room temperature, the supernatant completed to 25 mL with acidified ethanol and absorbance read at 535 nm using a spectrophotometer (UNICO SQ 2800, NJ, USA). Acidified solvent was used as a blank. The absorbance value was inserted into this equation:

$$C=(A/E) \times (TV/1{,}000) \times MW \times (1/SW) \times 10^6$$

where

C = Total anthocyanins content ($mg \cdot kg^{-1}$),
A = Absorbance at 535 nm,
E = Molar absorption coefficient (cyanidin 3-glucoside= 25,965$cm^{-1}L/mol$),
TV = Total volume (mL) of anthocyanins extract,
MW = Molecular weight of cyanidin 3-glucoside (449 g/mol),
SW = Sample weight.

Samples were analyzed in a differential scanning calorimeter (Mettler-Toledo, 822e, Switzerland), calibrated with indium. A microsyringe (Hamilton Company, USA) was used to inject 16 mg distilled water to 4 mg sample into a 40µL aluminium capsule. Capsules were placed in a heating chamber ventilated with nitrogen (20 mL/min), and analyzed in a range of 30 to 130°C at the rate heating of 5°C/min. Enthalpy and onset peak and final temperatures were measured with the STARe Thermal Analyzer ver. 8.1 software.

Viscosity of extruded products was measured at 92°C with a Rapid Visco-Analyzer (RVA 3C, Newport Scientific PTY Ltd., Sydney, Australia), following manufacturer instructions. Briefly, 2 g sample was adjusted to 28 g with distilled water in an aluminium slide. The sample-water suspension was kept under constant agitation and heated in the following sequence: 50°C for 1 min; heated to 92°C at 5.5 °C/min; held at 92°C for 5 min; cooling to 50°C at 5.6°C/min; and held at 50°C for 2 min (Newport-Scientific 1992). The resulting viscosity profiles were used to measure initial viscosity (V_{ini}); viscosity at 92°C (V_{92}); minimum viscosity (V_{min}) (*i.e*. the lowest viscosity value during the 92°C constant temperature); and final viscosity (V_{fin}) (*i.e*. maximum viscosity during cooling period). Retrogradation viscosity (V_r) (*i.e*. V_{fin} - V_{min}) was calculated from these parameters [16].

Samples of the unprocessed meal, the pellet and the expanded product for each treatment were packed into a 0.5 mm deep glass slide and placed in a Rigaku x-ray diffractometer (Ultima D/Max-2100, RigakuDenki Co. Ltd, Japan). The X-ray source was CuK_α with a wavelength of 1.5406°, 30kV, and 30 mA. Data were collected of 0-40° on a 2θ scale (2θ is the angle of diffraction to the incident beam)

Samples were analyzed with a scanning electron microscope (INCAx-sight 6650, Oxford Instruments, Abingdon, Oxfordshire, UK) equipped with a secondary electrons detector. Ground samples (<60 mesh) were placed in a PIN-type aluminum of 12 mm diameter previously prepared with double-sided carbon tape. For the blue corn / corn starch mixture, micrographs were taken at 10 KV and 1200 magnifications, while for the pellets and expanded product they were taken at 67 magnifications.

RESULTS AND DISCUSSION

Optimization

Food processed by extrusion containing starch can expand from 2 to 9 fold, depending on starch type and processing conditions, including temperature and moisture content. Optimization criteria were a minimum EI value of 4.1 and a maximum PF value of 13, both of which are used for commercially available products. Based on reported SME values for blue corn products (<250 kJ/kg) [8], maximum acceptable SME was fixed at 200 kJ/kg. Minimum TAC was established as 70 mg anthocyanins/kg sample, since this is the value recorded for blue corn and corn starch expanded products with EI and PF values similar to those of commercial snack products.

Optimum extrusion conditions for the third-generation snacks made from blue corn and corn starch were 120-126 °C and 23.80-25.20% moisture content (Figure **1**). The central point (123 °C, 24.6% moisture) was selected and validated experimentally. Predicted values for this point were EI = 4.10±0.04; PF

= 12.42±0.31 N; SME = 169.08±1.85 J/g; and TAC = 71.09±1.10 mg anthocyanins/kg sample. Pellets produced using the central point extrusion conditions exhibited average values of EI = 4.47± 0.07; PF = 11.45±0.49 N; SME = 185±5.5 J/g; and TAC = 61 ± 1.74 mg anthocyanins/kg sample. Only anthocyanin content differed ($p<0.05$) between the central point and the experimental products. All other values did not differ ($p=0.05$). Therefore, the model is effective for producing a third-generation snack using extrusion technology.

