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HEALTH, NUTRITION, AND FOOD

Linking geographical origin with nutritional, mineral, and visual proprieties of *pinhão* (*Araucaria angustifolia* seed) from the south of Brazil

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Abstract: The effect of harvest location on cooked *pinhão* seeds *(Araucaria angustifolia)* was investigated with regard to its centesimal composition, minerals, and color, and later correlated with environmental and soil variables. Significant differences between cooked *pinhão* from various harvesting locations were seen; also, principal component analysis was performed for the minerals, protein, moisture, total starch, and color parameters. The geographic location was one of the most important factors. Caçador presented greater differences: lower values for moisture, minerals, geographic parameter, and color characteristics. However, nearby localities, such as Cruz Machado with Bituruna and Lapa with São João do Triunfo, presented similar overall values for minerals and geographic parameters. Each regional geographic location was able to present unique characteristics so that the principal component analysis categorized it in specific quadrants, which is also in agreement with the CIELAB color space. However, hierarchical tree exhibited that CAÇ was the most distinct, due to the most distant municipality, presenting a unique microbiome. The *pinhão* is a source of various nutrients, which contributes to healthy dietetic daily values. It provides from 20% to 30% of dietary fiber, Cu (42.2%), P (31.1%), K (23.5%), and Zn (22.1%), while also providing quantities of Mg (12.9%), Mn (12.4%), Fe (11.5%), and Ca (6.4%). Therefore, it is possible to obtain food products based on cooked *pinhão* that contain many nutritional components associated with human health benefits.

KEYWORDS
agrobiodiversity, dietary fiber, endemic species, food composition, mineral nutrients

Practical Application: The *pinheiro-do-paraná* is a conifer that is currently endangered. However, the commercial use of its seeds may be key to guaranteeing its preservation, in addition to strengthening the economies of households

and small producers. The *pinhão* collected from a large area of Araucária forests, after subsequent boiling, removal of the almond, grinding, and freezing provides large amounts of carbohydrates, higher dietary fiber content, resistant starch, and large quantities of Cu, P, K, and Z, in addition to significant amounts of Mg, Mn, Fe, and Ca. All of these are desirable characteristics that increase the value of *pinhão*.

1 | INTRODUCTION

The *Araucaria angustifolia* seed, popularly known as *pinhão*, is a seasonal product that is widely harvested, is exploited in an extractive way, and is an important source of income for agricultural families (Danner et al., 2012). Moreover, *pinhão* harvest is ranked sixth among Brazilian ethno-botanical foods in a consensus from 2019 (9342 t) (IBGE, 2021). In this context, *pinhão* production is stimulated by many agencies and the government, which succeeded in doubling annual production levels from 1986 to 2019 (IBGE, 2021). The *pinhão* produced in the municipalities, participating in the current study, represented 1.63% of the entire Brazilian production (Bituruna/PR [BIT]: 0.57%, Caçador/SC [CAÇ]: 0.32%, Cruz Machado/PR [CM]: 0.56%, Lapa/PR [LAP]: 0.15%, São João do Triunfo/PR [SJT]: 0.03%) (IBGE, 2021).

Pinhão has a high nutritional value (Barbosa et al., 2019; Cordenunsi et al., 2004; Gil et al., 2021), and the edible portion (almond) corresponds to 70% of its weight (Oliveira et al., 2015). Although the raw nut is not edible, it is softened and consumed after cooking by gellification of the starch, a nutrient that is presented in large content (de Siqueira et al., 2019), leading to a high energy value, but is able to maintain a low glycemic profile, a characteristic that is attributed to the presence of dietary fiber and resistant starch (Cordenunsi et al., 2004). The remaining nutrients, proteins, crude fiber, lipids, and minerals contribute to approximately 7% (wet basis) of its mass (Gil et al., 2021). This almond is also a source of healthy inorganic nutrients such as phosphorous (P), manganese (Mn), copper (Cu), molybdenum (Mo), and chromium (Cr) (Barbosa et al., 2019).

Due to the characteristics of its starch, as well as the presence of significant levels of both resistant and digestible starch, the *pinhão* almond is promising as a health-promoting ingredient, or as foods supplement, such as nutraceutical and nutritional supplements (Zortéa-Guidolin et al., 2017). They may also be used to produce gluten-free starch flours, a unique attribute useful for individuals who suffer from celiac disease (Ikeda et al., 2018; Peralta et al., 2016). Their high dietary fiber content also allows them to be used in the production of specialized breadmaking products, such as cakes (Ikeda et al., 2018).

So far, few studies have investigated the centesimal composition variations of cooked *pinhão* almond from various regions of the south of Brazil. A previous work investigated the nutritionl value with respect to different states (Barbosa et al., 2019). A detailed study from the southern part is needed to find characteristics of the microbiomes of this specific flora, which may help improve proliferation of this endangered species containing ideal quantities of ingredients (Cordenunsi et al., 2004; Schveitzer et al., 2014).

Previous studies in other vegetables showed that the location of the growing area, as well as the climatic conditions prevailing each year, affected most of the chemical components of pea varieties (Nikolopoulou et al., 2007). The genotype, another variable studied, also affected the proximate composition of almond [*Prunus dulcis* Mill. DA Webb] kernels (Ibourki et al., 2022).

Therefore, the objective of this study was to evaluate the centesimal composition, mineral quantities, and color of cooked *pinhão* almond collected from five separate locations distributed over a wide area within the region of *A. angustifolia* natural ecosystem, which were statistically compared to the climatic and soil parameters. Knowledge regarding the existence of nutritional variations, and their relation to such harvest locations, will help in the development of a composition map analysis that presents the most favourable characteristics, that can be used for the manufacture of innovative products with health benefits.

