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# Chemical and nutritional characterization of loengo (*Anisophyllea boehmii*) fruits as a source of important bioactive with impact on health

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**Introduction:** Fruits are vital for human health, providing nutrients and other compounds linked to protection against different diseases. Loengo (*Anisophyllea boehmii* Engl.) produces an edible stone fruit, plum-shaped (drupe), dark blue and maroon or carmine in the ripening stage, which is generally, consumed fresh in the production areas. Characterization of this fruit will contribute to estimate its nutritional and pharmaceutical potential impact. The present work aimed at the physic-chemical, nutritional and antioxidant activity characterization of loengo fruit.

**Methods:** Soluble solid contents (SSC) in the pulp were assessed in juice, at room temperature. Total protein was determined using the Dumas method. In what concerns the mineral composition were determined by ICP-OES. Organic acids identification and quantification was accomplished using a high-performance liquid chromatography (HPLC) system. The total polyphenol contents (TPC) were determined in 96-well microplate assays. Antioxidant activity were determined by 2,2-diphenyl-1-picrylhydrazyl (DPPH) and the ferric reducing antioxidant power (FRAP) assay.

**Results and discussion:** Composition, in terms of fresh matter is as follows: pulp soluble sugar content (refractometer) is  $19.5\% \pm 0.02\%$ , and pulp titratable acidity  $1.2\% \pm 0.1\%$  expressed in citric acid; total protein content is  $7.4\% \pm 0.02\%$ ,  $8.0\% \pm 0.1\%$ ,  $16.9\% \pm 0.2\%$ , for the peel, pulp and seed, respectively. In what concerns the mineral composition, the results obtained show that it is a good source of minerals, mainly potassium and phosphorus, but also calcium, sulfur, and magnesium, with the pulp richer in potassium, the seed in phosphorus, and the peel in calcium. As expected, the pulp showed the highest total soluble sugar content, whereas the seed had the lowest. The main sugars found in the pulp were glucose and fructose. In the peel citric acid was the main organic acid found, together with malic, oxalic, and ascorbic acids. Most antioxidant activity was found in the seed. The results of this work provide important information to

recommend the consumption of this fruit for its sweet taste, chemical and nutritional composition, and, most of all, for its richness in total phenolic compounds for its potential to formulate nutraceutical foods.

#### KEYWORDS

*Anisophyllea boehmii*, nutritional composition, phenolic compounds, antioxidant activity (AA), organic acids

## Introduction

The consumption of fruits is vital for the promotion of human health, as natural sources of nutrients and vitamin C, as well as of phenolic compounds (Junqueira-Gonçalves et al., 2015; Bjørklund and Chirumbolo, 2017; Rodrigues et al., 2021). Many of these compounds have been linked to protection against different chronic pathologies associated with oxidative damage, whilst also exhibiting antimicrobial and other biological activities (Gironés-Vilaplana et al., 2014; Junqueira-Gonçalves et al., 2015; Ma et al., 2018). Some edible fruits are rich sources of minerals, as well as of phenolic compounds, which are responsible for their red, violet, purple, and blue colors (Maro et al., 2013; Redondo et al., 2021). They exhibit a wide range of antioxidant protective activities and other preventive benefits, including protection of genomic DNA integrity and potent cardioprotective, neuroprotective, anti-inflammatory and anticarcinogenic properties (Skrovankova et al., 2015; Graça et al., 2020). One of the most significant health advantages of these fruits is their capacity to prevent metabolic disorders, which are directly linked to dietary habits (Lima et al., 2016; Bjørklund and Chirumbolo, 2017; Paiva et al., 2024).

Climate change is widely recognized to have a significant negative impact on crop production and food safety everywhere in the world (Alam et al., 2011), clearly affecting and reducing fruit production and the quality of nutritional diet intake. The human population is continuously increasing, and the importance of food and nutritional security demands exploring alternative food resources. According to Iwatani and Yamamoto (2019), since the '90s, the Food for Specified Health Uses (FOSHU; Japan Ministry of Health, Labour and Welfare) has been searching for new types of foodstuffs, which alongside with nutrients, present positive effects on the consumer's health, as well as an opportunity to contribute to socioeconomic development. Recently, the global and individual consumption of functional foods and the interest in dark-colored fruits has increased considerably. The proposed way to improve food safety is to use all available safe, nutritious, and good-quality foods. Therefore, knowledge of the nutritional values and potential benefits that can be provided to humans by edible but as-yet-unexplored novel foods is a critical factor (Jacobs and Steffen, 2003; Das et al., 2012; Maro et al., 2013).

