Nanosuspension of pinhão seed coat development for a new high-functional cereal bar

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ABSTRACT

The seeds from *Araucaria angustifolia* – commonly named as pinhão - are important culturally, and viable regional food from Brazil. Although now considered an endangered species, methods to increase the awareness of this tree have been called upon attention. In light upon increasing such awareness, we utilized the seed coats of pinhão, which is considered a residue and discarded, in the development of cereal bars. Although the raw seed coats have a very high astringent flavour and impair the sensory taste; if considered as a nanoformulation, this strong astringent flavour is masked. Therefore, studies were also carried out with an experimental design to understand the effect of nanosuspension as a binding agent. In addition to physicochemical and nutritional characteristics performed, the results propose that nanosuspension of pinhão seed coats can be a potential ingredient for functional cereal bars due to high fibre content, proteins and phenolic compounds.

Keywords: cereal bars, *Araucaria augustifolia*, pinhão seed coat, nanosuspension, antioxidants.

Practical Applications

Nanosuspension of pinhão seed coats masks the astringent flavour of the raw seed coat, while also able to present high fibre content and is very resistant as cereal bar due to nanocellulose.

Cereal bars also present good acceptability index and high values of proteins, with presence of phenolics compounds extracted from the seed coat.

1. Introduction

Araucaria angustifolia with its common name, Paraná pine, is a tree with a natural occurrence in Brazil; distributed in the states of Paraná, Santa Catarina and Rio Grande do Sul, while also in some few areas in the southern region of the states of São Paulo, Minas Gerais and Rio de Janeiro in areas of higher altitude (Cladera-Oliveira and Marczak, Ligia Damasceno Ferreira Noreña, Caciano Pelayo Zapata Pettermann, 2011).

From this tree, it is possible to use its almond inside the seeds and its resin that, when distilled, provides several products with many industrial applications (Quinteiro *et al.*, 2019); among these products derived from Araucaria, stands out its seeds with its common name – pinhão (Cordenunsi *et al.*, 2004), whose harvest has been an important source of income in several regions in the southern part of Brazil (Tagliari and Peroni, 2018).

In this context, source of income from pinhão is the main incentive for the preservation of Araucaria – and its biome (Hanazaki *et al.*, 2018), since it is now considered an endangered species and shows important impact for the regional development of the Southern states of Brazil (Peralta *et al.*, 2016; Fichino *et al.*, 2017).

Therefore, methods to contribute with an increase, not only with an awareness of this tree, but to improve the monetary income from this species *Araucaria angustifolia* is through the use of pinhão in the cooperation with agri-food industry (Notarnicola *et al.*, 2012). One of these cooperation stands out the research onto cereal bars, which are products manufactured with cereal grains to provide dietary fibre intake and other ingredients and is now being fortified to provide vitamins and minerals (Ferreira *et al.*, 2015). They are easy to produce, practical and with a good acceptability (Appelt *et al.*, 2015). However, to obtain a cereal bar with good acceptability that meets nutritional benefits is not an easy task since there is a need to balance the various ingredients and to complement each other (Saleh *et al.*, 2019).

For this specific case, so far there is no scientific investigation on the use of pinhão seed coats for the production of cereal bars. Although our group exhibited a preliminary report on the effect of raw seed coats from pinhão in the formulation of cereal bars, they presented very low acceptability values (this report was presented in a master's thesis (Costa, 2013)) and it was mainly attributed to its seed coats possessing larger quantities of phenolic compounds – mostly tannins - and led to a very high astringent flavour (da Silva *et al.*, 2014).

Studies on techniques for debittering by-products, or residues, of natural origins have been gathering considerable attention since if this is removed, this threshold barrier, could be used for food technologies as various final products. Therefore, strategies that have been used to debitter includes mixing with different food additives (Bertelsen *et al.*, 2018), sweeteners , by the usage of Maillard reaction (Hong *et al.*, 2016) and most commonly by the encapsulation technology (Holgado *et al.*, 2019; Khan *et al.*, 2019) as it avoids exposure of bitter compounds to bitter taste receptors.

Nonetheless, another debittering approach is via particle size reduction, since the bitter sensation is associated with specific taste receptors (T2Rs), it has been shown that molecular weight of peptides influences the bitterness and the intensity increases with increasing peptide chain length (Fu *et al.*, 2019). Therefore, using ultra fine friction grinding, which has been demonstrated its importance in particle size reduction for bioactive compound extraction (Hatami *et al.*, 2017), could be exploited to debitter the astringent taste of pinhão seed coat.

Therefore, this study uses ultra-fine friction grinding on pinhão seed coats and formulate a cereal bar while measuring its influence on physicochemical and nutritional characteristics, by the usage of a nanostructured form. From this derived biopolymer, vegetal nanocellulose offers physical and chemical properties that allows its usage in a wide variety of hydrophilic and hydrophobic composite matrices and hybrid materials, including hydrogels and aerogels (Thakur and Thakur, 2015). Therefore, these attractive features can exhibit a potential improvement in acceptance for the development of cereal bar from the seed coat of pinhão. Furthermore, nanocellulose as a dietary fibre requires a thoroughly investigation since so far, insufficient studies have related its useful success in the implementation as a nutritional agent while only being used as stabilizer (Sanchez-Salvador *et al.*, 2015).