2 | MATERIALS AND METHODS

2.1 | Collection locations

Pinhão samples were collected from the following five municipalities from Paraná (PR) and Santa Catarina (SC) states, Brazil: *Bituruna*/PR (BIT): latitude: 26° 09′ 41 ″ S, longitude: 51° 33′ 09″ W; *Caçador*/SC (CAÇ): latitude: 26° 46′ 31″ S, longitude: 51° 00′ 54″ W; *Cruz Machado*/PR

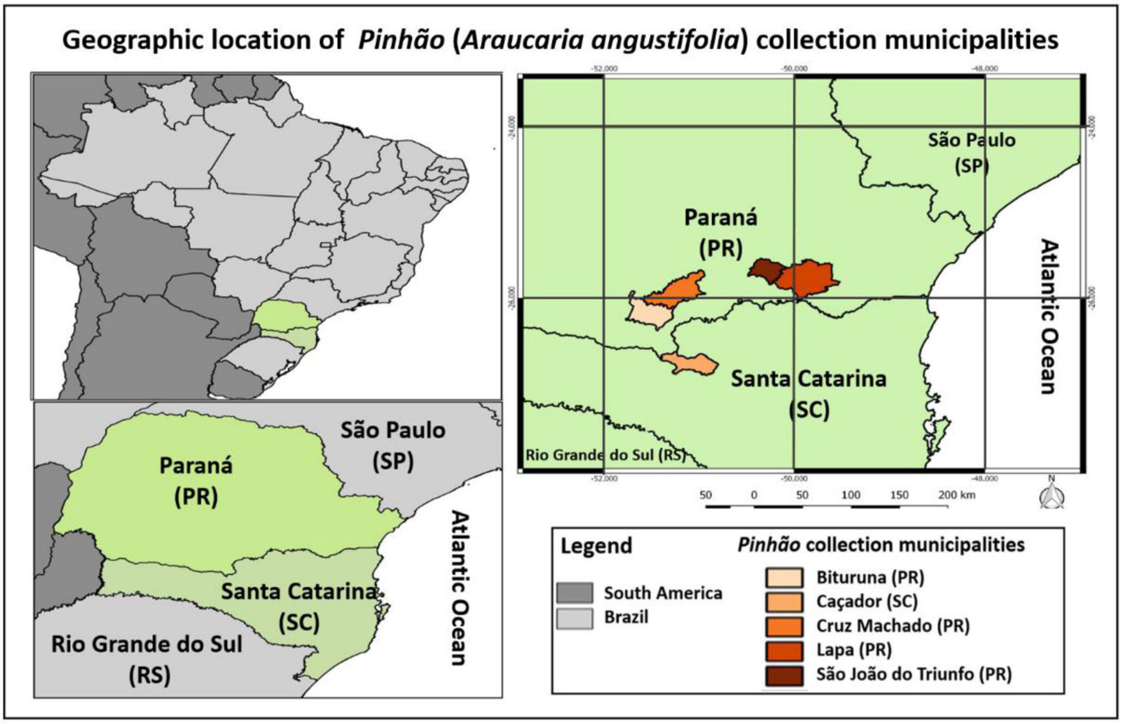

FIGURE 1 Geographic location of the five municipalities from which the *pinhão* was collected: Bituruna (PR), Caçador (SC), Cruz Machado (PR), Lapa (PR) e São João do Triunfo (PR)

(CM): latitude: 26° 01′ 03″ S, longitude: 51° 20′ 48″ W; *Lapa*/PR (LAP): latitude: 25° 46′ 11″ S, longitude: 49° 42′ 57″ W, and *São João do Triunfo*/PR (SJT): latitude: 25° 41′ 00″ S, longitude: 50° 17′ 50″ W). The five locations were chosen so as to have a well-distributed collection spanning regions, as *A. Angustifolia* flora covers a large area especially within the southern region of *Paraná* State and the northern portion of *Santa Catarina* State (Carvalho, 1994).

This study was carried out on 3 harvests on different days, with a maximum interval of 2 weeks. When the pinecones were ripe, they naturally dropped to the ground. Following from this, partner farmers collected these pine cones from at least 10 trees at different points from the farmer's property. Inside the pinecones, approximately 1 kg of pinhão seeds was collected, and later the quartering method of this total of 10 kg was carried out. In summary, we obtained 1 kg of homogenized sample from these collections, totaling 3 kg of pine nuts from each municipality, which were further stored in polyethylene bags and taken through further analysis. The analyses were performed in triplicate, and the mean values of each collection were used for statistical treatments. For better visualization of the location of the municipalities, a map was drawn using the Software Quantum Gis 2.18.9 (Figure 1).

2.2 | Collection of soil, geology, and climate data

Soil data were collected from the Embrapa Soils database and the FEBR Brazilian Soil Data Repository (Bdsolos.org, 2022; Pedometria.org, 2022). The geology was classified according to the Köppen climate classification, and the climatic variables were extracted from the climate.org database. More details in Supplementary Table S1 and S2.

Environmental variables were also calculated (supplementary material (1.1 to 1.5)). The Marsz Oceanity Index was in accordance with Andrade and Corte-Real (2016) while the other climatic variables were calculated with a similar procedure from Baltas (2007).

2.3 | Preparation of the *pinhão* almond samples

Pinhão samples from each region, approximately 1 kg of sample, were cooked individually for 30 min at 121°C (1 atm) in a vertical autoclave model 103 (Fabbe Center, São Paulo, Brazil). The *pinhão* almonds were removed manually using a wooden *pinhão* husk remover, and they were ground for 1 min in a grinder and sieved (Bertel, Caieiras, Brazil) with a 35 mesh, with an overall particle size lower

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than 425 μm. They were then conditioned in polypropylene flasks containing 100 g of *pinhão* sample and frozen in a vertical freezer Re 26 (Eletrolux, Curitiba, Brazil) at −20.0°C. The analyses were conducted after they had been defrosted to ambient temperature and fragmented with the aid of a mortar and pestle.

2.4 | Centesimal composition and minerals

The analyses were carried out in accordance with the AOAC (2016). The parameters evaluated were: moisture and ash (gravimetric method), protein (Micro-Kjeldahl method), and lipid (Soxhlet method). The total starch and resistant starch contents were measured by enzymatic methodology using the Megazyme Assay Kit K-TSTA and K-RSTAR, respectively (Megazyme International, Wicklow, Ireland). The dietary fiber contents (total [TDF], insoluble [IDF], and soluble [SDF]) were measured by enzymatic methodology using the Megazyme Assay Kit Total Dietary Fiber (Megazyme International, Wicklow, Ireland). The total carbohydrate content (%) was calculated based on the values obtained for moisture, protein, lipid, ash, and dietary fiber.

Mineral determination was performed after nitroperchloric digestion. The potassium (K) content was determined using a flame photometer (Quimis, Q398M2, Brazil) (Silva, 1999). The phosphorous (P) content was determined by spectrophotometry in the UV/VIS spectrophotometer of the visible region (Perkin Elmer, Lambda 20, England). The calcium (Ca), magnesium (Mg), iron (Fe), zinc (Zn), copper (Cu), and manganese (Mn) contents were determined using an atomic absorption spectrometer (Perkin Elmer, AA200, England) (AOAC, 2016).

