



Determination of bioactive compounds obtained by the green extraction of taioba leaves (*Xanthosoma taioaba*) on hydrothermal processing

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Abstract

The objective of this study was to obtain and characterize a freeze-dried powder of taioba leaves (*Xanthosoma taioaba*) as a source of bioactive substances and potential food applications. The fresh leaves were cooked and lyophilized for further analysis. Its bioactive contents expressed in flavonoids, ascorbic acid and total polyphenols were quantified. Total antioxidant activity (TAA) was determined by ABTS assays. The chemical composition via infrared spectroscopy (FTIR) and its microstructure were visualized by scanning electron microscopy (SEM). The bioactive contents of flavonoids, ascorbic acid and polyphenols were 17.15 mg/100 g, 58.3 mg/100 g and 24.15 mg Eq. of gallic acid/100 g, respectively. A high content of TAA was found. ABTS 37.35 ($\mu\text{g TE/g}$). The FTIR spectrum revealed high-intensity bands at 3350 cm^{-1} , 2928 cm^{-1} , 1637 cm^{-1} , and 1055 cm^{-1} related to vibrations associated with typical bands of $-\text{OH}$ groups present in cellulose membranes, hemicellulose, carbohydrates, lignin and water. The micrographs showed irregular structures of the ground leaves with a fibrous structure. These results indicate a high potential of this raw material in food formulations as a source of bioactives suitable for applications in various industrial segments.

Keywords: taioba (*Xanthosoma taioaba*); freeze-dry; antioxidant activity.

Practical Application: The leaves of Taioba (*Xanthosoma taioaba*) are a raw material with high standard of nutritional quality for food consumption after cooking, expressed in its fiber and protein composition. In the functional profile in bioactive compounds, it shows a high concentration of antioxidants such as vitamin C, chlorophylls and, with an important presence of carotenoids and phenolic compounds.

1 Introduction

Nonconventional food plants (PANCs) receive this nomenclature because their consumption is restricted to a certain region, state or small communities. However, PANCs are gaining increasing visibility among the public, aiming at food diversification and environmental preservation (Barbosa et al., 2021; Oliveira et al., 2019).

Among the PANCs present in the Amazon, one of the highlights is the taioba (*Xanthosoma taioaba*), a tuberous herbaceous plant belonging to the Araceae family. It has a dark green color, with leaves containing erect basal petioles of varying sizes, reaching 160 cm, in a sagittal shape (like the tip of a spear), have two collecting veins on the margin and subcoriaceous laminae. It can reach 2 meters in height and has robust stems, white exudate on the stem and green pseudostems (Barbosa et al., 2021; Croat et al., 2017).

Taioba has been related as a source of macro and micronutrients, in addition to chlorophylls, carotenoids, lycopene, and phenolic compounds, among other bioactives related to anti-inflammatory,

hypolipidemic, hepatoprotective, neuroprotective, antioxidant, diuretic, and immunomodulatory effects. Its consumption has been reported predominantly after hydrothermal processing (cooking) (Araújo et al., 2019; Sharma et al., 2021). This process can be defined as a binary interaction of water and heat on the constituents of plant matrices, promoting several changes in the nutritional, anti-nutritional and bioactive compounds of the matrices.

Research with thermal and hydrothermal processes has been applied to different types of food in order to avoid the toxicity of anti-nutritional compounds such as cyanide, the removal of glycosides, tannins, antitrypsin factors, among others, making it an indispensable operation in the processing of many foods, making them the safest for human consumption (Acquisgrana et al., 2022; Brandão et al., 2021; Sheikh et al., 2021, 2022).

Given the importance of the constituents present in the leaves of taioba, its preservation and expansion of its application in other food formulations is necessary. Thus, freeze drying can be used to preserve and prolong the potential effects of these

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constituents, as it is a process that uses sublimation as a base to minimize the effects of drying on the structural, nutritional and functional composition of foods (Nakagawa et al., 2021). In addition, freeze drying is associated with other advantages, such as the high rehydration capacity due to the preservation of the structure, composition and retention of the characteristic flavor of the food (Nakagawa et al., 2021; Ansar et al., 2022).