Loengo (*Anisophyllea boehmii* Engl.) produces an edible stone fruit (drupe), plum-shaped, dark blue, and maroon or carmine in the ripening stage. The mesocarp is fleshy with a pale reddish color, edible, and with a bittersweet flavor. The fruit is mainly consumed fresh (*in nature*), but it can also be processed into juice or jam, with the mesocarp (pulp) and exocarp (peel) as the most edible parts; the seed is rarely consumed. The stone (seed) comprises a considerable proportion of the fruit, the pulp layer is relatively thin and strongly

attached to the stone, whereas the peel is very thin and completely attached to the pulp (Ruffo et al., 2002; Ibrahim et al., 2015). The seeds may have nutritional and pharmaceutical value, but are usually discarded after consuming the pulp (Ibrahim et al., 2015; Nkengurutse et al., 2016).

Loengo occurs naturally in different African countries, namely, in high rainfall areas of Zambia, Tanzania, the Democratic Republic of the Congo, Burundi (Chen et al., 2015; Nkengurutse et al., 2016), and Angola (Sanfilippo, 2014; Chen et al., 2015). In Angola, loengo occurs mainly in the tropical climate regions which surround the provinces of Bié, Huambo, Huila, Lunda Sul and Malange (Chen et al., 2015). Nkengurutse et al. (2016) reported that fruits ripen between mid-November and early December. Loengo seeds may be used as a food ingredient or supplement; they are rich in L-arginine and exhibit healing and anti-inflammatory activities in the human body, in addition to being effective in diseases of the cardiovascular system (Kurhaluk, 2023). In Zambia, loengo fruits are consumed in juices and porridge and are also used in folk medicine (Kalaba et al., 2009). In Angola, these fruits are often consumed *in nature*. They are one of the traded wild fruits in Angola and constitute a source of income for farmers (Sanfilippo, 2014). In addition to consumption as a fruit, it may be prepared in juices or cooked in jellies and sweets to avoid the large losses observed during the harvest season (Curi et al., 2016; Zhang and Shao, 2015). The characterization of this fruit could contribute to a detailed estimate of the nutritional and pharmaceutical value of loengo, thus contributing to alternative nutritional food sources. The present work aimed at the physico-chemical, nutritional, and antioxidant activity characterization of the loengo fruit.

## Materials and methods

### Chemicals

All the reagents used were of analytical or HPLC grade. Methanol and ethanol (96% v/v) were purchased from Fisher Chemicals (Fair Lawn, NJ, United States). Folin-Ciocalteu reagent, sodium carbonate, gallic acid, tris(hydroxymethyl)aminomethane, L (+)-ascorbic acid, DL-malic acid and anthrone reagent (9,10-dihydro-9-oxoanthracene) were purchased from Sigma-Aldrich (St. Louis, MO, United States). D (+)-Glucose and D (–)-fructose were purchased from VWR International (Radnor, PA, United States). DPPH (2,2-diphenyl-1-picrylhydrazyl), Trolox (6-hydroxy-2,5,7,8-tetramethylchromane-2-carboxylic acid), acetic acid, sodium acetate, TPTZ (2,4,6-tris(2-pyridyl)-s-triazine), hydrochloric acid, iron (III) chloride hexahydrate, sodium hydroxide and acetone were purchased from LabChem (Zelienople, PA, United States). Ultrapure water was obtained from a Synergy® Water Purification System (MilliporeSigma, Burlington, MA, United States).

## Plant material

Loengo fruits were collected in the Huambo province, central plateau of Angola, at an altitude of 1,774 m. The harvested ripe fruits were selected to avoid the presence of spots or symptoms of disease. The fruits were thoroughly washed with water to remove impurities and stored frozen at  $-80^{\circ}\text{C}$  until use. All tests were performed in triplicate.