2.5 | Nutritional value

The total caloric value (TCV) in kJ per 100 g was estimated by adding the products of the protein, lipid, and carbohydrate values per gram (16.72 kJ [4 kcal], 37.62 kJ [9 kcal], and 16.72 kJ [4 kcal], respectively) and their respective dry basis energy index units. The Recommended Dietary Allowance (RDA) is the level of daily dietary intake sufficient to meet the nutritional needs of healthy individuals in a given gender and lifestage group. The RDA was used to predict the estimated demands of the inorganic components and proteins (IOM, 2006; WHO, 2012; WHO/FAO, 2004). Portions containing 100 g of *pinhão* almonds (wet basis) were used, considering that they are normally consumed with a moisture content of 45%, and, in order to obtain better consumption conditions, a 55 g portion (dry basis) was used (Barbosa et al., 2019).

2.6 | Analysis of the colorimetric components $L^* a^* b^*$

The color analysis was performed using a digital colorimeter MiniScan XE Plus (HunterLab, Reston, VA, USA), in the CIE system $L^* a^* b^*$, with the coordinate L* (brightness: 0 = black; 100 = white), a^* (red/green intensity: $+a^*$ = degree of red; $-a^*$ = degree of green), b^* (yellow/blue intensity: $+b^*$ = degree of yellow; $-b^*$ = degree of blue), with illuminant D65 and 10° observation angle. For the test, ~3 g of sample was placed on an acrylic plate with 4 cm in diameter and sample thickness of 5 mm (Malta et al., 2021). The readings were performed on the cooked and grinded *pinhão* almonds samples, and finally plotted using the "colorspace" R package.

Equation 1 presents the color variation (ΔE), which was calculated from the L^* (luminosity), a^*, and b^* (chromaticity) values. Δ is the difference between each color parameter of the control sample and the test sample. These assays were performed in triplicate.

$$\Delta E = \left[\left(L^* - L_0{}^*\right)^2 + \left(a^* - a_0{}^*\right)^2 \left(b^* - b_0{}^*\right)^2\right]^{\frac{1}{2}} \quad (1)$$

where L_0 and a_0, b_0 are parameters from the control sample (locality) and L, a, and b are parameters from the test sample.

2.7 | Statistical analysis

In each case, *pinhão* analyses were carried out in triplicate, except for the dietary fibers (total, insoluble, and soluble) and lipids, which were performed in duplicate. Analysis of variance (ANOVA) was used, followed by the Tukey method, to test the significance of the means of each variable among the study areas ($p < 0.05$), using the STATISTICA 13.2 version software (StatSoft Inc®, Tulsa, OK, USA).

To test for any potential correlations between the general environmental characteristics of the sampling areas and nutritional variables, physiographical and climate variables based on precipitation, annual temperature, and temperature of the month of collection were examined. Due to the lack of site-specific data for these variables, as well as the lack of climate stations in some municipalities, the authors used data that was modeled for each site using a webline application (Climate), which models

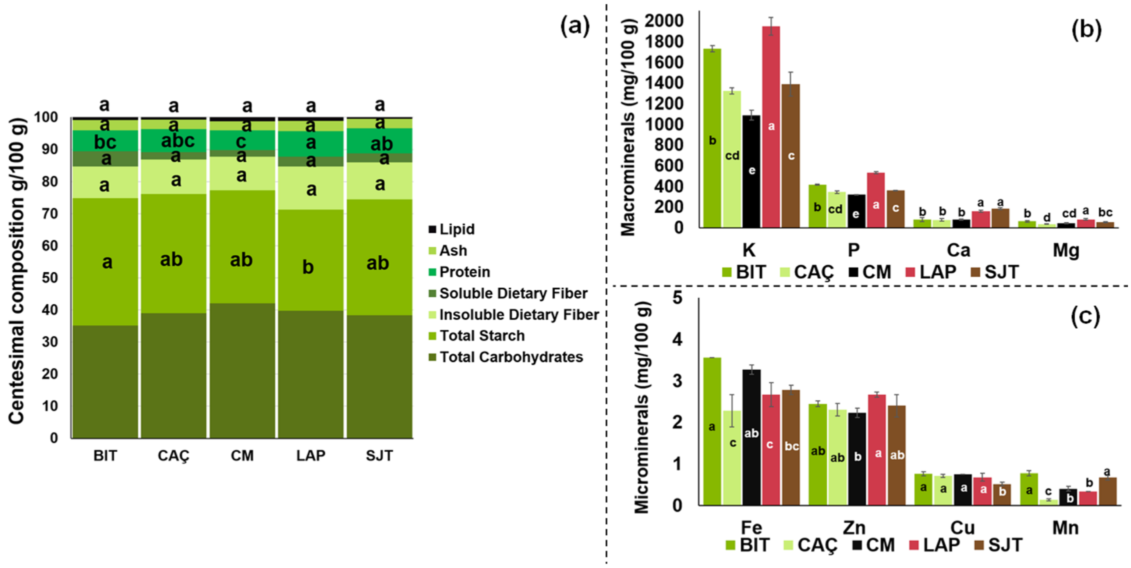

FIGURE 2 Centesimal composition (a), macrominerals (b), and microminerals (c) of *pinhão* almonds from five locations in the southern region of Brazil, on dry basis*. The superscript letters are Tukey's statistical data, in which same letters do not differ statistically from the same measurement attribute ($p < 0.05$). BIT: Bituruna; CAÇ: Caçador; CM: Cruz Machado; LAP: Lapa; SJT: São João do Triunfo

climatologic parameters to a scale of approximately 1 km, using an artificial intelligence dataset that spans 30 years. Precipitation, elevation, and temperature values from the data obtained were used as variables from the A.I. software data (Table S1). The authors initially tested the environmental variables using principal component analysis (PCA) in order to examine interrelationships among the variables (Hair et al., 2010) and to provide a simplified variable structure.

The statistical analysis of PCA and cluster analysis after PCA were carried out using R Studio software (R Core Team, 2020). Analysis of PCA and Hierarchical Clustering on Principal Components were calculated using the "Factominer" R package (Lê et al., 2008). Data analysis was conducted according to the concentration of compounds from nutritional and environmental variables depending on the collection sites.

3 | RESULTS AND DISCUSSION

3.1 | The effects of *pinhão* origin on the nutritional composition of the cooked *pinhão* almonds

In order to understand the influence of each collection site (Bituruna (BIT), Caçador (CAÇ), Cruz Machado (CM), Lapa (LAP), and São João do Triunfo (SJT)) on the nutritional variables of almonds, the nutritional characteristics (Figure 2) and moisture were assessed.

Detailed information Table S1.

The moisture of the *pinhão* samples ranged between 49.38 and 45.94 g/100 g, in which BIT presented the highest value and CAÇ the lowest. SJT, LAP, and CM showed intermediate values, with significant differences ($p < 0.05$). In order to carry out the evaluation without the interference of moisture, the following data were expressed on a dry basis.

