Effect of Açaí Powder and Chitosan Incorporation on Bread Quality

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Abstract—**The food industry trend is to produce foods with better nutritional quality. Within this proposal, the objective of this work was to study the inclusion of açaí powder and chitosan in bread formulations and to evaluate their quality. For the development of the bread formulations, the central rotational design was used putting the açaí power and chitosan as independent variables. Flour quality analyzes were performed (water hydration properties), dough and bread quality analyzes (fermentation time optimization, maximum expansion factor, crumb structure, crust and crumb color and specific volume). The quality analyzes were studied through regression and correlation analysis and response surface methodology at the 5% level of significance. After the determination of the optimized condition of açaí powder and chitosan was carried out the evaluation of the centesimal composition and scanning electron microscopy. The results showed that both açaí powder and chitosan significantly influence the flour properties. It was verified that the açaí powder promotes greater modifications in the physical parameters of the dough and the bread than the chitosan, which was confirmed by correlation analysis. The optimum conditions the inclusion of açaí power and chitosan was of 8.5 g and 0.75 g respectively. There was a improvement in the nutritional quality of the bread optimized, in comparison to the control, with the increase of the protein content and ashes. Microscopy showed an interaction between starch and chitosan, as well as the harmful effect of açaí powder on gluten network.**

Index Terms—**Açaí, chitosan, bread, quality**

I. Introduction

In recent years, there has been a noticeable increase in the application of vegetable proteins in the food industry owing to their unique functional properties [1].

Euterpe oleracea Mart. is a palm tree that occurs in the North of Brazil, with a high socioeconomic importance for the Amazon region. Its fruits are spherical, constituted by a slightly hard seed, involved by a greyish, oleaginous pulp, covered by a dark purple epidermis. From such fruits, the açai pulp is obtained; a product with high energetic value, nutritive potential, with high fiber content, proteins, lipids and anthocyanins [2]. The açai phytochemical composition (high polyphenols content, mainly anthocyanins and flavonoids) and its high antioxidant capacity have being linked to a range of health-promoting benefits, such as anti-ageing, anti-inflammatory, ant proliferative and cardio protective properties [3].

Chitosan, the *N*-deacetylate derivative of chitin, as one of the most promising biological macromolecule polymers has been widely studied and used because of its distinctive biomedical properties such as biocompatibility, biodegradability, antibacterial activity, nontoxicity [4], and are positively charged dietary fiber [5].

Cereal products could be one of the most important sources of bioactive compounds, which could make food functional [6].

Thus, the inclusion of açaí powder and chitosan can improve the nutritional quality of the bread. However, these ingredients not forming gluten network, that may affect quality of the bread. The aim of this study was to evaluate the effect of açaí power and chitosan inclusion through the response surface methodology in bread quality properties.

II. Material and Methods

A. Materials

Commercial Wheat flour enriched with iron and folic acid (composed of 72.5% carbohydrates, 12.5% protein, 14.0% water, 0.6% fat, and 0.4% ash), refined sugar; Dry yeast (*Saccaromyces Cerevisae*), Hydrogenated Vegetable Fat (HVF), refined salt, Chitosan (84% degree of deacetylation, Polymar, Brazil) and açaí powder (Sun Foods, Brazil) were used in the bread formulations. The particle size of the chitosan and açaí powder was set at 20 mesh (0.84 mm) by sieving.

B. Methods

1) Bread formulation

The bread dough control formulation comprised 300.0 g of wheat flour, 120.0 g water, 30.0 g hydrogenate vegetable fat, 15.0 g refined sugar, 11.0 g dry yeast, 2.0 g salt and the inclusion of chitosan and açaí powder was based on the Central Composite Rotational Design (CCRD).

2) Experimental design and bread making process

Bread samples were prepared with chitosan and açaí powder at different proportions as presented in Table I. Second order design matrix used for the evaluation of the

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effects of process variables on some physical properties of dough and bread.