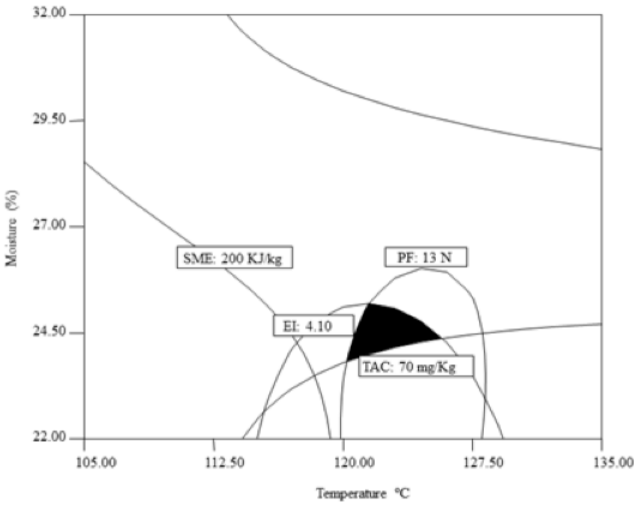

Figure 1: Optimum processing area (black zone) for a third generation extruded snack made from a whole blue corn / corn starch mixture.

Anthocyanin Content

Anthocyanins content in the mixture was 248.67mg/kg mixture prior to extrusion. Processing under the optimum conditions caused the loss of 75% of anthocyanins, which was expected since anthocyanin degradation is a function of temperature and exposure time [17]. These results are similar to those for breakfast cereal made with extruded corn and blueberry or grape concentrates in which anthocyanin loss was 70% in the samples containing grape and 90% in those containing blueberry [18]. Use of raw materials with anthocyanins in their structure could be used to produce colored products, thus reducing the need for artificial colorings in extruded products and providing health benefits. Anthocyanins are very effective at trapping free radicals due to their OH^- groups, and consequently can provide an anti-inflammatory effect, protection against liver damage, reduction of blood pressure and improved vision [19]. Using higher screw speed and feed moisture higher may help to retain higher anthocyanin in the extrudate, due to a decrease in the residence time in the extruder barrel.

Differential Scanning Calorimetry

Calorimetric techniques are used to study the structure and phase transitions in pure starch in food systems, and help to identify structural changes caused by heat and moisture during processing [20]. The DSC thermogram for the blue corn/corn starch meal showed values (onset = 64.4 ºC; peak = 69.45 ºC, end = 73.67 ºC) indicating the starch had not been submitted to hydrothermal processing, exhibited no structural damage and had the capacity to form a gel in the presence of excess water and high temperature (Figure **2**). The pellet, and expanded product, not showed thermal signal, meaning that their structures contained no starch with a crystal organization. The thermomechanical process completely gelatinized the starch, probably degrading it by the applied shear force. These results agree with the statement of Chinnaswamy and Hanna [21] that processed starch does not exhibit a gelatinization peak because the starch's crystal structure has been transformed or the starch completely gelatinized. In a study of corn products extruded at 90 and 100 °C, no endothermal peak was observed since at these temperatures the starch is completely gelatinized [22]. No thermal signal is observed in extruded products with high EI values [23], however, absence of a gelatinization peak does not guarantee that a starch has completely lost its granular and crystalline structure [24].

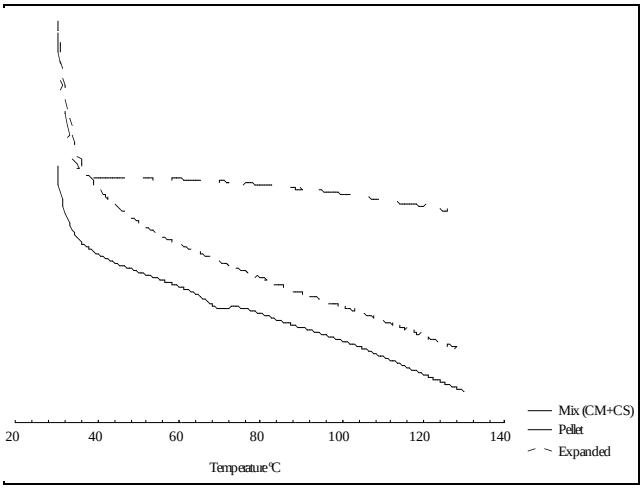

Figure 2: Differential scanning calorimetry thermograms for the whole blue corn (CM)/corn starch mixture (CS), extruded pellets and expanded products.