2. Materials and Methods

2.1 Cereal bar flavour and formulation market analysis

For the development of this cereal bar, it is essential to obtain market information, in order to optimize the desired results. Therefore, before the practical part of the study, a market survey was carried out with the objective of characterizing and analysing the most established commercial cereal bars.

A table was elaborated to collect information such as the marketplace, brands of cereal bars and the best-selling flavours; this study was carried out in supermarkets from the city of Blumenau in the state of Santa Catarina, Brazil and this information was provided by representatives from the cereal bars industry.

Parallel to this market research, an online questionnaire was elaborated and applied to 100 consumers of both genders with questions related to the habit of consumption, factors that influence its purchase, flavour and most preferred brand (Supplemental information Questionnaire).

The market research allowed to compare cereal bars commercialized according to their consumer preference. Therefore, the data shown in Table S.1 reports the supermarket research and Figure S.1 is related to the questionnaire made to the consumers which exhibits a tendency to consume cereal bars containing chocolate.

Based on the data obtained with the survey, chocolate flavour was chosen for the cereal bars, while it was also compared with the most common composition from commercialized cereal bars (Table S.2).

2.2 Production of the cereal bar

Cereal bar of the present study contains the common ingredients found in the labels of these commercial products (Table S.2). Oat flakes were roasted to decrease moisture and produce a crispier bar; quinoa was roasted to inactivate possible antinutritional factors (Carciochi *et al.*, 2016) and thus, also contributed to the crunchiness.

Cereal bar was produced in the proportion of 30% of liquid phase and 70% of solid phase. The ingredients in the liquid phase were mixed and heated in a stainless-steel baking pan until it reaches a desired consistency. Subsequently, solid phase ingredients (rice flakes, coarse and fine flaked oats, flaxseed, mixed quinoa, sodium bicarbonate, skim milk powder, cocoa powder, chocolate powder) were manually homogenized in a mixing bowl of stainless steel. Next, they were added into the liquid phase which were further mixed and subjected to dry heat cooking (90 ºC) for about 3 minutes – resulting in homogeneous formulation with a uniform mass. Finally, the dough was evenly distributed in stainless steel baking pan and pressed with the aid of a spatula to a final thickness of 1.5 cm. After cooling to room temperature, the dough was cut to obtain cereal bars of 2.5 cm wide, 10 cm long and approximately 25 g each, packed with foil and sealed in plastic container (Figure 1).

2.3 Production of nanosuspension from pinhão seed coat

Pinhão seeds were manually peeled, with the help of a pinhão cutting knife and the seed coats of pinhão were cooked to be easily grinded. For the cooking process, 100 g of seed coats of pinhão and 1000 mL of water was heated for 10 min at 100 °C (boiling).

This pulp was initially mixed in a 450 W blender for 10 minutes. Subsequently, cellulose suspension was subjected through a mechanical defibrillation process in a mechanical mill (Model MKCA6-4; Masuko Sangyo Co.Ltd.). The mechanical mill consists of two defibrillation stones, wherein from one adjustment it is possible to control the size of the produced fibres via compression and shear forces; through the pulp passage between the stone walls forms the separation of the fibre - process known as defibrillation - and after many repeating steps through the mill, there is formation of nanocellulose. After this process, the suspension mixture stabilizes between the many steps, to give a gel-like appearance as shown in Figure A.2 for the specific nanosuspension of the pinhão seed coats produced. The technical parameters related to mechanical mill were: rotation 1500 rpm; number of steps 30 and distance between discs 0.1 mm.

2.4 Experimental design for nanosuspension cereal bar formulation

A new formulation was developed for the addition of the nanosuspension to the cereal bars to evaluate the influence of this material and its behaviour as a binding agent. The experimental design adopted for the elaboration of the cereal bars was the 2^2 factorial, with the dependent variables being the nanosuspension and the mixture of gelatine - binding agent - with water (Table 1); while also maintaining the basic ingredients for the formulation of the bars, section 2.2.

To evaluate these bars produced from this design, a scale from zero (0) to ten (10) points with a manual mechanical analysis methodology was used, whereas the point zero (0) represents a bar without a binding agent (undesirable); point five (5), as a homogenous binding (desirable); and lastly, ten (10) as an extremely rigid, rubbery (undesirable) binding agent. This sensory aspect was performed by touch, while the reference for a desirable bar was the commercialized cereal bar. The design sought to obtain an ideal binder, in which the texture, crunchiness and sorting of the grains from the cereal bars were kept as the basic formulation, in order to produce a product sensory acceptable.

Table 1. Experimental design for the production of cereal bars with specific levels and concentrations for the variables tested as binding agents.

Variables *	Levels		
	-1	0	1
Nanosuspension (g)	0	14	28
Gelatine + water (g)	0	7.3	14.6

* Portion of 100 grams.