All ingredients were mixed in a semi-industrial mixer (Lieme, Brazil), following a preliminary mixing of dry ingredients for 1 min at low speed. Mixing was done for 3 minutes at medium speed and for 6 minutes at high speed. A quantify of water was added in the mixer at the beginning of the medium speed period. Dough temperature at the end of mixing was T = 26.0 ℃ ±1.0 ℃. After the mixture the dough was left to rest for 5 min and then divided in pieces of 100.0 g and hand-molded in an ellipses form. Dough fermentation was performed in fermentation chamber (Perfecta, Brazil) at 28.0 ℃±2.0 ℃ and 70.0% relative humidity for two hours in fermentation process. The doughs were baked without stream at 220.0 ℃ for 20 minutes and cooled at room temperature (28.0 ℃±2 ℃).

TABLE I. SECOND ORDER DESIGN MATRIX USED FOR THE EVALUATION OF THE EFFECTS OF PROCESS VARIABLES ON SOME PHYSICAL PROPERTIES OF DOUGH AND BREAD

Runs	Chitosan (x_1)	Açaí powder (x_2)	Chitosan (g)	Açaí powder (g)
1	-1	-1	0.45	5
2	+1	-1	0.45	15
3	-1	+1	1.35	5
4	+1	+1	1.35	15
5	-1.41	0	0.9	0
6	+1.41	0	0.9	20
7	0	-1.41	0	10
8	0	+1.41	1.8	10
9	0	0	0.9	10
10	0	0	0.9	10
11	0	0	0.9	10

3) Water hydration properties

The water hydration properties of white wheat flour and wheat flour added of chitosan and açaí powder were characterized according [7]. Each flour sample (0.5 g) was mixed with distilled water (30 mL) at 100 ℃ for 30 min. After centrifugation at 15.000 *g* for 20 min, the supernatant was oven-dried at 105 ℃. Three water hydration parameters (water absorption index, water solubility and swelling power) were calculated based on the following equations:

Water Absorption Index (WAI) = wet sediment weight/dry sample weight. (1)

Water Solubility (WS, %) = (dry supernatant weight/dry sample weight) x 100. (2)

Swelling Power (SW) = wet sediment weight/[dry sample weight x (1 – WS/100)]. (3)

4) Fermentation time optimization

Realized according [8] where Dough (50 g) was placed in a 500 mL graduated cylinder with a plunger mobile and inserted in a fermentation cabinet (Perfecta, Brazil) at 28 ℃ for 240 min. Dough volume (mL) was recorded during 4 h: every 10 min the first 2 h and the ulterior 2 h, volume was measured every 30 min. Measurements were performed in duplicate. The increase in volume (ΔV) was registered as a function of time and curves were adjusted by the STATISTICA software (version 9.0, Statsoft, Tulusa, UK).

$$\Delta V = V_{max} \text{ x } c \, [1 - \exp(-bt) \ . \quad (4)$$

where ΔV is the volume increment, t is the time and V_{max} correspond to the maximum volume increment achieved; b and c are constants. Fermentation time (t_f) is the time required for achieving 3/4 of V_{max}, because during the beginning of the baking process the fermentation continues until the structure is established.

5) Maximum expansion factor

Maximum Expansion Factor of dough was determined according [9], considering the dough like a truncated ellipse.

6) Specific volume

Specific volume of bread was determined as a ratio of volume and weight [10]. Five breads of each formula were analyzed.

7) Crumb color

The color of the crumb was determined using the CIELAB parameters (L*, a*, b*). The visual sensations that are sent to the brain create the three dimensions of colour judgment response that is often referred to as three-dimensional colour space. In the CIE Lab system, these dimensions are expressed as: L* related to lightness varying from black (zero) to white (100), and the other two are related to chromaticity: a* from green (-a*) to red (+a*) and b* from blue (-b*) to yellow (+b*). The crumb color analysis was conducted in the center bread slice with thickness of 20mm. Forty measurements were averaged for each sample using Colorimeter Minolta CR-300 (Osaka, Japan), with a measurement area of 8 mm and geometries d/0°, 10° viewing angle and standard illuminant D65.

8) Crust color

Color measurements of bread crust (20 measurements/runs) were performed. Results were expressed as browning index (BI) according to the following equations, according [11] and [12].

$$BI = 100 \text{ x } (X - 0.31)/0.172 \quad (5)$$

$$X = a^* + 1.75L^*/5.645L^* + a^* - 3.012b^* \quad (6)$$

9) Crumb structure image analysis.