The total carbohydrate content presented varied from 35 to 42 g/100 g. The total starch (Figure 2a) of the samples showed a significant difference ($p < 0.05$) between the different localities, with BIT (39.72 g/100 g) showing the highest value, while LAP the lowest (31.43 g/100 g). The other municipalities ranged between these two localities and were not statistically significant. On the other hand, the resistant starch contained within the total starch did not vary statistically ($p < 0.05$), and the values obtained were on average 4.3 ± 0.5 g/100 g. The differences in total starch values can be attributed to the higher protein content present in the LAP sample (8.00 g/100 g), as can be seen by (Silva et al., 2022) whose data corroborate that the *pinhão* almonds present higher carbohydrate content, but a significant protein content. Foods containing resistant starch are of importance for human consumption, as this nutrient is a kind of soluble dietary fiber and is resistant to digestive enzymes of the upper digestive tract, bringing health benefits such as preventing disease processes, including cancer of the colon diabetes and obesity (Bai et al., 2021).

Regarding dietary fiber, no significant differences were observed between different sources ($p < 0.05$) (Figure 2a). The total fiber was around 14.18 g/100 g, which corresponds to ~11.22 g/100 g of insolubles and ~2.95 g/100 g of solubles. There were no differences ($p > 0.05$) between the ash (2.9 ± 0.2 g/100 g) and lipid (0.9 ± 0.3 g/100 g) contents of the cooked *pinhão* almond samples from different regions (Figure 2a). On the other hand, the protein content was significantly different ($p < 0.05$) among the *pinhão* samples from different localities, with higher values for LAP (8.00 g/100 g) and lower values for CM (6.17 g/100 g), representing a variation of ~29% in its content. This may be attributed to different plant metabolisms among the different plants, variations in soil nutrients such as nitrogen, or a combination of these factors. A previous study (Barbosa et al., 2017) indicated that the availability of P and K in the soil affected the accumulation of these elements in the epidermis, whereas the type of tissue and crystal formation were key characteristics for the dynamics of the nutrients in the epidermis of *A. angustifolia*.

Some of the variations in nitrogen content were also previously attributed to climate and genetics, reported to be due to the average temperatures that exceeded 22°C during the Brazilian summer in the studied region, which favors an increase in organic material (Alvares et al., 2013), resulting in the increasing of the seed filling during the formation of *pinhão* almonds (Barbosa et al., 2019).

The dehydration of its seeds in the final stages of maturation during the months of April to May also increases the protein content (Peralta et al., 2016). However, it is possible that the maturation stage did not contribute to these variations, since the *pinhão* almonds were collected when the seeds were already mature, on the ground below the trees.

The total caloric value of the samples was 1367.03–1440.93 kJ/100 g ($p < 0.05$), with LAP with the lowest value and CM with the highest (Figure 2b). The high content showed that almonds can be a source of energy, mainly attributed to the high amounts of carbohydrates and total starch, revealing that *pinhão* is an essentially source of starch, in addition to not containing gluten (Cordenunsi et al., 2004). The *pinhão* is also a significant source of total dietary fiber, predominantly the insoluble type (between 2.15 and 5.10 times greater than that of the soluble fiber). The nutritional composition values of cooked *pinhão* almonds are close to those reported in the literature (Gil et al., 2021; Schveitzer et al., 2014; Silva et al., 2022). Only one study (Cordenunsi et al., 2004) has evaluated the total and resistant starch content of cooked *pinhão* almonds, and the values found are twice that of the present study.

When comparing cooked *pinhão* and cooked white rice, a cereal consumed worldwide, these almonds contain 80% of protein and present higher levels of lipids (2.4 times) and ash (9.7 times). In addition to having an important characteristic, that is, a higher content of total (4.9 times), insoluble (13.3) and soluble fibers (1.6 times) (Walter et al., 2008), which leads to a significant impact on the functioning of the human body. Some types of fiber can improve blood cholesterol levels, lipid profiles, and insulin sensitivity. Futhermore, they are able to prevent various chronic diseases, such as diabetes, obesity, and colon cancer (Li & Komarek, 2017). It also provides the same amounts of minerals as present in rice (Walter et al., 2008). Thus, the consumption of cooked *pinhão* can provide high amounts of nutrients with good human health benefits and is associated with a low glycaemic profile (Cordenunsi et al., 2004); therefore, *pinhão* is an excellent option for consumption.

The mineral content of the *pinhão* almonds was different ($p < 0.05$) for seeds from different localities (Figure 2b,c). Mineral contents of K varied from 1088.87 to 1950.55 mg/100 g, and P from 320.37 to 534.23 mg/100 g but these minerals presented similar behavior in localities with a Pearson correlation of 0.86, that is, when one is increased, the other one is also increased. The greatest difference occurred between LAP and CM, which were 79% and 67% for K and P. These differences may be attributed to differences in the availability of the elements in the soil, in a manner similar to the effect on the composition of the epidermis of *A. angustifolia* (Barbosa et al., 2017).

The values of Ca were between 77.07 and 186.80 mg/100 g and for Mg between 36.16 and 80.79 mg/100 g, in which the lowest values for both minerals in *pinhão* were from CAÇ and the highest values in *pinhão* were from SJT for Ca and LAP for Mg. Variations in Ca (142%) and Mg (123%) in the *pinhão* have been attributed to a reduction in phloem transportation of Ca due to reduced transpiration from the seeds, as compared to that of the leaves, as well as to reduced movement, since it is primarily associated with cell walls (White, 2012) and oxalate crystals (Barbosa et al., 2017).

The microelements (Figure 2c) (Fe [2.28–3.56 mg/100 g], Zn [2.23–2.67 mg/100 g], Cu [0.52–0.76 mg/100 g], and Mn (0.14–0.78 mg/100 g]) also exhibited differences for all samples depending on the origin, varying between 19 and 472%, and in 75% of the BIT sample data, it had higher values. The variations obtained in the present study were smaller than Barbosa et al. (2019), possibly attributed to the smaller number of samples collected, since the authors covered more locations.