## Measurement of physical and chemical properties

A digital caliper was used to measure the equatorial and polar diameters of 21 loengo fruits under optimal temperature and humidity conditions ( $20^{\circ}\text{C}$ – $22^{\circ}\text{C}$  and 40% humidity). The fresh weight of the whole loengo fruit was determined using an OHAUS CS200 electronic balance; the loengo fruit components were separated into peel, pulp, and seeds, and analyzed separately.

## Determination of pH, titratable acidity and soluble solid contents

The pulp pH was determined using an Orion potentiometer, model 420 (Orion Research, Inc., Boston MA). The titratable acidity of loengo pulp was obtained using method 942.15 of the [Association of Official Analytical Chemists \(2000\)](#). The titration results were expressed in g citric acid/100 g of sample. Pulp soluble solid contents (SSC) were assessed in triplicate, at room temperature, in juice, using a pocket refractometer ATAGO PAL-1 0.0%–53.0% BRIX, (3810) Digital Hand-HELD, Tokyo, and expressed as g sucrose/100 g of sample.

## Determination of weight, moisture and ash

To determine the moisture content of the loengo fruit peel, pulp, and seeds, the method used by the [Association of Official Analytical Chemists \(2003a\)](#) was followed; these materials were first placed in a filter box and weighed. After rigorous weighing, the boxes were placed with the lid open in a forced-air oven at  $105^{\circ}\text{C}$  during 48 h (until the weight became constant). Afterwards, the boxes were covered to minimize atmospheric water absorption, cooled inside a desiccator, and weighed. The water content of the loengo fruit was calculated as the difference between the fresh weight and the dry weight divided by the fresh weight. The total ash content was determined by weighing the ashes left after combustion at  $500^{\circ}\text{C}$  in a muffle furnace for 24 h. The ash content was calculated by dividing the ash weight by the fresh weight of the sample ([Association of Official Analytical chemists, 2003b](#)).

## Determination of crude fiber, lipids and total protein

Determination of crude fiber and lipid content were carried out following the Weende method, as previously described by ([Duarte et al., 2021](#)). Crude fiber was measured using 1 g of dried biological material. The extraction of sugars and starch was achieved through acid hydrolysis with 1.25% (v/v)  $\text{H}_2\text{SO}_4$ , followed by alkaline

hydrolysis with 1.25% (w/v) NaOH, which removed proteins and some hemicellulose and lignin. Subsequently, 150 mL of 0.2 M KOH was added to the sample, boiled for 30 min and the sample was washed with acetone in a Kitasato flask. The sample was then dried in an oven at  $105^{\circ}\text{C}$  and subsequently in a muffle furnace at  $550^{\circ}\text{C}$  during 8 h. Lipids were extracted from dried 1 g biological material samples with ethyl ether in a Soxhlet apparatus ([IPQ, 2009](#)). Following solvent evaporation, fat residues were dried at  $105^{\circ}\text{C} \pm 2^{\circ}\text{C}$  until a constant weight was achieved. Total protein content was determined using the Dumas method ([Kirsten, 1979](#)), assuming an average of 16% (w/w) nitrogen content in the loengo fruit proteins. These tests were carried out in triplicate.

## Determination of mineral composition

The mineral composition of the fruit samples was determined using acid digestion, according to the methodology outlined by ([Martins et al., 2020](#)), followed by absorbance readings in an Inductively Coupled Plasma Optical-Emission Spectrometer (ICP-OES; Thermo Scientific™ iCap Series 7000; Thermo Fisher Scientific, Waltham, MA, United States). A sample of 0.25 g was digested with a mixture of nitric acid and hydrochloric acid (ratio 1:3) at  $105^{\circ}\text{C}$ . The mixture was cooled and, upon reaching room temperature, filtered and diluted to 50 mL with distilled water. The resulting extracts were subsequently analyzed for potassium, phosphorus, calcium, sulfur, magnesium, sodium, manganese, zinc, iron, boron, and copper.