To evaluate the effects of the hydrothermal process followed by freeze-drying, regarding the presence and maintenance of bioactive compounds, the application of aqueous extraction follows the premise of replacing solvents harmful to health and the environment with solvents inert to them, acting in line with the principles of green chemistry (Płotka-Wasyłka et al., 2017; Pacheco-Fernández & Pino, 2019).

In this context, maintaining the quality of food matrices with the addition of freeze-drying processes added to ultrasound-assisted green extraction aims to promote the search for better results, such as increased yield and quality maintenance. In view of the above, the objective of this research was to evaluate the proximate composition, the content of bioactive compounds and antioxidants, and the spectroscopic and morphological profile of the leaves of taioba (*Xanthosoma taioba*) powder.

2 Materials and methods

2.1 Raw material and sample preparation

To carry out the present research, approximately 5 kg of the Taioba (*Xanthosoma taioba*) leaf was obtained on the market in the city of Belém, Pará, Brazil. The samples were transported in plastic bags of low-density polyethylene (LDPE) and stored at a temperature of 20 °C until use in the Food Science Laboratory at the Faculty of Nutrition (FANUT), Federal University of Pará (UFPA). The leaves were sanitized in a sodium hypochlorite solution (150 ppm) for 10 min before-cooking in water for 10 min, following the traditional procedure in Northern. After cooking, the leaves were freeze-dried (Solab, model SL-404, São Paulo, Brazil) for 48 h, ground in a knife mill (Tecnal, model Willye TE-650, São Paulo, Brazil), vacuum-packed (Cetro, DZ 300T) and stored at -7 °C, protected from light, until use. Another part of the leaves was used in the biometric characterization.

2.2 Determination of the biometric composition of taioba leaves

The biometric characterization of the leaves was carried out according to the analytical methods of Association of Official Analytical Chemists (1992). Fifty leaves were used to determine the following parameters: length of the fruit (cm), diameter of the fruit (cm), and weight (g) with the aid of an analytical scale (Bel brand, model L303i).

2.3 Chemical composition

Determination of the composition of macronutrients of the taioba leaf was based on physicochemical analyses, including water activity: DECAGON's AquaLab Series 3TE instrument; pH: according to the AOAC method (Association of Official Analytical Chemists, 2016); humidity: according to AOAC

method no. 934.06 (Association of Official Analytical Chemists, 2016); crude protein micro Kjeldahl method no 920,152 from Association of Official Analytical Chemists (2016); total lipids method no 983.23 of Association of Official Analytical Chemists (2016); fixed mineral waste with AOAC method 940.26 (Association of Official Analytical Chemists, 2016); total fibers 985.29 enzymatic-gravimetric method Association of Official Analytical Chemists (2016); total carbohydrates calculated by difference and application of total energy value: atwater factors 4 - 9 - 4 kcal/g for proteins, lipids and total carbohydrates, respectively. All of the analyses were executed in triplicate, and the final results are presented as their means.

2.4 Analyses of bioactive compounds

Extract preparation

The extracts were prepared using lyophilized leaf samples using an ultrasound (Solid Steel São Paulo, Brazil), and the powder samples were suspended in 70% ethanol solution (w/v). After sonication and a frequency of 20 kHz for 10 min at 20 °C, the material was subsequently centrifuged (Sigma model 6-15H) at 3,900 rpm for 15 min. To obtain the crude extract, the supernatant was recovered, filtered and concentrated on a rotary evaporator, model Laborota 4000 (Heidolph, Schwabach, Germany), under low pressure and controlled temperature (40 ± 5 °C). The extracts were stored in amber glass vials, added to nitrogen gas (N₂), hermetically closed and stored at -18 °C until the time of analysis.