2.4.1 Nanosuspension evaluation by Transmission Electron Microscopy

The characteristic morphology of the nanosuspension pinhão seed coat was investigated using a transmission electron microscope (TEM) JEOL, model JEM-1200 EXII. For this, the nanosuspension was diluted to 0.1% w/v, dispersed in an ultrasonic bath for 30 min, and then casted on a copper TEM sample grid. The images were obtained from three different suspension solutions.

2.4.2 Sensory analysis

In order to evaluate the acceptability of the cereal bars produced, the acceptability index (AI) was measured and thirty tasters assessed if they liked or disliked the cereal bars developed in this work and were fully aware of the use of nanosuspension of pinhão seed coat as a cereal bar ingredient. For the evaluation, a nine-point structured hedonic scale ranging from "extremely liked" (9) to "extremely disliked" (1), was used to evaluate the following parameters: colour, aroma, taste, texture and overall acceptance (Teixeira, 2009) with a descriptive statistical analysis to determine the frequency of answers for each value. The taster team, made up of men and women aged 18 to 50 years, was selected because of their consumption of similar products, availability and interest in participating in the test. The product was served in 10 g white polyethylene containers at room temperature.

To calculate the Acceptability Index (AI) of the product, the following expression was used: AI (%) = A x 100/B, where A is the average grade obtained for the product and B is the maximum grade given. According to Teixeira et al (2009) for a product to be accepted in terms of their sensory properties, it is necessary to obtain an acceptability rate of at least 70%.

2.5 Physicochemical and nutritional tests

The physicochemical and nutritional characteristics of cereal bars were evaluated with the formulations from the basic formulation and with the specific binding agents to better understand the effect of the nanosuspension.

2.5.1 Moisture test

To quantify the moisture content of the samples, the gravimetric method was used at 105 ° C. About 5 g of different bars were weighed in porcelain crucibles (50 mL), followed by oven drying for 12 hours. Samples were withdrawn from the oven and stored in a desiccator at room temperature until cooled and weighed.

The calculation for moisture content was performed according to the following formula:

$$\text{Moisture (\%)} = \frac{m_3 - m_2}{m_1} * 100 \qquad 01$$

Where m_3 = crucible weight of dried samples (g); m_2 = weight of previously dried crucible (g) and m_1 = weight of sample immediately before drying (g).

2.5.2 Water activity:

Water activity in the cereal bars was determined with a 3TE AquaLab Water activity meter, series 3B, v 3.0 (Decagon Devices Inc. WA, USA) and a standard water activity of 0.50, at 25°C (Chemists, 2005).

2.5.3 pH measurement

The pH was measured in cereal bars containing nanosuspension with gelatine and water using the Potentiometric method (Tecnal pHmeter). Ten grams of cereal bar was weighed and dispersed in 100 mL of distilled water, stirred for 10 min and the pH was immediately measured with a previously calibrated pHmeter.

2.5.4 Acidity index

Acidity index was determined by weight 5 g of sample into 125 mL conical flask and 100 mL of reverse osmosis water was added. The solution was homogenized and filtered on qualitative filter paper. Afterwards, a 25 mL aliquot of the filtrate was separated and titrated using a 0.1 mol / L sodium hydroxide burette and phenolphthalein as indicator.

2.5.5 Total soluble solids

The content of soluble solids (° Brix): was obtained, expressed in Brix grade, by measuring 10 g of the cereal bars and/or dried seed coat, and mixed in 100 ml of water; a bench refractometer was used for reading.

2.5.6 Phenolic activity

The total phenolic compounds of the extracts of dried pinhão seed coats were determined by the method described by Singleton and Rossi (1965), with minor modifications. The colorimetric method is based on the reaction of phenolic compounds with Folin-Ciocalteu reagent. Gallic acid was used as a reference standard. Results are expressed as gallic acid equivalent.

2.5.7 Nutritional composition

Determinations of lipids and protein content were performed according to the procedures of the Adolfo Lutz Institute (Sanitária, 2005). Total fats (ether extract) were determined in the sample through the analysis of moisture and using ethyl ether as a solvent extractor for six hours. The ether extract obtained was placed in an oven at 70°C for one hour to remove residual solvent, followed by cooling in a dry area and measuring its weight. The total protein was determined in a sample of 0.5 g by using the micro-Kjeldahl method, which quantifies the nitrogen content. The protein concentration was calculated by multiplying the percentage of total nitrogen by the conversion factor 6.25. The total dietary fibers were determined through the official enzymatic method from Prosky et al. (1992) (Prosky and DeVries, 1992) and used about 1g of the sample, in quadruplicate. The method consists in hydrolysing protein using a protease, followed by hydrolysis of starch with thermal stable alpha-amylase and amyloglucosidase (glucoamylase) enzymes. The products obtained from hydrolysis that are the total fibres were removed from the hydrolysate and constituted the residual fibrous mass. The residue obtained from the hydrolysate was oven dried at 70°C, cooled in desiccators to room temperature and weighed. The percentage of fibres can be calculated by subtracting the protein and minerals masses. The caloric value, kcal and kJ, was determined considering the conversion factor for kcal of 4.0 for protein and carbohydrate and 9.0 for total fats.