Bread crumb structure was determined by digital image. Images were obtained from digitalization at a 550 dpi resolution on a HP ScanJet 2400 scanner, on the crumb's central area with a resolution of 900x900 pixels. Images were analysed by the ImageJ® 1.47v software (National Institute of Health, USA). Images were salved on jpeg format and cut to a field view of 900x900 mm, captured colored images were converted to 8-bit in shades of gray and thresholded by the Otsu algorithm. It was possible to obtain the number of alveolus and pore circularity values [13].

10) Proximate composition of chitosan, açaí powder and breads

Proximate composition of the control and optimized formulation was determined using AOAC Methods [14]. Moisture content was determined using the oven drying method, 925.10; crude protein by Kjeldahl digestion and distillation (N x 5.7 for bread; N x 5.66 for chitosan and açaí powder) was measured according to the Method 920.87. Crude fat was determined by hexane extraction using Method 945.16 and ash was determined by dry-ashing at 550 °C according to the Method 923.03. Total available carbohydrates were calculated by difference, i.e. 100 − (% moisture + % protein + % fat + % ash).

11) Scanning electron microscopy (SEM)

The surface of the polymer encapsulated formulation was observed using a FEI Inspect S50 Scanning Electron Microscope (at low energy of 10 kV). The samples were deposited on carbon tapes and coated with gold, using vapor deposition techniques. The surface was scanned using a magnification between 5.000 and 40.000×.

12) Statistical analysis

In the study, a 2-factor-5 level Central Composite Rotational experimental design was chosen for the modeling of processing variables (chitosan and açaí powder). The predictive regression models were constructed for all parameters of analysis. Second-order polynomial equation of function X_i as stated below was fitted for each response analyzed:

$$Y = \beta_0 + \beta_1 X_1 + \beta_2 X_2 + \beta_{11} X^2 + \beta_{22} X^2 + \beta_{12} X_1 X_2$$

where Y is the estimated response; b_0, b_i, b_{ii}, b_{ij} are constants. X_i and X_j are processing variables (Chitosan and Açaí Powder). Real values were used for analysis. The experimental combinations were implemented in triplicate in the center point of the model for the estimation of the experimental variance. The response surface analysis was carried out using STATISTICA software (version 9.0, Statsoft, Tulusa, UK), ANOVA, regression surface response and correlation analysis were performed.

III. Results and Dicussion

A. Water Absorption Properties and Browning Index

Central Composite Rotational design of response surface methodology was chosen to show the main effects of chitosan and açaí powder addition and their interactions on the water hydration and color properties. The Water Absorption Index and Water Solubility of samples increased significantly with increasing of chitosan and açaípowder addition.

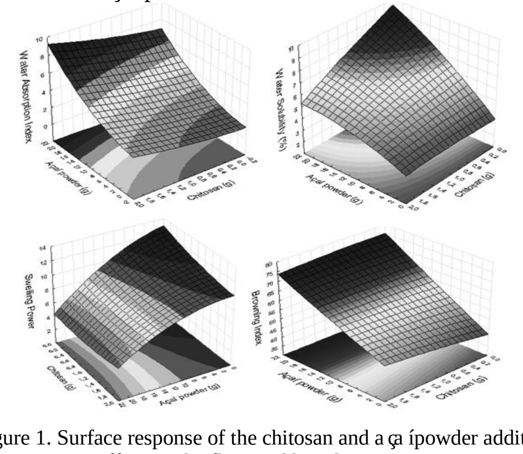

Figure 1. Surface response of the chitosan and açaípowder addition effect on the flour and bread properties

Effect of chitosan and açaí powder levels on water hydration properties of flour and browning index of crust is shown in Fig. 1.

WAI of flour increased with increase in açaí powder level, due to presence of hydrophilic compounds. According [14], the açaí have many compounds in your composition, among them stand out Phenolic Compounds and Anthocyanins. These compounds are, chemically, polar molecules and have affinity for water, increasing the capacity of water absorption of flour.

Addition of chitosan provided smaller variations in WAI. The same behavior occurs to WS, this parameter increased to values more 7.0% with 10 g of açaípowder without chitosan addition. The repulsion to water can be explained by the electron pair repulsion of water molecule with the electron pair of the nitrogen atoms of monomeric units of chitosan, featuring two molecules as strong Lewis base.