Viscosity Profiles

Viscosity profiles are analyzed by adding water to ground or powdered products and monitoring changes

in viscosity during a temperature cycle. Paste viscosity is another way of evaluating degree of degradation in starchy materials during thermal and mechanical treatments. Severe treatments destroy starch granular structure, consequently diminishing hot paste viscosity [25]. The viscosity values (V_{ini}, V_{92}, V_{min}, V_{fin} and $V_{setback}$) of the studied meal, pellets and expanded products confirm this since they indicate that starch granule integrity had been affected by the heat and shear force of extrusion (Table **2**). This damaged the starch's water absorption capacity at high temperatures, lowering its gelling capacity and consequently affecting paste viscosity. In a study of puffed cereal grain meals, paste viscosity decreased due to starch structural changes [26]. There was a clear difference visible in the present results between the paste forming capacities of the native and processed starches. Processing also favored amylose precipitation, affecting V_r since a native or unprocessed starch has a greater capacity to retrograde.

Table 2: Viscosities at Different Heating Times for Whole Blue Corn/Corn Starch Mixture, Extruded Pellets and Expanded Products

Viscosity (cP)					
Material	**V_{ini}**	**V_{92}**	**V_{min}**	**V_{fin}**	**V_r**
Mix (CM+CS)	76	4797	2825	6664	3839
Extruded Pellets	220	824	617	878	261
Expanded Products	194	365	326	387	61

V ini = Initial viscosity; V92 = 92 ºC viscosity; V min = Minimum viscosity; V fin = Final viscosity; Vr = Retrogradation viscosity.

In the viscosity profiles, the meal exhibited viscosity until heated, whereas the pellets and expanded products had water adsorption capacity when cold and developed viscosity, although with values lower than for the native starch (Figure **3**). This agrees with Becker *et al*. [27], who stated that thermal processing of starches produces a viscosity peak at cold temperatures, but when the starch is heated in water this peak is lower than in intact starch granules. In the present results, this behavior can be attributed to changes in starch internal structure caused by thermo-mechanical treatment of the blue corn/corn starch mixture. In this restructuring, hydrophilic starch groups are exposed to water, and the principal changes occur when granule crystalline regions become amorphous as the granule fuses, thus favoring water uptake in cold temperatures.

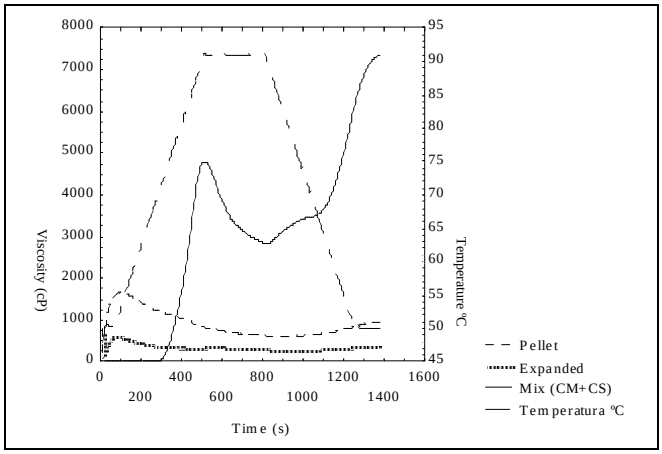

Figure 3: Viscosity profiles for the whole blue corn (CM) / corn starch (CS) mixture, extruded pellets and expanded products.

Native starches require water and heat to swell, a phenomenon, which favors hydrogen bond rupture and loss of packing among polymer molecules within the granule. If it's crystalline structure is broken by mechanical methods or heating in water, the granule exhibits paste viscosity at cold temperatures, which is indicative of severe starch granule disruption during processing [28].