2.5.8 Statistical analysis

Data from the evaluation of the produced bars were subjected to analysis of variance (ANOVA) at a level of 5% significance.

3. Results and Discussion

Following the experimental design, produced bars were analysed in order to evaluate the effect of the nanosuspension addition to better understand the potential binding effect and the combination of concentration from the variables - nanosuspension and gelatine with water (Figure 2).

With this methodology (Figure 2a) the results indicates that value five (5) is attributed to a bar with desirable binding agents, it can be observed that the ideal formulation occurs for gelatine content with water is in between the values eight and six, indicated in the graph, without any nanosuspension since this addition exceed the good range in the cereal bars so that there is lack of binding for complete union of the grains, without some other characteristics such as the crunchiness of the bars. Therefore, in this specific case the ideal sample is within a concentration of zero (0) nanosuspension and gelatine with water in 7.3 grams.

From these results it can be deduced that the mixture of gelatine with water and nanosuspension produced undesirable cereal bars. This can be explained by the high-water content existing in this composition with nanosuspension, which presents a weak binding agent with the presence of water and gelatine. This behaviour can be further seen with the Pareto diagram (Figure 2b), with a statistic significant influence when there is only gelatine with water. In addition, the values found for the binding agents produced with the addition of nanosuspension pinhão seed coats exhibit that it plays a weak binding agent role in cereal bars when incorporated into the mixture of water with gelatine.

Therefore, further studies were focused on the combination of nanosuspension, since both (water + nanosuspension) have the same behaviour in the composition acting as binder. In addition, an optimal quantity of gelatine was added to this suspension, based on the experimental design, and replacing the water in the basic formulation of the cereal bar with mixed nanosuspension pinhão seed coats.

Cereal bars containing nanosuspension notably presents a higher resistance and; although nanosuspension behaves poorly as a binding agent, it contributed to the cereal bars in some other properties obtaining a set of ideal factors, such as uniformity, texture, crunchiness, colour, gloss and without imparting their original taste, resulting in a bar with high commercial impact and desired characteristics, as can be seen in figure 1.

Due to the visible increase in the viscosity from the dough formulation before the preparation of the cereal bar, and aiming at higher fibre content, the addition of 10 g of nanosuspension was made in order to obtain a cereal bar with desired characteristics. Furthermore, it is important to remind that binding agents help in agglutinate the ingredients; and to achieve a chewy texture such bars have high levels of binder components. Another important aspect is that cereal bars may fail to form without traditional binding ingredients as a self-supporting because the binder cannot retain the bar in a desired shape.

Nanocellulose from defibrillation of pinhão seed coats was confirmed by TEM (Figure 3) exhibiting varying degrees of defibrillated cellulose on the order of nanometric size. The effect of defibrillation was already exposed before by our group (Missio *et al.*, 2018) with many attractive features such as a successful intimate interconnection between two forest-based resources, but also improve the bioavailability of the compounds. Nanocellulose can also act as a reinforcement due to the high elastic modulus and strength obtained from the defibrillation process leading to reinforced nanometric cellulose fibres, which is one of the reasons why this process was used (Klemm *et al.*, 2011).

With the production of the nanosuspension cereal bars, sensory analysis was performed and the produced bars presented satisfactory results with scores mostly above 5 in the hedonic scale, as shown in Figure 4. Within these responses and data, the acceptability index was calculated and presented mean value of 82.5%, as it is above of 70% it represents an index with good acceptance.

As the bitter sensation is associated with specific taste receptors (T2Rs), hydrophobic chains of bitter peptides are examples of trigger mechanism to the T2Rs. The perception of bitter taste initiates binding these bitter compounds to T2Rs which induces a neural signalling cascade whose intensity is dependent on the hydrophobic recognition region of T2Rs (Fu *et al.*, 2019). If these compounds are not within the size range of these receptors, the bitterness sensation could be masked which could have been occurred within the nanosuspension of the pinhão seed coat. Studies on ultra-fine friction grinding have already shown that for curcuma suspensions, there is a decrease on particle size with disruption of the vegetable material (Serpa Guerra *et al.*, 2020).

Having achieved a high degree of acceptance, this product may be promising for the cereal bar consumer market, the general mechanism between the production of this bar is that the nanosuspension may weakly impart the sensory taste – astringent flavour – while also possessing phenolic compounds presenting a healthier cereal bar for the consumer market. These phenolic compounds are present in the water-extractable fraction of the pinhão seed coat, corresponding to approximately 18% (wt) (Table 2). The analysis of these water-extractable, showed that the concentration of phenolic compounds, in gallic acid equivalent, is 87 ± 9 mg per gram of extractives (approximately 16 mg per gram of seed coat). These substances have been increasingly used in the development of functional foods providing health benefits beyond nutritional value (Seidel *et al.*, 2007; Alu'datt *et al.*, 2012). Consumption of these compounds may reduce the risk of cardiovascular disease, cancer and other age-related chronic diseases (Russo, 2007).

Table 2. General characteristics of pinhão seed coats.