Swelling Power decrease with açaí powder addition, this behavior is relation with the dilution of starch amount and the inclusion of proteins that may have been attached to the starch. The variation of flour swelling power was 13.20 for the control formulation to 5.11 for run 6 added 20 g açaí power and 0.9 g chitosan. [15] reports that interactions between these polymers could also be rearranged. During heating the gelatinization of starch and the denaturation of gluten occur due to heat-induced conformational changes, thus the smaller amounts of starch are starch available to promote the swelling power of flours. The reduction of the starch available for gelatinization process can be explained by forming the protein-starch [16] and chitosan-starch [17] complex.

The crust of bread presented browning as increases the amount of Açaí powder added. The run 7 obtained the largest value of browning index (77.30), due to the addition of 20 g Açai powder without chitosan inclusion. Coloring pigments present in açaí such as flavonoids and anthocyanins, may have contributed to the browning of the bread crust. [18] found L* values near to 25.97 indicating that the product is very dark, which contributed to the browning of bread crust.

Other ingredients can be promotes the increase of browning in breads and bakery products: oat fiber [19], wheat muffins [20] and Maillard reaction [21].

Table II shows the mathematical models that represents the dependent variables in function of independent variables.

B. Bread and Dough Properties

Fig. 2 show the surface response for Vmax, alveoli number, specific volume and maximum expansion factor of dough.

Maximum volume and maximum expansion factor of dough, Alveoli Number and Specific volume of bread was reduced significantly ($p \leq 0.05$) by the ingredients addition. In general, the açaí powder produced greater effects on these quality parameters, as shown by the mathematical models presented in Table II.

The mathematical model that represents the fermentation time behavior depending on the quantities of açaí and chitosan was the only one who did not show high correlation coefficient ($R^2 = 0.5889$). For others parameters was possible to establish a satisfactory statistically relationship.

TABLE II. MATHEMATICAL MODEL BY REGRESSION ANALYSIS ($p \leq 0.05$)

Water Hydration properties	Model	R^2
WAI	$Y = 3.65 + 0.398X_1 + 1.254X_2 + 0.047X1^2 + 0.2024X_2^2 + 0.030X_1X_2$	0.9930
WS	$Y = 5.87 - 0.423X_1 + 1.005X_2 - 0.029X_1^2 - 0.091X_2^2 - 0.216X_1X_2$	0.9070
SWP	$Y = 9.31 - 0.358X_1 - 1.512X_2 + 0.088X_1^2 - 0.279X_2^2 + 0.168X_1X_2$	0.9881
Color Properties		
L*	$Y = 62.20 + 0.138X_1 - 5.241X_2 - 0.375X_1^2 + 0.218X_2^2 - 0.368X_1X_2$	0.9963
a*	$Y = 14.66 + 0.16X_1 + 1.683X_2 - 0.35X_1^2 + 0.301X_2^2 - 0.44X_1X_2$	0.9204
b*	$Y = -19.39 + 0.409X_1 - 2.732X_2 - 0.09X_1^2 - 0.563X_2^2 + 0.409X_1X_2$	0.9436
Browning Index	$Y = 58.54 + 0.786X_1 + 6.65X_2 - 0.101X_1^2 + 0.101X_2^2$	0.9869
Dough and bread properties		
Maximum Volume	$Y = 73.78 - 2.703X_1 - 10.502X_2 - 1.007X_1^2$	0.9537
Fermentation Time	$Y = 59.99 - 5.681X_1 + 2.201X_1^2 + 0.022X_2^2$	0.5889
Alveoli Circularity	$Y = 0.811 - 0.013X_1 - 0.055X_2 + 0.011X_1X_2$	0.9437
Alveoli Number	$Y = 951.99 - 64.21X_1 - 56.23X_2 - 19.02X_2^2 + 27.01X_1X_2$	0.9237
Specific Volume	$Y = 2.75 - 0.236X_1 - 0.246X_2 + 0.06X_1^2$	0.8590
Maximum Expansion Factor	$Y = 1.78 - 0.090X_1 - 0.109X_2 + 0.049X_1^2$	0.8518

X_1: Chitosan; X_2: Açaí powder.