3.2 | Nutritional contribution of the *pinhão* almonds to the diet

Figure 3 shows the contribution of macro and micronutrients upon consumption of 100 g of *pinhão*, with ~45% moisture per day, based on pre-established maximum and

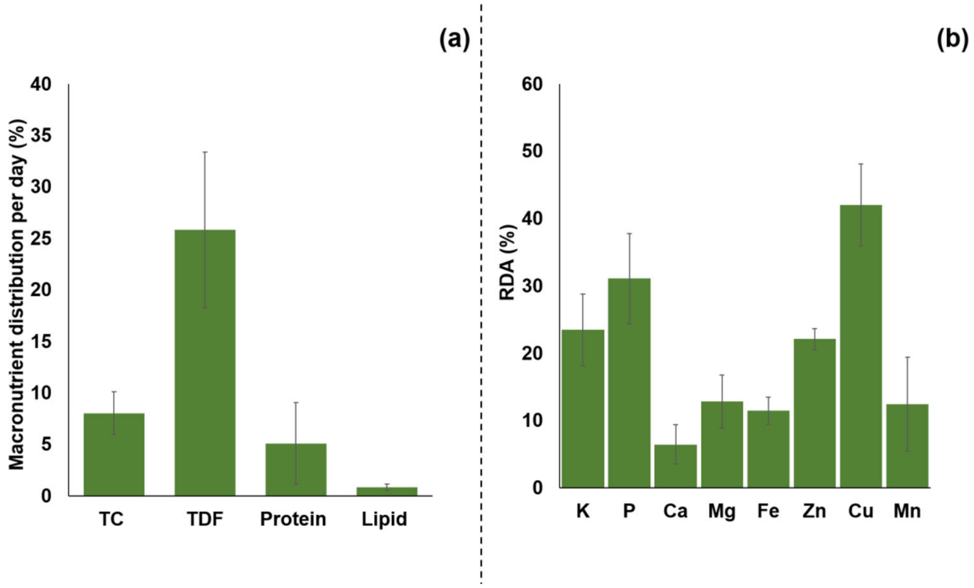

FIGURE 3 Macronutrient distribution per day (a) and Recommended Dietary Allowance (RDA) (b). Macronutrient distribution per day, considering diet with 2000 kcal/day. TC: total carbohydrates (45%–65% of energy); TDF: total dietary fiber: adequate intake (AI) of dietary fiber 25–38 g of fiber per day for females and males, respectively; protein (10%–35% of energy), and lipid (20%–35% of energy). RDA Mean value: P (700 mg), K (3510 mg), Ca (1000 mg), Mg (240 mg), Cu (0.9 mg), Fe (14 mg), Mn (2.1 mg), Zn (6 mg) (WHO/FAO, 2004; WHO, 2012)

minimum average values (IOM, 2005). In this sense, the *pinhão* almond provides an average of 8.04% of carbohydrates, 25.85% of fibers, 5.07% of proteins, and 0.84% of lipids of the daily nutrients that is part of a healthy diet (IOM, 2005). The IOM (2005) gives an acceptable range of macronutrient distribution of *pinhão* for carbohydrates (45–65% of energy), protein (10–35% of energy), and fat (20–35% of energy). These ratios provide acceptable limits in order to cover the major portion of the macronutrient requirements for the majority of active individuals.

Pinhão almond is considered a "source of high dietary fiber content" since its dietary fiber value per 100 g solids of 7.48 g is greater than the required 6 g, according to Brazilian country regulations (Brasil, 1998). It should be noted that an adequate intake (AI) of dietary fiber for adults (19–50 years old) is 38 g of fiber per day for males and 25 g of fiber per day for females (WHO/FAO, 2004; WHO, 2012).

The mineral values for the RDA are continually updated in order to reflect the demands of the human body, considering the interactions among the elements, age range, and sex. According to Directive n° 27 from January 13, 1998(Brasil, 1998), a food product whose values are greater than 15% of the daily mineral demand, according to the RDA, is considered a food source rich in such a component. In this way, the *pinhão* is a source of Cu (42.2%), P (31.1%), K (23.5%), and Zn (22.1%). The results of Barbosa et al. (2019) indicate that raw almonds are a source of Cu (43.6%), P (32.8%), Mn (25.0%), and K (18.5%), whereas the results of Schveitzer et al. (2014) indicate that the *pinhão* are a source of Fe (81.4%), Mn (52.4%), P (29.4%), and Cu (22.2%). This reveals several differences, which may be attributed to their origin and temporal variations.

It is noteworthy that some of the samples of *pinhão* can be classified as good sources of Mg, and also show significant differences (Figure 2c) that depend on their place of origin, revealing the importance of studying the composition and origin of the raw material.

In relation to Ca, all samples showed low availability, and on average, they were 4 times higher than those reported in previous studies (Barbosa et al., 2019; Cordenunsi et al., 2004). The relatively low availability of Ca may not be critical, for it may be supplemented with the consumption of other food sources rich in this component (Ikeda et al., 2018). Thus, consumption of the *pinhão* almonds may contribute to a significant ingestion percentage of Cu, P, and Zn nutrients, because, on average, the population of Brazil (both men and women) ingest only 21.2% of the necessary Cu content, 12.0% of P, and 22.5% of the necessary Zn content (Araujo et al., 2013). In addition, the almond contains small quantities of Ca and Mg, which could complement the 88.6% of Ca and 72.6% of Mg of the adequate daily recommended ingestion levels that are, on average, consumed by the Brazilian population (Araujo et al., 2013).

Therefore, the direct consumption of the cooked *pinhão*, as well as its use in the preparation of foods, can produce a supplemental food that is rich in carbohydrates and dietary fiber, as well as a source of the minerals Cu, P, K, and Zn; moreover, geographic origin is a differential factor that potentially stimulates its commercialization.

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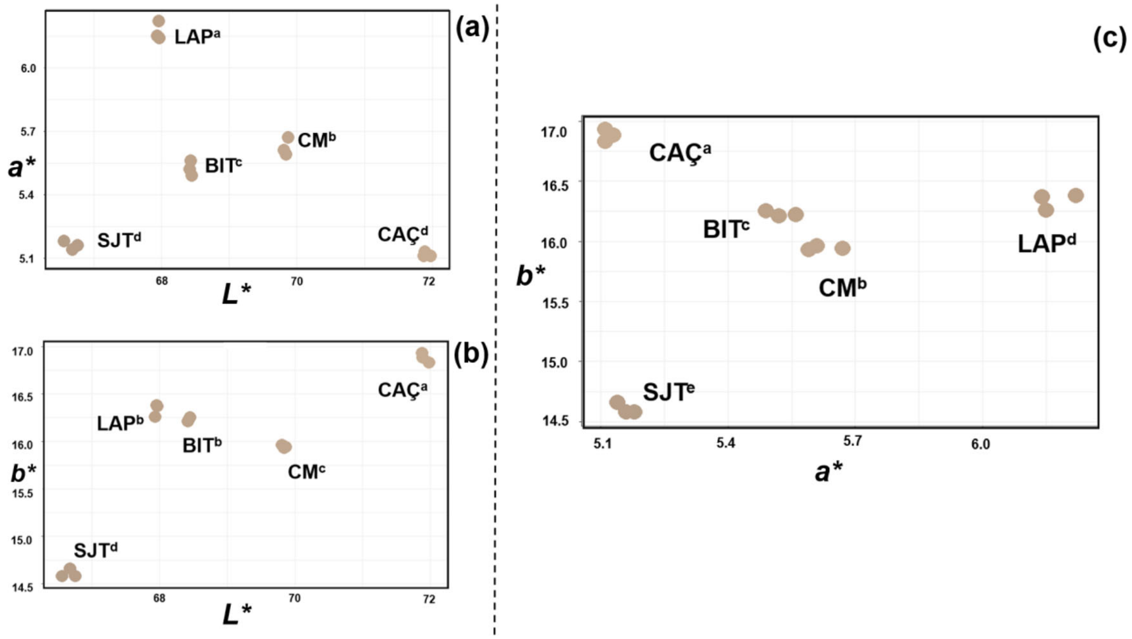