Analysis	**Pinhão seed coats (*)**
Moisture (%)	5.0 ± 2.0
STT (oBrix)	2.1 ± 0.4
Proteins	1.8 ± 0.1 g
Total Fat	6.0 ± 0.8
Fibres	46.1 ± 0.2 g
Carbohydrates	44.0 ± 1.0 g
Calories	237.0 ± 3.0 kcal
Water extraction fraction (%)	17.9 ± 0.4
Phenolic concentration (**)	87.0 ± 9.0

(*) Where applicable, values are presented in g/100g. (**) (mg equivalent of gallic acid/g)

The analysis of the nanosuspension from pinhão seed coats was already reported by other groups and hold many beneficial ingredients with flavonoids and phenolics mainly from the group of tannins (De Freitas *et al.*, 2018).

After the production of this bar with nanosuspension, the nutritional composition was obtained (Table 3) and compared to cereal bars from the market containing chocolate flavour and from other works found in literature.

The commercial cereal bars used as comparison parameters show fibre content on average from 1 to 4 grams per 22 g of product; therefore, inferior to the value found in the bars produced herein. Cereal bars containing nanocellulose reached approximately 6.38 grams of dietary fibre per 22 g of product, due to the higher values of cellulose in the seed coats (approximately 46 g/100 g for dried pinhão seed coats – Table 2), their addition potentially increases the fibre value.

Table 3. Nutritional Facts table of the produced cereal bar containing nanosuspension.

Nutritional Facts Serving Size 22 g (1 bar)		
	Amount per serving	% VD (*)
Calories	134.20 kcal = 563.20 kJ	6.71%
Carbohydrates	18.15 g	6.05%
Proteins	6.60 g	8.80%
Total Fats	3.92 g	7.12%
Dietary fibre	6.38 g	25.52%

(*) Percent daily values are based on a 2,000 kcal or 8,400 kJ diet. Your daily values may be higher or lower depending on your calorie needs.

Dietary fibre consumption is reportedly below the recommended in recent years (Jones, 2014). A recent study demonstrates the effect in digestion of a high fibre content bread compared to a control with normal fibre, there was a reduced digestion rates of 30% estimated for high-fibre bread compared to the control (Gouseti *et al.*, 2019). It is also important to notice that commercial cereal bars exhibit a dietary fibre average of 2-3 g in which the cereal produced in this work had almost the double nutritional amount for the same quantity. The Brazilian Health Regulatory Agency (Anvisa) reports that a cereal bar can be classified as a high content dietary fibres product if contains at least 6 g of fibres /100 g to act as a physiological functional fibre source, (Obrigatória, 2005) while also able reduce the risk of diseases, to be categorized as a functional product (Fuller *et al.*, 2016). In addition, fibre-rich foods are indicated against intestinal disturbances (Qi *et al.*, 2018), diabetes (Consortium, 2015), atherosclerosis (Wu *et al.*, 2003) and cancer (Kunzmann *et al.*, 2015). In this context, the cereal bars produced have a great market perspective, emerging as a new product with functional characteristics.

It was also possible to observe a small increase in energy value, carbohydrates and total fats of the cereal bar, which is due to the use of special ingredients and chocolate that confers its flavour to the cereal bar. Therefore, little represents to the achieved goals of this work. Nonetheless, their values are within the range of commercial chocolate bars.

Lastly, the amount of proteins obtained from cereal bars containing nanosuspension from seed coats of pinhão exhibits values three times higher than the available commercial cereal bars and, although they have lower values than protein bars used by athletes, the advantage comes from the cheaper cost in producing them. Even though pinhão seed coat possess protein in its nutrients (1.8 g/100 g of dried seed coat), and studies have shown that seed coats tends to also present higher protein values (Kalpanadevi and Mohan, 2013), the increase may be due to the specific ingredients - flaxseed and skim milk powder - that helped contributed to this quantity (Kaur *et al.*, 2018).

All the nutritional facts obtained for this cereal bar remained within the Brazilian national standards (2005), in which it describes that the recommended daily quantity of carbohydrates, proteins, total fats and Fibres should be 300, 75, 55 and 25 g, respectively, all below the threshold region considered as overnutrition.

Analysing the data obtained from the physicochemical properties of the cereal bars produced, it was possible to perceive that nanosuspension of pinhão seed coats does not change considerably when added in the formulation of cereal bars, as shown in Table 4.

Table 4. Results of physicochemical analyses of cereal bars with and without the nanosuspension of pinhão seed coats.

Analysis	**Bar with suspension**	**Bar without suspension**
Moisture (%)	11,0500 ± 0.47	11,7982 ± 0.97
w_a	0,5867 ± 0.003	0,6457 ± 0.02
Ph	7,4533 ± 0.10	7,4567 ± 0.05
STT (^{the}Brix)	2,2000 ± 0.90	3,2333 ± 0.42
ATT (mL NaOH 0, 1N)	0,3367 ± 0.06	0,0900 ± 0.03

In relation to moisture, the cereal bar produced is within the parameters of resolution of the ANVISA (ANVISA - National Health Surveillance Agency, 1978), lower than 15%, which is the maximum limit for the existence of the product in question. The Water activity (w_a) also obtained interesting results, presenting values below 0.80 and 0.88, since it has been reported that values above these favours the development of molds and yeasts, respectively. Therefore, the developed product has conditions for conservation.