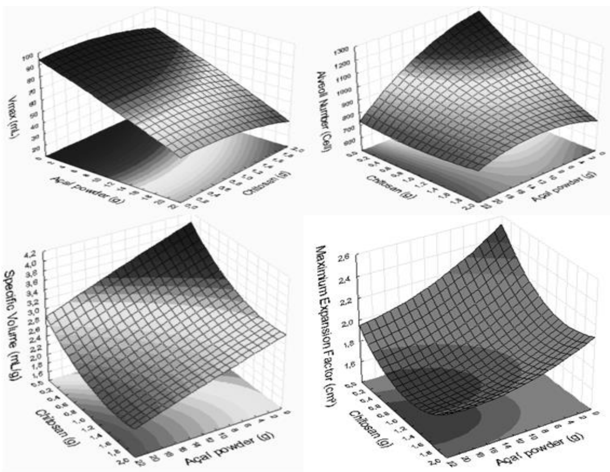

Figure 2. Surface Response of the chitosan and açaí powder addition on the dough and bread properties

This reduction in the expansion parameters is related to the presence of components as minerals and fibers presents in the composition of açaí power and chitosan promotes cracks in the gluten network, which reduces carbon dioxide retention capacity, compromising the dough expansion, specific volume and crumb structure of breads.

Table III presents the correlation analysis between independent variables and dependent variables.

TABLE III. CORRELATION ANALYSIS

Quality Variables	Açaí Powder	Chitosan
Water Absorption Index	0,97*	0,15
Water Solubility (%)	0,90*	-0,23
Swelling Power	-0,97*	-0,08
Vmax	-0,94*	-0,17
Fermentation Time	0,68*	-0,25
L*	-0,98*	0,04
a*	0,91*	0,02
b*	-0,91*	0,24
Browing Index	0,99*	0,09
Alveoli Number	-0,94*	-0,15
Alveoli Circularity	-0,77*	-0,45*
Specific Volume	-0,68*	-0,59*
Maximum Expansion Factor	-0,63*	-0,57*

*Show statistical significance of correlation coefficient at reliability level of 0.05. The number of observations was 33 for each property.

Açaí powder showed correlation significantly with all variables. Higher positive correlation was found to WAI, WS, a*, fermentation time and Browing Index. The Sweeling Power, Vmax, L*, b*, alveoli number and circularity, Specific volume and maximum expansion factor was obtained higher negative correlation with açaí powder addition in bread formulations. The chitosan was presented significantly correlation for alveoli circularity, specific volume and maximum expansion factor only. This shows that Açaí powder has greater influence on the quality parameters of flour, dough and bread.

Among food matrices in particular, dough is a complex matrix of different, closely associated components. Induced by mechanical or thermal energy input, its polymers can be cross-linked creating the macroscopic structure through inter and intramolecular forces. The matrixes of wheat dough mainly consist of starch granules, gluten proteins and lipids [16], the inclusion of açaí powder promoted a greater amount of substances to interact with the starch and gluten proteins, more than chitosan due its diversity of nutrients, as shown in Table IV.

Optimum conditions the inclusion of chitosan and açaí powder that maximized bread physical properties; it was found that the addition of 0.75 g chitosan and 8.5 g of açaí powder provides better quality parameters of products.

C. *Proximate Composition of Chitosan, Açaí Powder, Control and Optimized Bread*

TABLE IV. PROXIMATE COMPOSITION OF CHITOSAN, AÇAÍ POWDER, CONTROL AND OPTIMIZED BREAD FORMULATION

Component	Chitosan	Açaí Powder	Control Bread	Optimized Bread
Moisture	8.17[b]±0.05	7.33[c]±0.03	32.44[a]±0.05	32.88[a]±0.04
Protein	Nd	13.44[a]±0.08	7.34[c]±0.05	9.27[b]±0.08
Fat	Nd	28.19[a]±0.05	2.05[c]±0.08	3.99[b]±0.04
Ash	0.05±0.02	5.95[a]±0.04	1.75[c]±0.03	3.42[b]±0.03
Carbohydrate (%)	91.78[a]±0.03	45.09[d]±0.08	56.42[b]±0.06	50.44[c]±0.04

nd: not detected.

Chitosan is mainly composed of carbohydrates (91.78%). Açaí powder presented high levels of protein, fat and ash.

Moisture content between control and optimized bread did not show significant differences. However, the addition of chitosan and açaí powder promotes significant increase in the protein, fat and ash content. In addition, reduces the carbohydrate content, improving the nutritional value of bread.