Ascorbic acid content (AA)

Determined by titration by the reduction of 2,6-dichlorophenol-indophenol (DCFI) compound by ascorbic acid (Cunha-Santos et al., 2019). The lyophilized leaves (5 g) were diluted with 40 mL of a 4% aqueous oxalic acid solution and mixed for 30 min at 3,900 rpm on a magnetic stirrer (Solab brand, model SL-91/3) in a dark room and then vacuum filtered. The filtered component was titrated with the addition of the 2,6-dichlorophenol-indophenol solution until a pink color persisted. L-ascorbic acid was used to prepare the standard solution (0.5 mg/mL), and the concentration was calculated by comparison to the standard and expressed in mg/100 g of fresh mass.

Flavonoid content

The flavonoid content was analyzed following the assay reported by Francis (1982) using a UV-Vis spectrophotometer (model UV-1800, Shimadzu, Tokyo, Japan) at a wavelength of 374 nm. W were extracted from 1 g of lyophilized leaves with 30 mL of 95% ethanol/1.5 M HCl (85:15, v/v) mixed for 15 min at 3,900 rpm on a magnetic stirrer (Solab, model SL-91/3). The extract was transferred to a 50 mL volumetric flask, completing the volume with ethanol-HCl (1.5 M) and stored for 12 h at 4 °C (Francis, 1982). After filtration, the absorbance was measured in a UV-vis spectrophotometer (model UV-1800, Shimadzu, Tokyo, Japan) at a wavelength of 374 nm. The total flavonoid content was determined by applying the Lambert-Beer law and was calculated as mg/100 g using the following formula (Equation 1):

$$\text{Total flavonoids content} = \frac{A_{374} \times \text{dilution factor}}{E_{1\text{CM}}^{1\%} 374\text{nm}} \quad (1)$$

where A_{374} is the absorbance in the diluted sample and $E_{1\text{CM}}^{1\%}$ cm, 374 is the value factor (76.6) of molar absorptivity for the acid-ethanol solvent measured in a 1 cm cell at 374 nm at a concentration of 1% (w/v).

2.5 Chlorophyll content

The percentage of chlorophyll was determined by the Lichtenthaler method (Lichtenthaler, 1987); an aliquot (1 g) of leaves was macerated with 10 mL acetone solution (80%) (v/v) until all pigmentation was extracted and then centrifuged at 4,000 rpm for 10 min (Sigma model 6-15H). The supernatant was transferred to a 25 mL volumetric flask. The volume was completed with acetone solution (80%) (v/v). Absorbance readings were performed using a UV-Vis spectrophotometer (UV-1,800, Shimadzu, Tokyo, Japan) with 647 nm and 663 nm wavelengths. P.A. Acetone was used as a negative control. Each sample was analyzed in triplicate. The results were expressed in mg of chlorophyll by 100 g of sample.

2.6 Total phenolic content

Total phenolic content (TPC) were determined using the Folin-Ciocalteu assay as reported by Aliakbarian et al. (2011) and were measured at 725 nm using a UV-Vis spectrophotometer (UV-1800, Shimadzu, Tokyo, Japan). The TPC results were standardized against gallic acid equivalents per 100 g of oil (mg GAE/100 g). The method was based on the linear equation (Equation 2):

$$Y = 0.0017X \quad (2)$$

where Y is the TPC expressed in mg GAE/100 g of oil and X is the read absorbance, with $R^2 = 0.9966$.

2.7 Total antioxidant activity

The total antioxidant activity (TAA) was analyzed through the capture of the [2,2'-azino-bis(3-ethylbenzthiazoline-6-sulfonic)] (ABTS) radical (Rufino et al., 2010). The absorbance was measured at 734 nm using a UV-Vis spectrophotometer (UV-1800, Shimadzu, Tokyo, Japan). The total antioxidant capacity was calculated in triplicate against a calibration curve of ethanolic solutions of known Trolox concentrations, based on the equation 3.

$$Y = -0.3364X + 0.6239 \left(R^2 = 0.997 \right) \quad (3)$$

where Y is the TAA (expressed in $\mu\text{g TE/g}$ of Trolox concentration) and X is the read absorbance.