X-ray Diffraction

At the 2θ scale used here, the blue corn/corn starch mixture exhibited XRD values of 14.92, 17.62 and 22.76 º (Figure **4**). These values correspond to an A-type diffraction pattern found in cereal starches like those in the present study. Both the pellets and expanded products had values of 13 and 20 °, the latter corresponding to a V-type pattern indicating amylose-lipids complex formation [28]. Extrusion involves high temperatures, and at high temperatures complexes may form between amylose and free lipids in the raw material, which favor formation of type-II lipid-starch complexes [29]. These diffraction patterns have also been observed during amylose molecule re-association into double helices within the starch's three-dimensional network. These results also agree with those of Miyoshi [30], who observed peak formation in hydrothermally-treated corn and potato starches at a 2θ angle of 19.5 º. This corresponded to a V-type pattern caused by amylose complex formation and amylose recrystallization. High temperatures can completely destroy starch structure, producing an amorphous state on XRD graphs, or can induce formation of a new structure [31]. Formation of starch/lipid complexes is favored when lipid chains are small. Formation of these complexes can provide health benefits because it reduces the amount of lipids available for

metabolization by the organism. It also reduces sugar availability, as shown in in lower water-soluble solids values, a measure of starch dextrinization [32].

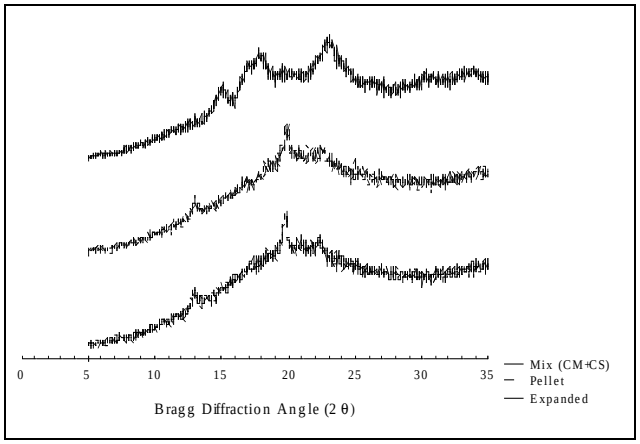

Figure 4: X-ray diffraction patterns for the whole blue corn (CM)/corn starch (CS) mixture, extruded pellets and expanded products.

Scanning Electron Microscopy

Extrusion caused a number of visible changes in starch structure related to loss of granule shape, such as gelatinization, plasticization and rupture of intramolecular bonds. Native corn starch granules exhibited a polyhedral shape with some surface marks, probably due to the extraction process (Figure **5a**). The round starch granules and aggregates, which are bonded by protein, are from the blue corn.

The extruded pellet produced using optimum processing conditions (122 °C, 24.60% moisture) contained no intact starch granules, probably because these either melted or plasticized due to the heat and moisture of extrusion (Figure **5b**). Cavities on the pellet surface were probably formed by water evaporation at the moment the sample exited the barrel. Interior pressure in the extruder barrel and matrix is higher than room temperature, creating a decompression