D. Scanning Electron Microscopy (SEM) of Dough and Bread

Electron microscopy scanning of dough shows that gluten network was damaged by the presence of açaí powder and chitosan (Fig. 3, Fig. 4 and Fig. 5).

Fibers and minerals present in açaí powder composition can affect the dough expansion capabilities, compromising the specific volume of bread because the interactions of these components with the gluten network. According [1], chemical interactions involved in protein gels are mainly ionic bonds, hydrogen bonds and disulfide bonds, as well as hydrophobic interactions. Several studies report the reduction in the specific volume of breads as a result of fiber dietary addition [22], [23], and [24], the chitosan has the ability to act as fiber, that interacts with the gluten network causing its weakening, which explains its negative correlation with the crumb structure and dough expansion during fermentation.

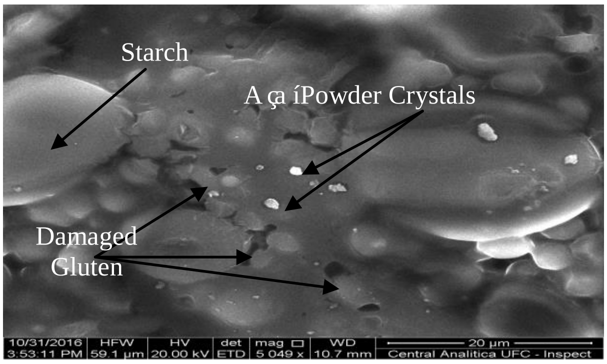

Figure 3. Scanning electron microscopy of dough added with 7.5 g of açaí powder and 0.85 g of chitosan.

Fig. 4 show the Açaí Powder Crystals and Fig. 5 the chitosan-starch interaction.

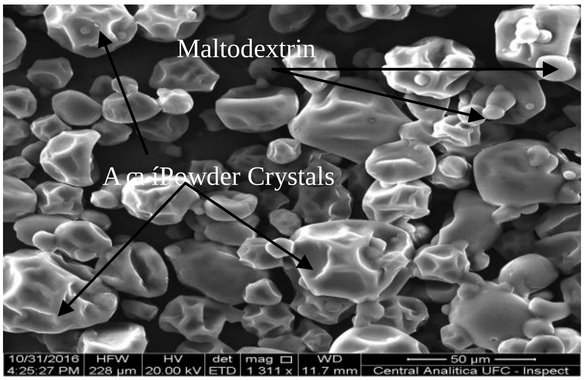

Figure 4. Scanning electron microscopy of açaí powder.

The crystal morphology is similar to those found by [25] for açaí juice dried by spray-dryer. The particles produced with all types of carrier agents showed spherical shape and various sizes, which is typical of materials produced by spray drying.

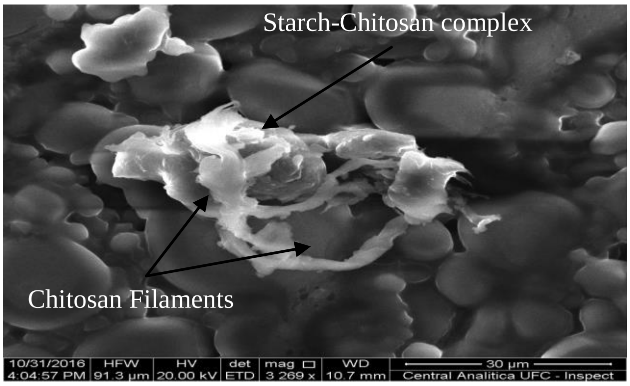

Figure 5. Scanning electron microscopy of dough added with 7.5 g of açaí powder and 0.85 g of chitosan.

In the Fig. 5, it is possible to check chitosan filaments involving a starch molecule, promoting the formation of a complex between the two polysaccharides.

IV. Conclusion

Açaí powder and chitosan inclusion in bread formulations promote significant changes in the technological parameters of flour, dough and breads. The correlation analysis showed greater influence of açaí powder in decreasing quality of breads than chitosan.

The optimized formulation was incorporated of these ingredients in 8.5 and 0.75 g, respectively. This condition produced breads with better nutritional quality, with emphasis on increasing of protein, ash and fat content.

Electron microscopy analysis suggests that açaí powder has the ability to damage the gluten network and chitosan to form complex with starch.

In this way, these ingredients can be added together for the improvement of the nutritional quality of breads.

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