2.8 Infrared spectroscopy

Fourier transform infrared spectroscopy (FT-IR) analysis was performed using a Frontier 98737 spectrometer (Perkin Elmer, Waltham, MA, USA) in transmission mode in the

4000 – 400 cm^{-1} range with 4 cm^{-1} resolution, and the data were analyzed with Origin 8.0 software (OriginLab Corporation, Northampton, MA, USA). The sample analysis was carried out as potassium bromide (KBr) disks at room temperature.

2.9 Morphological analysis by scanning electron microscopy

The degreased samples of JL were deposited on a sample holder with the aid of carbon tape and metallized with Au/Pd using a metallizer, model SC7620 (Quorum Technologies, Lewes, UK). Metallization was performed for 2 min with a 5 mA current. Electromicrographs were obtained using a scanning electron microscope, model VEGA 3 (Tescan, Cranberry Township, PA, USA), with an electron beam current of 85-90 μA and an acceleration voltage of 10.0 kV. The micrometric scales were designed under the same optical conditions.

3 Statistical analysis

The analysis was performed in triplicate (mean \pm standard deviation). using Statistica version 10.0 software (Statistica for Windows, 2010).

4 Results

Biometric data of Taioba leaves are presented as the mean and standard deviation in Table 1.

The biometric parameters of the taioba leaf show high standard deviations. The results of the wide variety of weights and dimensions of the leaves. When compared to the studies by Costa et al. (2020) with hydroponic cultivation, an average of 17.84 cm in transverse length and 16.87 cm in longitudinal length were obtained, which are lower than the current research (Figure 1).

This fact may be related to several factors, such as species varieties, soil nutrition, edaphoclimatic differences, and cultivation method, since one is a hydroponic system and the one in the present work is a conventional vegetable garden. The size of the leaves is an important characteristic for marketing leafy vegetables Costa et al. (2020). The nutritional composition of powdered taioba leaves is shown in Table 2.

AW is an intrinsic factor that interferes with the durability of the product and is directly proportional to its greater risk of degradation. In the present research, Aw and pH were below the levels conducive to the growth and development of microorganisms as well as enzymatic reactions (Rahman, 2019).

Table 1. Biometric characterization of the in natura taioba leaf.

Parameters	Maximum	Minimum	Average and Standard Deviation
Weight (g)	79.64	27.26	48.15 \pm 16.07
Transverse Length (cm)	45.0	27.4	35.55 \pm 5.59
Longitudinal length (cm)	62.3	41.7	48.11 \pm 6.82

The results are expressed as the mean \pm standard deviation.

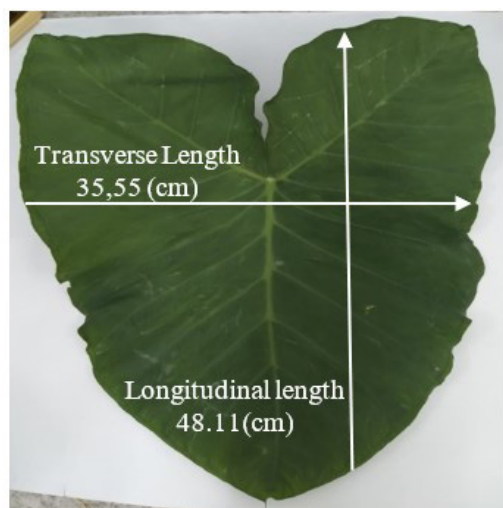


Figure 1. Biometric characterization of the in natura taioba leaf.

However, studies by Costa et al. (2020) compared leaves in natura under hydroponic cultivation and found a higher pH (6.31). These data show that the drying method positively influences the microbiological and enzymatic safety of powdered taioba.