4. Conclusion

The cereal bars containing nanosuspension of pinhão seed coats in its formulation was developed to be a product with quality assurance, within the dietary parameters. There is the possibility of becoming a food of functional character as to the fibre content having almost two times higher than commercial cereal bars in the category. The physicochemical and nutritional analysis presented results adequate to a commercial cereal bar and is within the standards from Brazilian Health Regulatory Agency. The most important aspect of this cereal bar is from the good acceptability tests, which is a high achievement since the seed coat of pinhão presents a high astringent flavour and preliminary tests with the macroscale size of the seed coats resulted in negative viable outcome for the production of cereal bars. These positive results were due to the defibrillation of the seed coats, increasing its acceptance while being now able to investigate further into a commercial product of this natural product.

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Conflicts of Interest

The authors declare that there are no conflicts of interest regarding the publication of this manuscript.

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Figure 1. Standard production of chocolate cereal bars with or without nanosuspension of pinhão seed cots in its composition

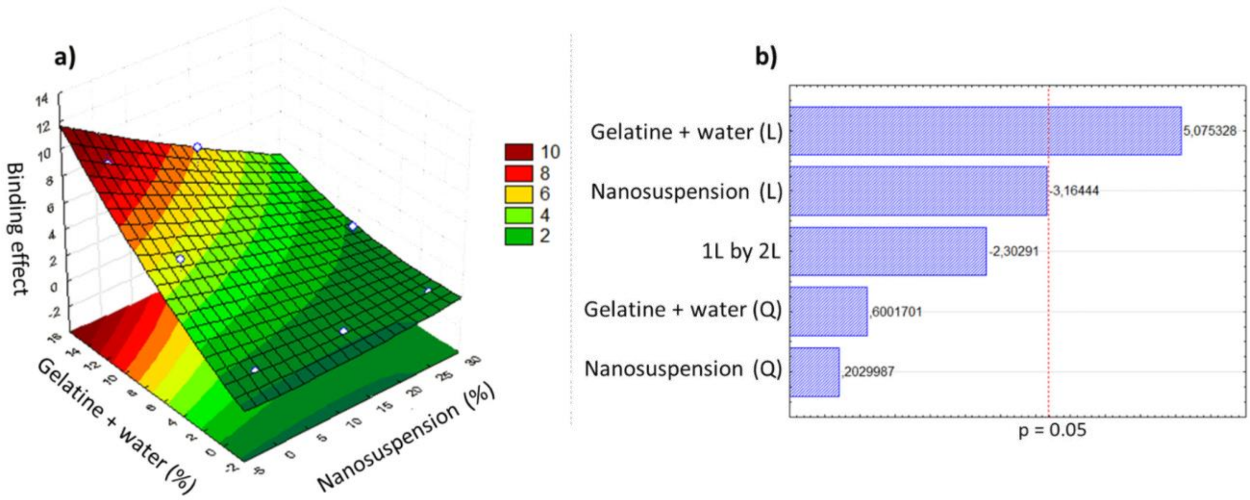

Figure 2. (a) Surface response chart for various combinations and concentrations of nanosuspension and gelatine with water in the evaluation of binding agent for the cereal bar; (b) Pareto diagram; influenced parameters in the binding agents of the cereal bar. 1L by 2L, linear interactions effects between Gelatine + water and nanosuspension of pinhão seed coats; L, linear main effects of input values; Q, square main effects of input values