## Characterization of fruit sugars

Sugars were extracted from the loengo fruit peel, pulp, and seed with an aqueous solution containing 50 mM Tris-HCl buffer, pH 7.5, for 1 h, at  $4^{\circ}\text{C}$  and continuous agitation. The mixture was centrifuged at  $10,000 \times g$  and  $4^{\circ}\text{C}$  for 10 min. The supernatant was collected. Subsequently, the sugars were separated from the macromolecules by size exclusion chromatography. The filtration process was conducted using exclusion chromatography in PD-10 columns (prepacked with Sephadex G-25 M). The gel contained in these columns comprises dextran granules (glucose polymers) with a molecular weight cut-off (MWCO) of 5 kDa (solution 1).

## Carbohydrates determination

Total carbohydrates were quantified by the anthrone method, as proposed by [Trevelyan et al. \(1952\)](#), using solution 1 (see above, section “Characterization of fruit sugars”). The fruit samples were mixed with concentrated sulfuric acid (75% v/v) and anthrone reagent (9,10-dihydro-9-oxoanthracene) and then boiled until the reaction was complete. With this treatment, glycosidic bonds were hydrolyzed, and the monosaccharides were dehydrated, forming furfural or hydroxymethylfurfural, which then reacted with the anthrone reagent, producing a blue-green colored compound. Total sugars were then quantified by spectrophotometry (Bio-Tek synergy spectrophotometer) at a wavelength of 630 nm, employing a standard curve of sucrose (0–100 mg/mL).

## Organic acids identification and quantification

Sample extracts (solution 1; see above, section “Characterization of fruit sugars”) were diluted (1:20 v/v) in 25 mM sulfuric acid. Separation of the organic acids and sugars from all the extracts was accomplished using a high-performance liquid chromatography (HPLC) system (Chromaster, Hitachi, Tokyo, Japan) equipped with a UV–visible detector 5,420 (Chromaster, Hitachi, Tokyo, Japan) monitored at 210 nm for the organic acids, and a Refractive Index (RI) Detector 5,450 (Chromaster, Hitachi, Tokyo, Japan) for the sugars. The organic acids and sugars were separated using an ionic exclusion column (Rezextm ROA Organic Acid H+ (8%) column, 300 mm × 7.8 mm, Phenomenex, Torrance, CA, United States) at 65°C. Sulfuric acid (5 mM) was used as the mobile phase at a 0.5 mL/min flow rate. Organic acids identification and quantification were achieved using malic, citric, oxalic and ascorbic acids as standards, at concentrations ranging from 0 to 20 g/L. The results were represented in g/L of the corresponding organic acid (calibration curves:  $y = 4.45 \times 10^{-7}$ ,  $R^2 = 0.9996$  for malic acid;  $y = 4.12 \times 10^{-7}$ ,  $R^2 = 0.9994$  for citric acid;  $y = 3.87 \times 10^{-8}$ ,  $R^2 = 0.9995$  for oxalic acid; and  $y = 5.37 \times 10^{-8}$ ,  $R^2 = 0.9992$  for ascorbic acid). Sugar identification and quantification were achieved using glucose and fructose as standards, at concentrations ranging from 0 to 40 g/L. The results were expressed in g/L of the corresponding sugar (calibration curves:  $y = 1.48 \times 10^{-6}$ ,  $R^2 = 0.9996$  for glucose and  $y = 1.43 \times 10^{-6}$ ,  $R^2 = 0.9996$  for fructose). All experiments were performed in triplicate.

## Assessment of total phenolic content

The procedure described by [Rufino et al. \(2010\)](#) was followed to determine the content in phenolic compounds. Each fruit part crushed was weighed (1.0 g) and mixed with 10 mL of methanol/water (50:50 v/v). The mixture was stirred vigorously for 30 s until complete homogenization and incubated for 1 h at room temperature. Then, the samples were centrifuged at  $25,400 \times g$  for 20 min at 4°C, and the supernatant was retrieved. Subsequently, 10 mL of acetone/water (70:30 v/v) was added to the pellet, and the process was repeated under the same conditions described above. The methanol and acetone supernatants were combined, and distilled water was added until a total volume of 25 mL was reached.