The low moisture contents presented by the lyophilized material show the efficiency of the applied drying process, markedly reducing the moisture contents presented in the comparative bases (Table 2). The inorganic composition with the fixed mineral residue was double the value, and the same trend was observed for the macronutrients proteins, lipids, and carbohydrates.

The total protein contents were lower than those reported in the literature for fresh leaves and dehydrated leaves (Jackix, 2015). However, higher than the values obtained in the research by Botrel et al. (2020) and Moura et al. (2021) and similar to the data presented for taioba starch (Ramos et al., 2020). The lipid content results are superior to the values reported in the references of Table 2 for taioba in natura. However, these results are superior to the data presented for taioba starch (Ramos et al., 2020). Such differences may be related to different sample conditions, origins, growth, climates, soils, plant genetics, nutrient concentrations, and phenological stages.

The total fiber content obtained for the powder sheet shows the prevalence of insoluble fibers in relation to soluble fibers. Its total and insoluble contents were higher than those reported by Botrel et al. (2020), similar to the values presented in research by Jackix (2015). Fibers are considered functional constituents. The higher proportion of insoluble fibers may be related to cellulose, hemicellulose and lignin, which are important for human health, as they act by improving the intestinal microbiota, reducing systemic inflammation and controlling type II diabetes (Ayua et al., 2020).

The amount of carbohydrates and the high energetic value found in freeze-dried taioba leaves were much higher than the data presented in the in natura leaves reported in the research by Botrel et al. (2020) and Moura et al. (2021) which was expected

Table 2. Nutritional composition of powdered taioba leaves.

Parameters* (%)	Powdered taioba Current search	Taioba in natura Botrel et al. (2020); Moura et al. (2021)	
AW	0.23 ± 0.15	ND	ND
pH	5,15 ± 0.60	ND	ND
Moisture	8.35 ± 3.20	86.58	90.77
Lipids	1.91 ± 0.75	0.62	0.51
Protein	5.15 ± 0.55	3.05	3.08
Total fiber	14.5 ± 0.63	3.89	ND
Soluble fiber	3.50 ± 0.85	ND	ND
Insoluble fiber	11.55 ± 0.33	ND	ND
Ashes	3.45 ± 0.71	1.74	1.57
Carbohidrates	66.64	4.12	4.07
Energetic value	294.8	34.26	33.19

The results are expressed as the mean ± standard deviation. ND – not determined AW - Water activity *Dry base.

due to the drying technique applied and the calculation of the contents expressed by the other components being on a dry basis. The different energy densities in the leaves can be the result of some aspects, such as the size presented by the leaves (Figure 1), the need for energy reserve tissue for foliar nutrition (leaf blades) fundamental to plant metabolism, nutritional differences in the soil and climate.

Compared with other leafy vegetables such as jambú (*acmella oleracea* L) in the research by Neves et al. (2019) carbohydrate contents and energy value, on a dry basis, were lower than taioba. However, in cariru (*Talinum triangulare*) and amaranth (*Amaranthus hybridus*) leaves, the total carbohydrate and energy inputs were higher than those obtained in this research.

These data show that the application of drying technologies, such as lyophilization, increases the concentration of its nutrients and the degree of durability and conservation. Since these are one of the biggest obstacles to its commercial expansion, I have restricted its commercialization in natura.

Another constituent evaluated was the presence of calcium oxalate, a constituent of several hardwoods and considered an anti-nutritional factor, due to the negative interference in the availability of calcium (Liu et al., 2018). The high solubility of oxalate in water causes its content to be eliminated during hydrothermal processing (cooking), making taioba leaves safer for consumption and processing (Liu et al., 2018; Lima & Krupek, 2016). The content of calcium oxalate in the powdered leaves of taioba was 697 mg/100 g. The content of bioactive substances and antioxidant activity of the freeze-dried taioba leaf extract are presented in Table 3.