FIGURE 4 CIELAB color space plots of the *pinhão* almonds cooked from different origins. (A) Plots of a^* against L^*, (B) b^* against L^*, (C) b^* against a^*. In which: L^*(degree of brightness: 0: black; 100: white); a^* ($+a^*$: degree of red color; $-a^*$: degree of green color); b^* ($+b^*$: degree of yellow; $-b^*$: degree of blue color). The superscript letters are Tukey's statistical data, where same letters do not differ in the same plot ($p < 0.05$). BIT: Bituruna; CAÇ: Caçador; CM: Cruz Machado; LAP: Lapa; SJT: São João do Triunfo

TABLE 1 Cooked pinhão almonds color parameters

Parameters	BIT	CAÇ	CM	LAP	SJT
L^*	68.43 ± 0.02^{c}	71.92 ± 0.06^{a}	69.84 ± 0.03^{b}	67.96 ± 0.02^{d}	66.67 ± 0.10^{e}
a^*	5.51 ± 0.04^{c}	5.12 ± 0.01^{d}	5.62 ± 0.04^{b}	6.17 ± 0.04^{a}	5.16 ± 0.0^{d}
b^*	16.23 ± 0.02^{b}	16.88 ± 0.05^{a}	15.94 ± 0.01^{c}	16.34 ± 0.07^{b}	14.61 ± 0.05^{d}
ΔE *BIT*	–	1.56 ± 0.05^{b}	0.32 ± 0.03^{c}	0.33 ± 0.03^{c}	1.74 ± 0.03^{a}
ΔE *CAÇ*	1.56 ± 0.12^{c}	–	1.41 ± 0.06^{c}	4.21 ± 0.17^{a}	2.29 ± 0.06^{b}
ΔE *CM*	0.32 ± 0.04^{d}	1.41 ± 0.03^{b}	–	1.10 ± 0.09^{c}	1.99 ± 0.01^{a}
ΔE *LAP*	0.33 ± 0.02^{d}	4.21 ± 0.08^{a}	1.10 ± 0.06^{c}	–	2.17 ± 0.06^{b}
ΔE *SJT*	1.74 ± 0.02^{d}	2.29 ± 0.05^{ab}	1.99 ± 0.11^{ac}	2.17 ± 0.08^{a}	–

Notes: Means ± standard deviation. The superscript letters in line are Tukey's statistical data, where same letters do not differ in the same plot ($p < 0.05$).
ΔE: color variation; BIT: Bituruna; CAÇ: Caçador; CM: Cruz Machado; LAP: Lapa; SJT: São João do Triunfo.

3.3 | Colorimetric test

The values for the components identified in the colorimetric tests were obtained after grinding the cooked *pinhão* almonds; yellow-brown tones can be seen in all graphs (Figure 4). Localities CM and BIT presented parameters L^*, a^*, and b^* that were closer, although were statistically different ($p < 0.05$). When calculating the color variation (ΔE), yet with a color difference not perceptible by the naked eye, a condition to overcome this barrier is when the $\Delta E > 2$ (Malta et al., 2021; Francis & Clydesdale, 1975). The values in this work were, however, 0.32 ± 0.03. The SJT sample presented the greatest difference, with $\Delta E < 2$ only for BIT. While CAÇ presented $\Delta E > 2$ for LAP and SJT (Table 1). Most ΔE values were statistically different ($p < 0.05$) for each municipality. Only CM and LAP samples were equal ($p < 0.05$) when compared to BIT, as well as CM, LAP to CAÇ, when compared to SJT.

Variations in these parameters may be associated with the cooking process, and not the different localities. This is because cooking by immersion of the *pinhão* almonds with the bark causes part of the bioactive compounds, and those that give the bark color, to migrate to the edible portion (Cordenunsi et al., 2004), turning it into darker tones.

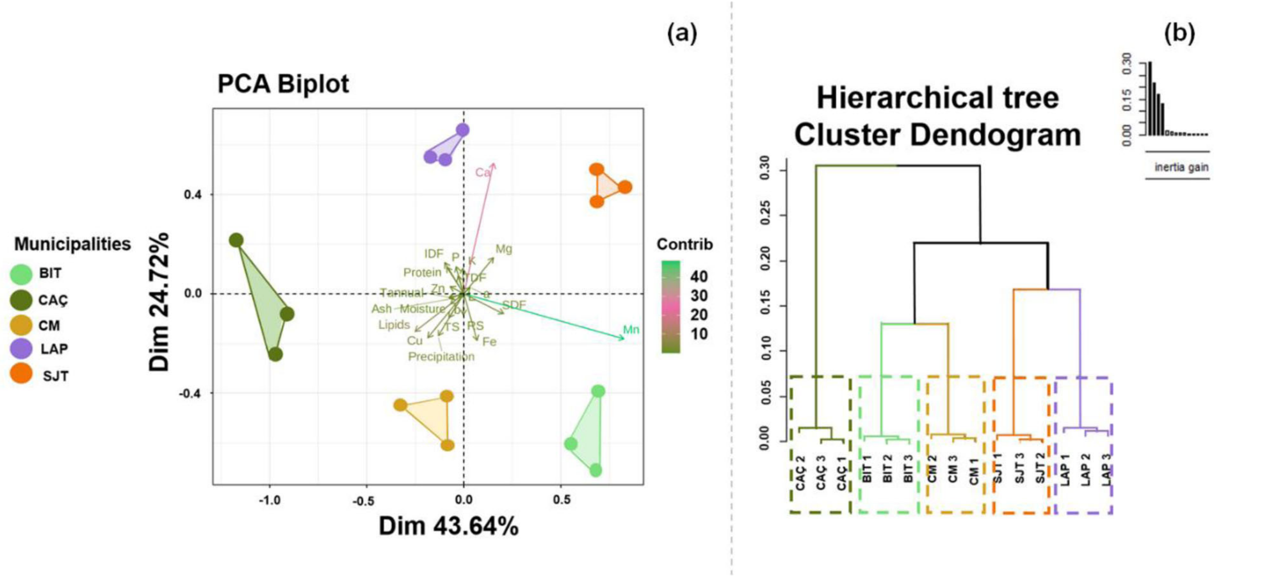

FIGURE 5 Principal component analysis (a) and hierarchical tree: cluster dendogram (b) of nutritional and environmental variables of *pinhão* almonds from different origins. BIT: Bituruna; CAÇ: Caçador; CM: Cruz Machado; LAP: Lapa; SJT: São João do Triunfo