## Determination of total polyphenols

The total polyphenol contents (TPC) were determined in 96-well microplate assays according to the method described by [Graça and colleagues \(2020\)](#). The standard curve was prepared based on a acetone (70%) solution of gallic acid with concentrations ranging from 0 to 9  $\mu\text{g mL}^{-1}$ ,  $R^2 = 0.9984$ . The peel, pulp and seed extracts, or gallic acid (150  $\mu\text{L}$  at 100  $\mu\text{g/mL}$ ), were added to Folin–Ciocalteu reagent (150  $\mu\text{L}$ , at 0.1 M) and, after 10 min, were thoroughly mixed with sodium carbonate (300  $\mu\text{L}$  at 7.5% w/v). The mixture was then incubated at room temperature in the dark for 2 h. Absorbance (760 nm) was measured, and the TPC results are reported in mg of gallic acid equivalents (GAE) per g of peel, pulp and seed extract.

The 2,2-diphenyl-1-picrylhydrazyl (DPPH) experiment was conducted according to ([Nunes et al., 2020](#)) by combining 100  $\mu\text{L}$  of sample extract with 3.9 mL of DPPH radical solution to assess the radical scavenging capacity. The samples were shaken vigorously and incubated for 40 min at room temperature, protected from light; the absorbance was measured using a UV–visible spectrophotometer (Cary 100, Agilent Technologies, Santa Clara, CA, United States) at 515 nm against methanol. The standard curve was prepared based on a methanolic solution of Trolox (6-hydroxy-2, 5, 7, 8-tetramethylchroman-2-carboxylic acid) with concentrations ranging from 0 to 1,000  $\mu\text{g mL}^{-1}$ ,  $R^2 = 0.9958$ . The samples were analyzed in triplicate, and the results were expressed as mg of Trolox per 100 g dry weight (DW).

The ferric reducing antioxidant power (FRAP) assay was performed according to the previously described procedure ([Nunes et al., 2020](#)). FRAP test solution was prepared by mixing at a ratio of 10:1:1 (v/v/v) 0.3 M acetate buffer at pH 3.6, 10 mM 2,4,6-tris(2-pyridyl)-s-triazine (TPTZ) prepared in 40 mM HCl, and 20 mM aqueous iron (III) chloride hexahydrate solution. A total of 90  $\mu\text{L}$  of sample extract, 270  $\mu\text{L}$  of distilled water, and 2.7 mL of test solution were vortexed and incubated for 30 min in a water bath (Precisiontm 2,864, Thermo Scientific, Waltham, MA, United States) at a temperature of 37°C, protected from light. The absorbance was measured using a UV–visible spectrophotometer (Cary 100, Agilent Technologies, Santa Clara, CA, United States) at a wavelength of 595 nm against the test solution. The standard curve was prepared based on a methanolic solution of Trolox with concentrations ranging from 0 to 800  $\mu\text{g mL}^{-1}$ ,  $R^2 = 0.9971$ . The samples were analyzed in triplicate, and the results were expressed as mg of Trolox per 100 g DW.

## Statistical analyses

All experiments were performed in triplicate, and the data are expressed as the mean  $\pm$  standard deviation (SD). RStudio (version 2022) was used to compare different treatments, using one-way and two-way analysis of variance (ANOVA). Tukey’s test was used to compare differences between groups, and statistical discrepancies with a  $p$ -value less than 0.05 were considered statistically significant.

## Results

The average weight of the loengo fruit is approximately 9.4 g, with an oval structure measuring  $20.01 \text{ mm} \pm 2.7 \text{ mm}$  in equatorial diameter and  $34.8 \text{ mm} \pm 1.8 \text{ mm}$  in polar diameter. One of the most notable characteristics of these fruits is their high soluble sugar content ( $19.5\% \pm 0.02\%$ ) and low titratable acidity ( $1.2\% \pm 0.1\%$ ), which strongly contribute to a distinctive flavor. The loengo fruit primarily comprises pulp and peel, which collectively accounting for 72.88% of the fruit total weight. The pulp, comprising 74.26% water, is the tissue with the most significant weight in the fruit. The peel, in contrast, is rich in ash and crude fiber, while the seed is abundant in protein and lipids.