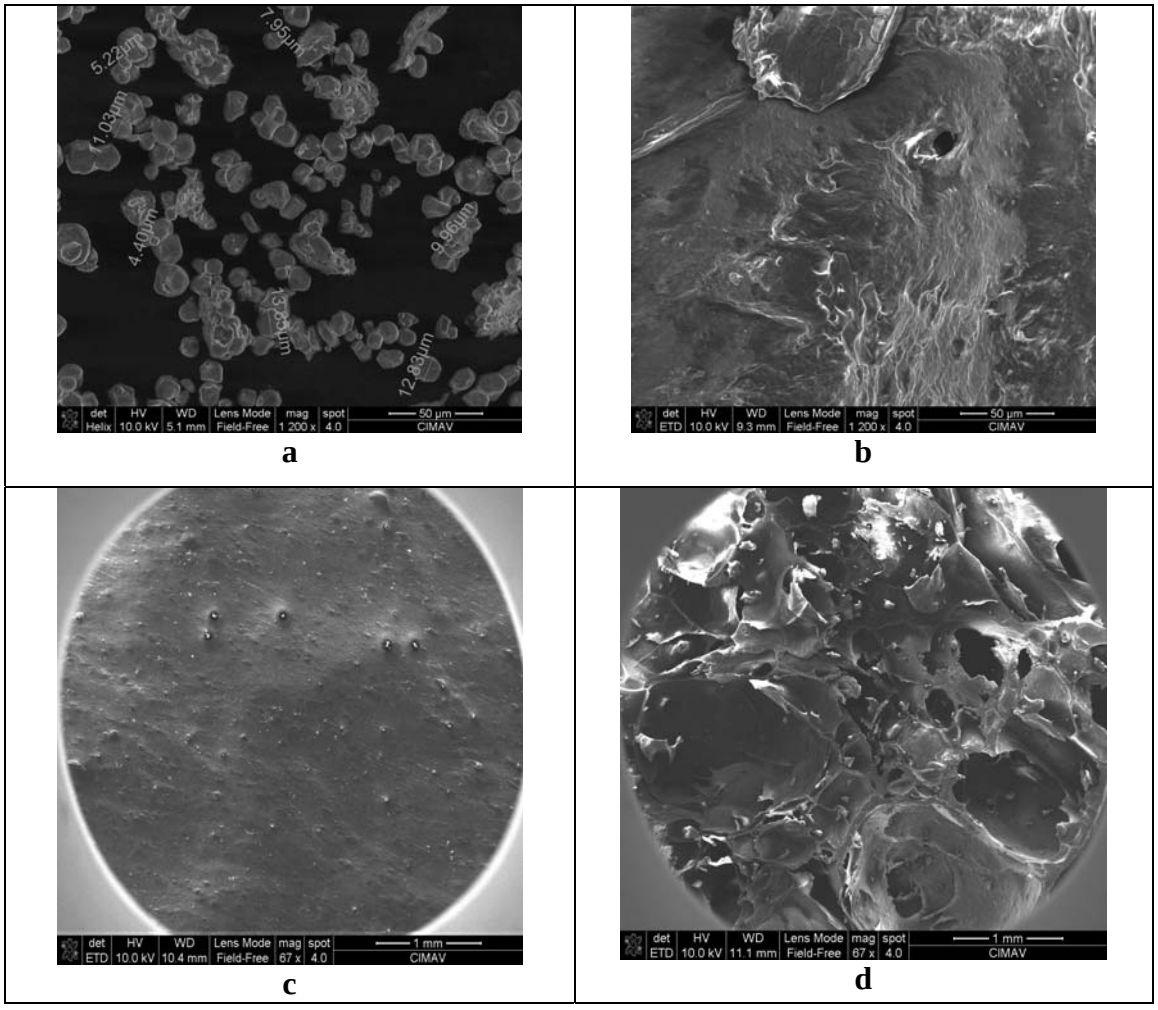

Figure 5: Scanning electron micrographs (1200X) of **a**: whole blue corn/corn starch mixture; **b**: extruded pellet; **c**: extruded pellet (67X) and **d**: microwave expanded products.

effect and rapid water evaporation as the material exits the barrel. This is supported by Aguilar-Palazuelos [33], who stated that a liquid mass tends to cool as the water it contains evaporates and as it reaches a certain cooling temperature the material acquires its final physical form, with marks from water vapor transit.

Transversal cuts were made across the optimum processing region of the pellets and expanded products. At 67X magnification, the pellets exhibited a smooth structure with white dots in the surface (Figure **5c**). These dots were probably endosperm fragments that grinding had not completely reduced and did not melt during extrusion due to their larger size. The expanded product had a porous structure with thin-walled gas cells of different sizes throughout the analyzed area (Figure **5d**). This structure is similar to that reported for a third-generation snack produced with sorghum and expanded in hot oil [34]. These cells in the expanded pellet were created by sudden pressure release of water vapor within the pellet. This is a process similar to rupture of the pericarp in popcorn [35] or puffed rice [26], in which vaporized water creates excess pressure inside the grain, resulting in rupture. In the present case, microwaves raised water temperature by creating friction between the water molecules. As pellet internal temperature and pressure increased, it reached a point when the internal structure could support no more pressure, after which it ruptured and expanded, giving the cells an elongated shape [36].

CONCLUSION

The analyzed optimum processing conditions were adequate for producing a third-generation snack from a mixture of whole blue corn meal and corn starch. Physicochemical characteristics were acceptable and process energy use as measured by SME was low. Anthocyanins content was lower than predicted by the optimization model. The extrusion cooking destroyed the starch granular structure, and favored bonds between starch and free lipids in the raw material and between starch and added monoglycerides. Formation of these complexes may provide health benefits since it reduces available lipids and sugars content.

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