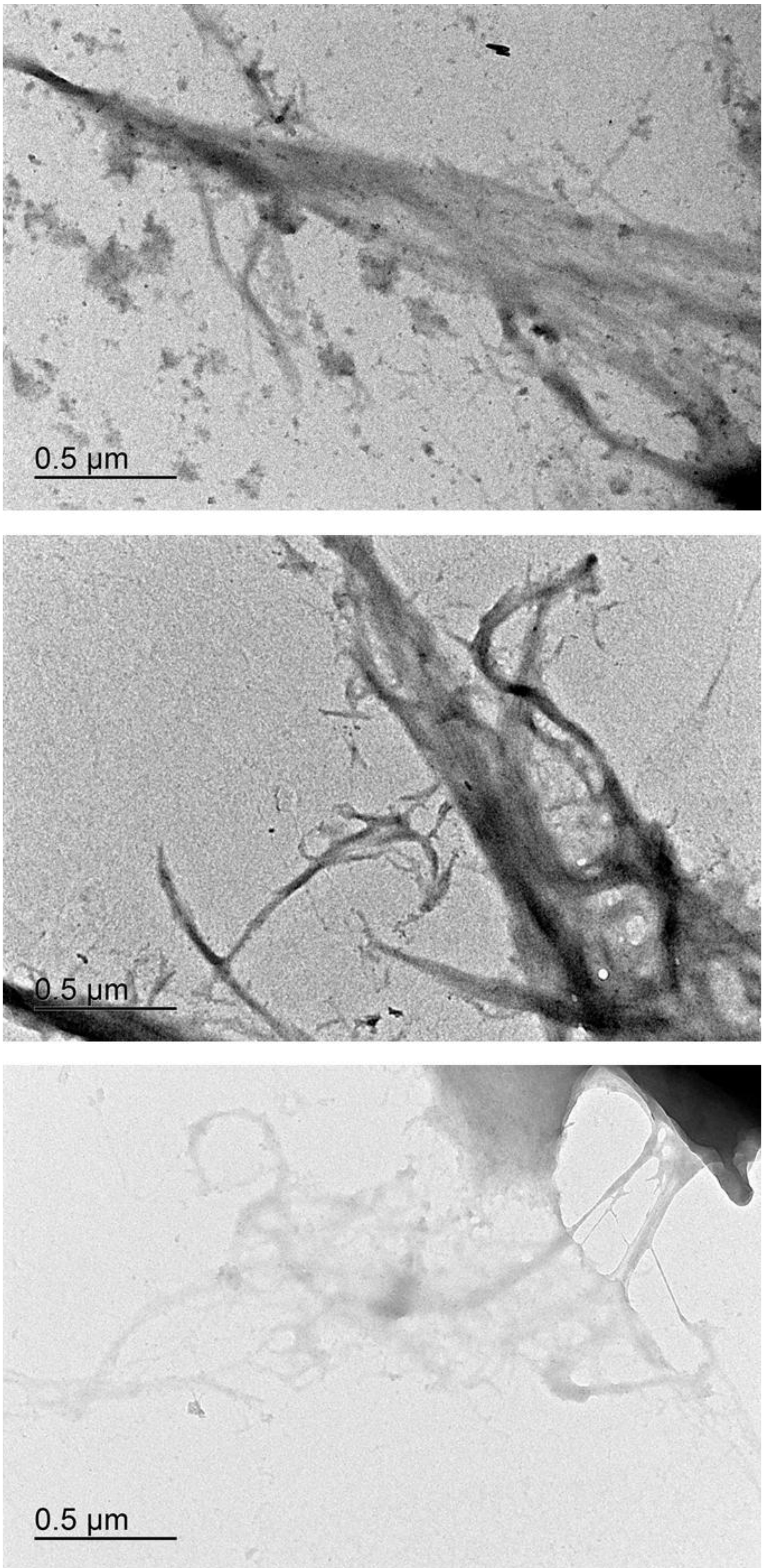

Figure 3. Morphology of the nanocellulose presented on nanosuspension of pinhão seed coat from three independent solutions

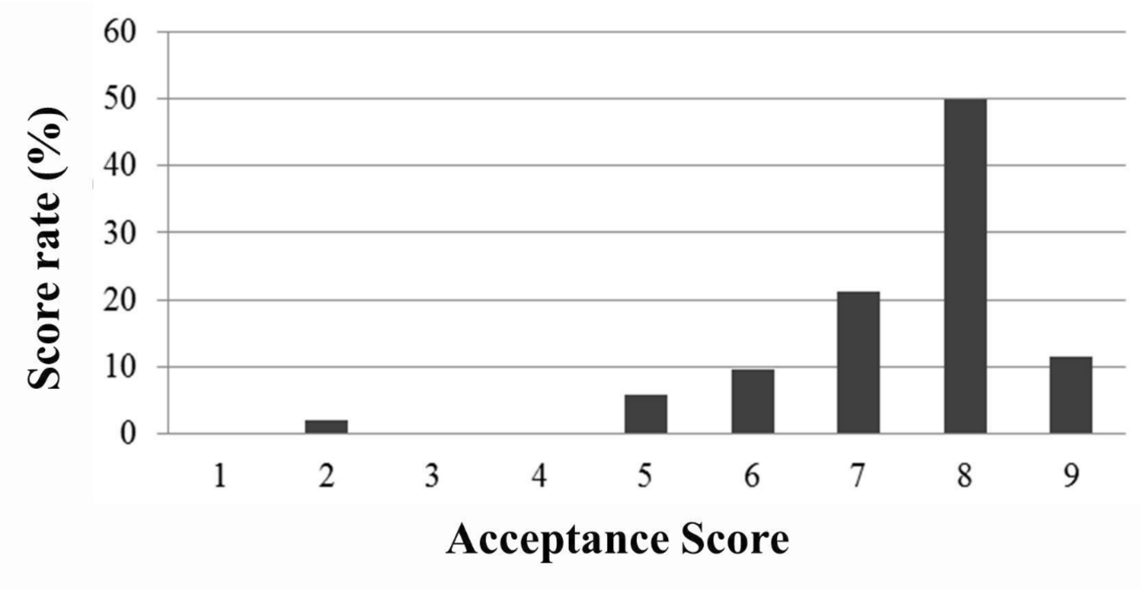

Figure 4. Relationship graph of the score rate of acceptance attributed to the cereal bars containing cellulose of pinhão seed coats

Table S.1. Market research on commercialized cereal bars.