The vitamin C content of freeze-dried taioba leaves was lower than that of fresh leaves, with a mean of 87 mg/1000 g. However, it is superior to other hardwood species, such as jambu (*Acmella oleracea* L), cariru (*Talinum triangulare*) and amaranth (*Amaranthus hybridus*) (Araújo et al., 2019; Santos Filho et al., 2021). Chlorophyll levels in the powder of taioba leaves were lower than those in fresh leaves, as shown in the research by

Table 3. Content of bioactive substances and antioxidant activity of the freeze-dried taioba leaf extract.

Assays	Content of bioactive Substances
Ascorbic acid content (mg/100 g)	58.30 ± 0.82
Total chlorophyll (mg/100 g)	7.54 ± 0.52
Flavonoids content (mg/100 g)	17.15 ± 1.25
Polyphenols content (mg GAE/g)	24.15 ± 0.85
ABTS (µg TE/g)	27.35 ± 0.77

Data represent the mean ± standard deviation of triplicate determinations.

Araújo et al. (2019), who showed averages of 8.94 mg/100 g. However, higher than the hardwood species chicory (*Eryngium foetidum* L.) and cariru with averages of 30 and 21.25 µg/mL, respectively, in the studies by Santos et al. (2021).

These variations are related to the differences imposed in the treatment of the sample (cooking), the different species, the stage of plant development, and the soil and climate characteristics, among others.

The presented contents of phenolic compounds and flavonoids (Table 2) were lower than those presented by the leaves of taioba in natura reported in the studies by Jordan et al. (2021) and Avellar et al. (2018). In comparison with other vegetables such as chicory

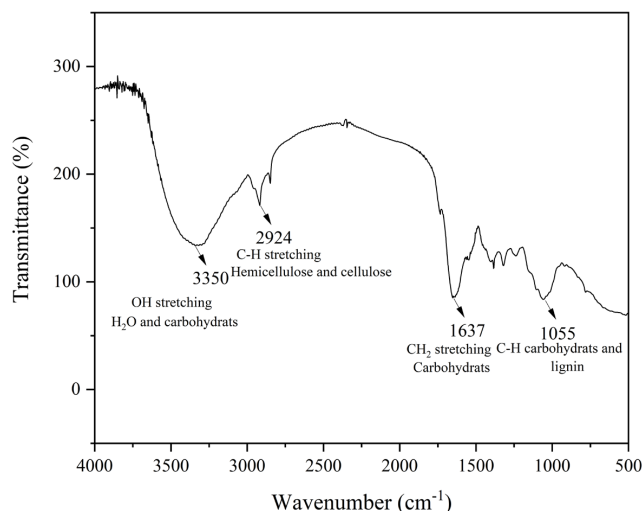
(*Eryngium foetidum* L.) and cariru (*Talinum triangulare* Jacq), the results of this research were superior to the findings of Santos Filho et al. (2021), with means of 10.8 and 21.41 (mg GAE/g) for phenolics and 9.12 and 15 mg/g for flavonoids, respectively.

These results show a relationship with the research by Kourouma et al. (2019) in evaluations of the effects of the hydrothermal process with proportional reduction of phenolic compounds and ascorbic acid, a fact related to the water-soluble characteristics of these compounds being solubilized in water during cooking (Chen & Roca, 2019) showed a reduction in flavonoid contents by 60% in cooking for an average time of 10-15 min, similar to the time applied in this research.

The antioxidant activity of powdered taioba leaves showed higher antioxidant activity than fresh leaves (26.48 µM trolox/g) evaluated in the research by Araújo et al. (2019). When comparing the antioxidant activities by ABTS in powdered jambú leaves (13.65 µmol Trolox/g), the results of this research are also higher (Santos et al., 2021) revealing the high potential for antioxidant action of this material in powder.

The FTIR spectrum of taioba leaf powder is shown in Figure 2. The application of this tool allows obtaining information related to functional chemical groups and their vibrational states according to interactions and changes in the structure and composition of materials (García-Salcedo et al., 2018; Ramos et al., 2020).