3.4 | Principal components analysis and hierarchical clustering on principle components

In Figure 5a, the principal components analysis (PCA) is presented to understand the behavior of the nutritional color and environmental variables statistically, while also considering the different harvest locations. The results exhibit that the PCA is able to explain 68.36% of the variation between samples, considering the nutritional composition, minerals, color, and environmental variables such as mean temperature in July (month of collection), mean annual temperature, as well as annual precipitation. The *pinhão* from each municipality exhibited distinct characteristics that led, in its majority, to be in each specific quadrant, except for the CAÇ, which was observed in the left area, among CM and LAP, in its majority, to be in each specific quadrant, a unique case is the CAÇ in which some of the samples were in the lower left quadrant, together with CM.

Figure 5b exhibits the cluster analysis performed after the PCA (hierarchical clustering on principle components). As expected, clustering followed the characteristics of the PCA with more detailed information whereas CAÇ was the most distinct, while BIT and CM as well as SJT and LAP were grouped individually if only three divisions bars are considered (black lines in Figure 5b). Figure 6a–c shows the contribution of each variable in dimensions 1 and 2.

The characteristics of CAÇ, distinct from the other localities, may be related to its geographic position, as it is the most distant, since the other ones are neighbors in pairs (Figure 1). In addition, CAÇ had lower annual and month-of-collection temperatures than the others, and it is one of the highest and rainiest localities studied.

Dividing into 5 clusters, it can be noted that cluster 1 composed of CAÇ is characterized by high values for the colorimetric components (b^* and L^*), chemical composition (ash, moisture, total starch, and protein), environmental variables (precipitation and annual temperature), and Zn. On the other hand, it presented low values of Mg and Mn. Cluster 2, composed of CM, is characterized by high values of lipid, Fe, and low values of P and K. While in cluster 3, composed of LAP, it is characterized by high values for the minerals P, Mg, and K, in addition to presenting a characteristic a^* color parameter. However, it presented with low Fe contents, L^* color parameter, moisture, total and resistant starch, and annual temperature and precipitation.

Cluster 4, composed of BIT, is characterised by high values of soluble dietary fiber, while also exhibiting lower values of Ca, protein, insoluble dietary fiber, annual and July temperatures. Finally, cluster 5, composed of SJT, is characterized by high values of Ca and low values of lipid and Cu. These interactions found in the PCA can be related to a previous study by Yu et al. (2021) that evaluated the chemical composition of different genotypes of edamame [*Glycine max(L.) Merr.*] produced in different locations. The study presented that localities had an effect on protein content, moisture, lipids, starch, and neutral detergent fibers. The genotype presented no significant variation on the fiber content, possibly due to genetic proximity (Yu et al., 2021), but comparing localities, interaction was only significant in protein and starch. Variations in protein content were justified by the authors due to temperature changes, as in previous studies, whereas higher protein

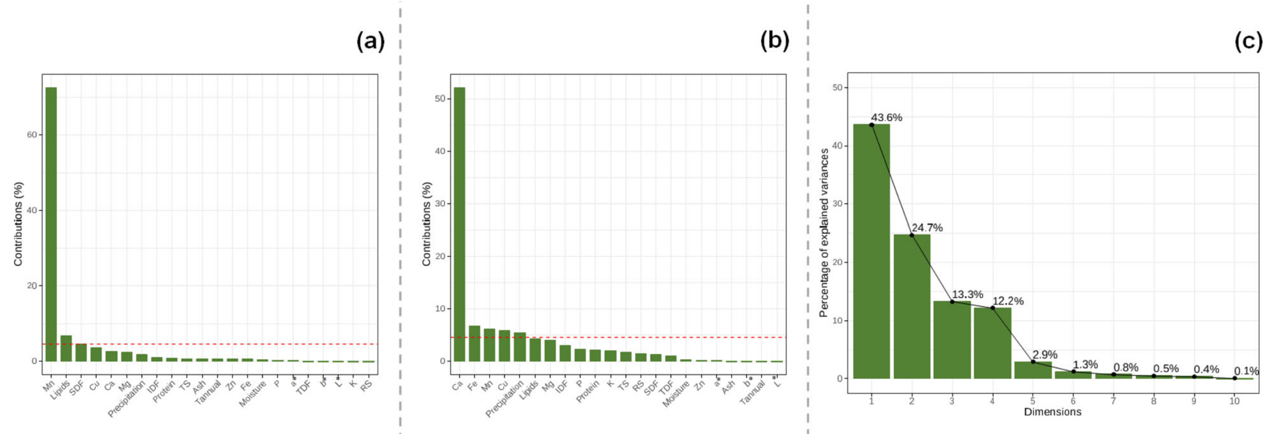

FIGURE 6 Contribuition PCA for variables Dim 1 (a), Dim 2 (b), and percentage of explained variances (c). TC: total Carbohydrates; TS: total starch; RS: resistant starch; TDF: total dietary fiber; IDF: insoluble dietary fiber; SDF: soluble dietary fiber; *a*, b*, L**: color parameters, elevation, precipitation annual; T July and T annual are environmental variables by Climate-data.org

content was found at daytime temperatures greater than 30°C (Wolf et al., 1982). The variations in moisture were related to maturation of samples, as moisture gradually decreases throughout the development of seeds (Xu et al., 2016). Nonetheless, the starch content, in places where the seeds had higher sucrose, glucose, and fructose, presented higher values than in cases with lower levels of these sugars.

These results reinforce the hypothesis, which has been already proposed by other researchers, that variations in the nutritional composition of *pinhão* are dependent on the genetic expression of the vegetable variety, development stage, soil and climate characteristics, irrigation techniques, and dehydration at the end of maturation (Astarita et al., 2003), but do not represent a critical factor for its widespread use as a raw agroindustrial material. However, this diversity may soon represent an opportunity to classify the origin of the *pinhão* according to its *terrain*; a concept that has already been applied to wine, coffee, chocolate, tea, beef, cheese, and other products that are influenced by their location of origin.