Local	Brands SOLD	Best Selling Brands	Best Selling Flavours
1	Jasmine, Levittá, Ritter, Hershey's, Nutry, Nestlé	Ritter, Nestlé	Cappuccino, “Brigadeiro”, strawberry with chocolate
2	Trio, Kobber, Nutrale, Nutry, Quaker, Ritter, Hershey's	Trio, Ritter	Strawberry with Chocolate
3	Quaker, Nestlé, Nutry, Trio, Ritter, Levittá, Nature Valley, GranPure	Nutry, Trio, Ritter	Flavours with chocolate
4	Nutry, Hershey's	Nutry	Strawberry with Chocolate
5	Quaker, Nestlé, Nutry, Trio, Ritter, Levittá, Parati	Nutry, Ritter, Parati	Brazil nut, strawberry and hazelnut with chocolate

Table S.2. Basic formulation of the cereal bars.

Liquid		Solid	
Ingredients:	**g (for 100 g)**	**Ingredients:**	**g (for 100 g)**
Unflavoured Gelatine	2.80	Oats in thick flakes	5.50
Water	9.00	Oats in fine flakes	16.00
Brown Sugar	5.00	Oat bran	7.00
Corn glucose	5.00	Rice flakes	9.00
Polydextrose	5.00	Flaxseed	3.80
Maltodextrin	7.00	Quinoa mix in grains	2.50
Soy lecithin	0.93	Skim milk Powder	5.00
Canola Oil	2.00	Cocoa powder	5.00
Chocolate flavouring	4 drops (0.37 g)	Sodium bicarbonate	0.56
Vegetable oil	3.50	Citric acid	0.04
		Chocolate Powder	5.00

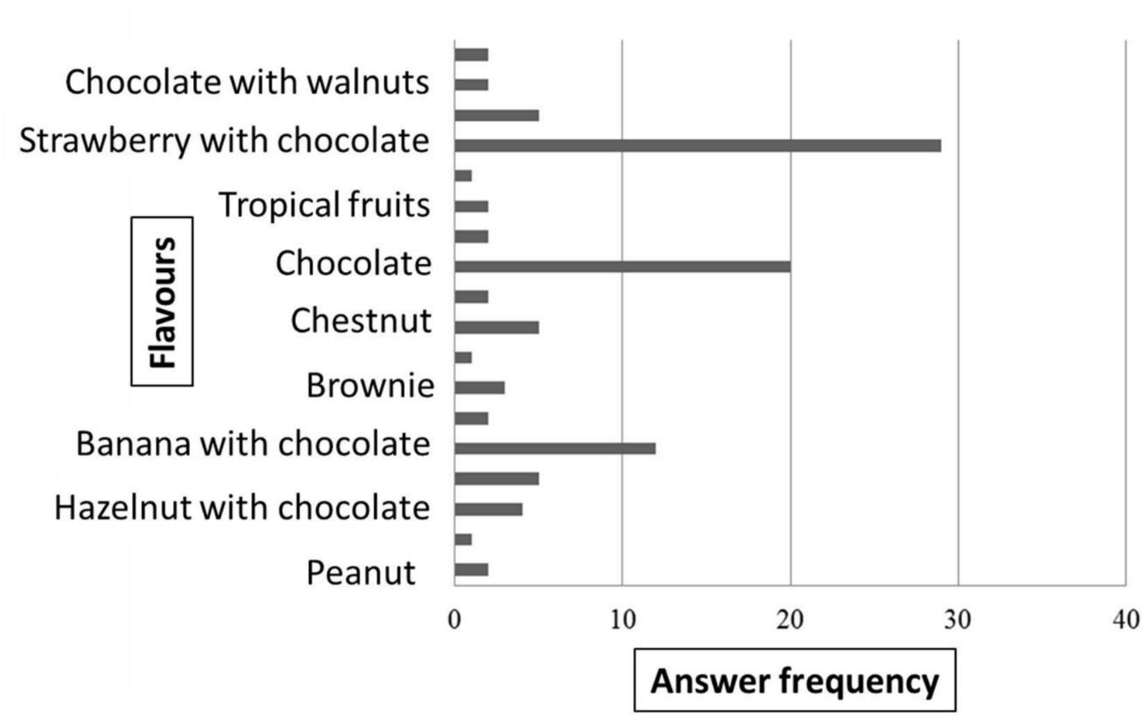

Figure S.1. Frequency of flavours preference responses for cereal bar survey.

Figure S.2. Nanosuspension of pinhão seed coats after 30 steps through the mechanical mill.

REGIONAL UNIVERSITY OF BLUMENAL
Food Processing Laboratory
Pinhão Project

Full Name:..   Date:___/____/____

Birth Date:___/____/____   Gender: ( ) Male   ( ) Female

School level: ....   Ocupation: ....

Market Research – Cereal Bars

1. How often do you consume cereal bars?
   ( ) Daily
   ( ) More than once a week
   ( ) Once a week
   ( ) Occasionally
   ( ) Rarely
   ( ) Never

2. What is the main factor influencing the purchase of cereal bars?
   ( ) Flavour
   ( ) Appearance
   ( ) Nutritional Value
   ( ) Composition (types of cereals and the like)
   ( ) Price
   ( ) Brand
   ( ) Variety of flavors

3. What flavor do you prefer? ....

4. What brand do you prefer? (optional) ....