Highlighted spectral peaks with broad bands at 3,350 cm⁻¹ and 1,637 cm⁻¹ are related to stretching vibrations of -OH groups present in cellulose membranes and in water. The presence of bands at 2,928 cm⁻¹ and 1,055 cm⁻¹ may be related to CH₂ bending in hemicellulose and CH vibrations, which commonly appear in carbohydrates and lignin. These findings confirm the high

**Figure 2.** FTIR pattern of powdered taioba leaves.

presence of fibers in the material (Table 2), reinforcing the functional aspects of powdered taioba leaves.

Comparing the data from this research with powdered jambú leaves (Santos et al., 2021) it is possible to notice the similarities of chemical constituents based on spectral peaks with high intensity bands at 3,400 cm⁻¹ and 1,063 cm⁻¹, related to vibrations, which are associated with typical bands of cellulose and organic acids. Similar to the findings in this research.

The morphology of the taioba leaf powder shows structural constituents that confirm the presence of the functional groups obtained by FTIR (Figure 3).

In the findings of the morphological structure of the powder of taioba leaves, it is possible to visualize the structure of the cell wall (plant parenchyma) with fiber bundles, possibly constituents of cellulose and hemicellulose. There are structures that may have lost their original architecture due to the drying process related to water loss during sublimation under vacuum in the freeze-drying process.

Compared with research by Pandey et al. (2014) in powdered leaves of *Spilanthes acmella* L. var. *oleraceae*, *S. calva* L., and *S. paniculata* Wall, showed the presence of deformed starch granules with different diameters, similar to the research by Santos et al. (2021) with powdered jambu leaves. Findings not visualized in this research.

5 Conclusion

The process of obtaining and characterizing the powder of Taioba leaves showed that the use of lyophilization is an adequate technique for the preservation and greater conservation of this raw material to maintain and concentrate its high content of nutritional and bioactive compounds resulting from its high levels. Fiber, carbohydrates, vitamin C and antioxidant compounds. Its FTIR spectral profile shows typical behaviors of matrices rich in cellulose, hemicellulose, fibers, carbohydrates and lignin. Its morphological pattern confirms the physicochemical and

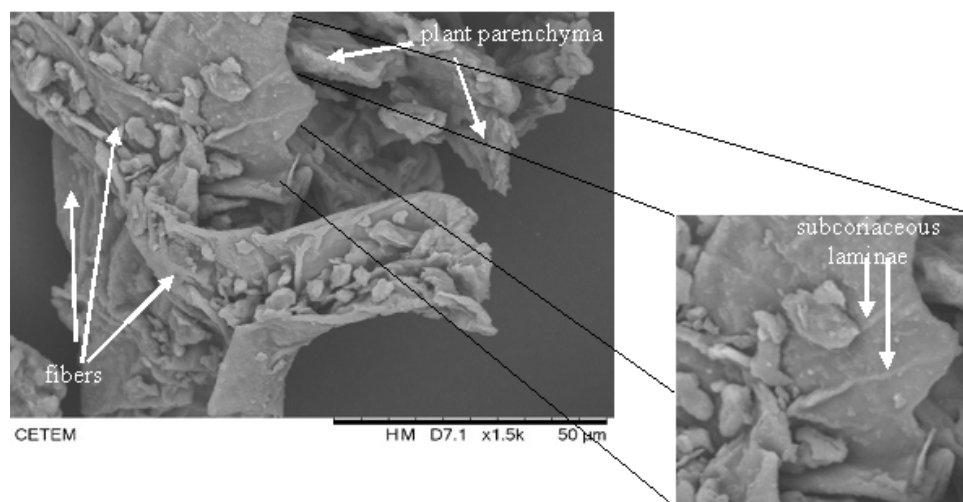


Figure 3. Morphological structure of taioba leaf powder.

spectroscopy findings, showing cellulosic-based structures and fibrous constitution. These data reveal that taioba leaf powder has a longer shelf life and maintains important nutritional and functional constituents to human health, implying an alternative raw material as a complement or supplement of nutritional and bioactive substances in foods.

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