3.5 | Relationship of soil, geographic, and environmental characteristics with the composition of *pinhão*

Geographic parameters from the harvest locations (Table 2) present a variation of 300 mm for annual precipitation with a variation of 131 m of altitude. Overall, these municipalities can be considered as continental with little effect of maritime climate. Values of JCI for continentality are normally considered in the northern hemisphere; so the ones found herein assigned as maritime need to be evaluated with some reservation. Nonetheless, both oceanity measurements presented that these harvest locations were considered as continental. Specifically, MOI was adjusted for King George Island, southern hemisphere, and may adapt better to the climate data attributed herein. Lastly, aridity measurements classified these locations as very, or extremely, humid and explain the specific ecosystem—mixed ombrophilous forest. Nonetheless, PCI that compensates the annual precipitation with monthly ones presents that LAP municipality was the most unique that showed a semi-humid environment.

Soil data collected in each municipality, at different depths, are provided in Table S2. Variations occur in the data collection. Overall, there is higher quantity of carbon and nitrogen and the nutrients within the soil at the ground level. The oxides varied depending on the soil depth. According to Silva et al. (2001), the araucaria gather nutrients from the deeper soil layer than shallow soils, and the depth of the soil is more important than its chemical characteristics, as greater growth in volume was observed, associated with greater survival, when the araucaria was present in deeper soils (average of 20 m^3/ha), compared to a growth in stony soils (16.6 m^3/ha) and shallow soils (average of 12.2 m^3/ha). Therefore, it is possible to observe that BIT presented the highest nutrients for deeper soils and could have helped in the absorption for the seeds followed by LAP and CAÇ. Moreover, pH of the soil remained overall constant from the measurement depth and is unique to each terrain. However, the municipality that presented the highest oxide constituents was CM that may present higher ash content.

The PCA analysis of the geographic and soil data was performed (Figure S1), and data for depth was used in PCA as intensity of individuals and variables. Overall, hierarchical clustering on principal component was not able to cluster the individuals correctly as some unique characteristics are seen only for CAÇ1, BIT1, CM1, and LAP attributed to the high content of nutrients on the low

TABLE 2 Environmental variables of the municipalities involved in the study

Parameters	BIT	CAÇ	CM	LAP	SJT
Precipitation annual (mm)	1751	1707	1451	1320	1451
Elevation (m)	881	914	786	917	786
Geology	Basalt (Igneous)	Basalt (Igneous)	Basalt (Igneous)	Sedimentary	Sedimentary
Continentality of JCI	Maritime	Maritime	Maritime	Continental	Continental
	20.95	16.08	24.64	39.94	47.16
Oceanity1 of KOI	Continental	Continental	Continental	Continental	Continental
	0.96	0.47	1.95	1.49	0.48
Oceanity2 of MOI	Continental	Continental	Continental	Continental	Continental
	1.26	1.31	1.22	1.24	1.18
Aridity (IDm)	Extremely humid	Extremely humid	Extremely humid	Very humid	Very humid
	65.83	64.9	62.74	49.62	52.96
PCI (Ip)	Very humid	Very humid	Humid	Semi-humid	Humid
	35.26	35.17	33.6	26.38	28.06

Note: Precipitation annual and elevation were obtained for website Climate.org; The Geology was classified according to the Köppen climate classification; Continentality por Johansson Continentality (JCI); Oceanity1: Kerner Oceanity Index; Oceanity2: Marsz Oceanity Index (MOI); Aridity by De Martonne; PCI: Pina Combinate Index by Zambakas (1992).

depth of the municipalities soils, which were not seen at more depths. Therefore, data from soil was only used as correlation with micro- and macro-nutrients from *pinhão* seeds.

Pearson correlation of the seed nutrients (.P) (Figure S1) with soil (.S) and geography (.G) demonstrates few similarities. For example, the standard ones—and not related to the plant—like oceanity and elevation are perceived. However, more importantly, for the seed nutrients (denoted as .P), like Cu and protein, there seems to be a negative correlation with metallic oxides from the soil (such as SiO_2 and Al_2O_3 which in turn are negatively correlated with oceanity index), which may have been due to a more stony soil. However, Fe was positively correlated with Al_2O_3 and C/N ratio. Mn from the seed was positively correlated with K from the soil, also a common phenomenon as a very high index can block Mn uptake. Additionally, some macronutrients of the seed, like Ca, seem to suggest some negative correlation with aridity index, as expected because the mixed ombrophilous forest consists of a more humid environment.

4 | CONCLUSION

This work investigated the variations between collection sites for *pinhão* almonds, which were more predominant for mineral content, followed by protein, moisture, and total starch. While, resistant starch, dietary fiber, lipids, and ash did not differ. The color parameters *L**, *a**, and *b** were statistically different, but the color variation in most samples was not noticeable to the human eye. In general, LAP and SJT were the places with the highest nutrient content, and BIT, CM, and CAÇ were the lowest. Caçador was the most distant municipality and showed the greatest differences in parameters, while the pairs CM and BIT as well as LAP and SJT, or nearby municipalities, had some similar parameters, which could be related to climate, elevation, and annual and average temperatures. These variations do not prevent its application as a raw material for food products, but can serve as differentials for the elaboration of different products according to the *pinhão* origin. Thus, evaluating variations in its composition is relevant to developing various products, in addition to highlighting the importance of its consumption in the diet, being an alternative for vegetarians and vegans, since the almonds have a high content of carbohydrates, which contain starch total and significant amount of resistant starch, benefiting human health, as well as being a source of dietary fiber and minerals, contributing to the recommended daily intake of total dietary fiber (25.85%), Cu (42.2%), P (31.1%), K (23.5%), and Zn (22.1%). In addition, it provides an average of 8.04% of carbohydrates, 5.07% of proteins, and 0.84% of lipids of the daily nutrients for a healthy diet.

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AUTHOR CONTRIBUTIONS

Danielle Specht Malta: Conceptualization; Data curation; Formal analysis; Investigation; Methodology; Writing

– original draft; Writing – review & editing. **Matheus Samponi Tucunduva Arantes**: Methodology; Writing – review & editing. **Alvaro Luiz Mathias**: Conceptualization; Funding acquisition; Project administration; Resources; Supervision; Writing – original draft; Writing – review & editing. **Washington Luiz Esteves Magalhães**: Writing – review & editing. **Cristiane Vieira Helm**: Conceptualization; Methodology; Resources; Writing – original draft; Writing – review & editing. **Maria Lucia Masson**: Supervision; Writing – original draft; Writing – review & editing

CONFLICT OF INTEREST

The authors declare that there is no conflict of interest.

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