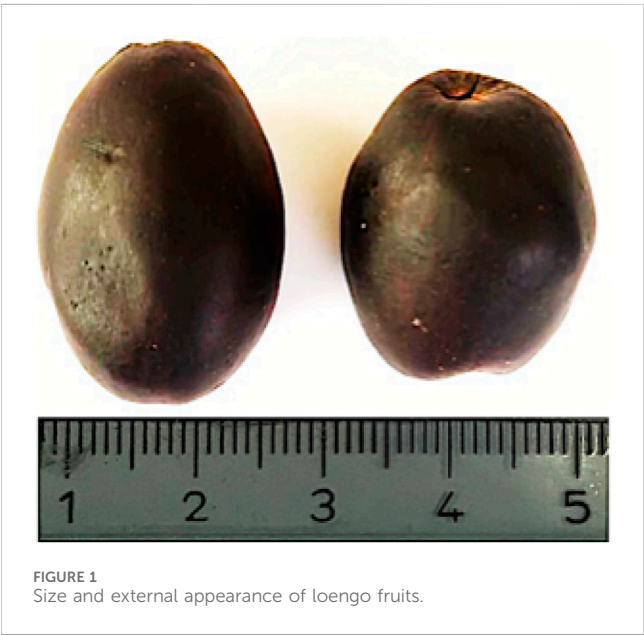
The results obtained demonstrate that the mineral composition of the peel, pulp, and seed presents a range of macro- and microminerals. The peel contains a large amount of K and Ca, the pulp contains a large proportion of K and S, whereas the seed



TABLE 1 Physicochemical properties characteristics of loengo fruit.

Sample	<i>A. boehmii</i>
Weight (g)	9.4 ± 0.4
Equatorial diameter (mm)	20.0 ± 2.7
Polar diameter (mm)	34.8 ± 1.8
pH	3.1 ± 0.1
SSC (%)	19.5 ± 0.1
Titrateable acidity (%)	1.2 ± 0.1

Values are represented as the mean ± standard deviation (SD). Soluble sugar content (SSC), pH and titrateable acidity were measured in the pulp.



contains a great abundance of K and P, these represent the main minerals found in this fruit. In addition to the aforementioned minerals, loengo fruits contain notable quantities of Zn, Mg, Fe, and Mn.

Loengo fruit total carbohydrates (including those derived from mono, oligo, and polysaccharides), quantified by the anthrone method, show a higher sugar concentration ( $\text{g} \cdot 100 \text{ g}^{-1} \text{ DW}$ ) in the pulp ( $70.28 \pm 1.48$ ), followed by the peel ( $34.99 \pm 0.23$ ), and finally the seed ( $6.47 \pm 1.43$ ), the component of the fruit that presents the lowest quantity of sugars. The sugars identified were glucose and fructose, which are present in similar amounts in the skin and pulp, with more glucose than fructose in the seed.

Citric acid is the most abundant organic acid present in the different tissues of the loengo fruit. Other organic acids, such as oxalic acid, ascorbic acid, and malic acid, were also present. The organic acids identified in the loengo fruit (citric, malic, oxalic, and ascorbic) exhibited a higher concentration in the peel, and the pulp contains a higher concentration of ascorbic acid than the seed.

The loengo seed contains a higher concentration of total phenolic compounds, with 117.09 mg GAE per 100 g of DW, compared to the

peel (38.07 mg GAE per 100 g of DW) and the pulp (25.46 mg GAE per 100 g of DW), which exhibited lower concentrations. However, there was no significant difference ( $p < 0.05$ ) between the peel and pulp phenolic contents. Not surprisingly, the seed higher enhanced antioxidant activity, while the pulp demonstrated an antioxidant activity with notable similarity to the peel.

## Discussion

### Physicochemical parameters

The quality of fruits may be assessed by some chemical and physical parameters that are crucial for consumer acceptability (Kitzberger et al., 2017). The variation in weight and in polar and equatorial parameters among loengo fruits collected from Huambo, presented in Table 1, is minimal, thus allowing the results to be accurately expressed as mean ± SD. The average weight of each fruit is  $9.4 \text{ g} \pm 0.02 \text{ g}$ . The fruits of the plum variety studied by Kitzberger and colleagues (2017) were about 4.5 times heavier than the loengo fruit, with an average weight of 42.3 g. Stanley cultivar plums are larger than loengo fruits (Ertekin et al., 2006). In turn, loengo fruit weights are higher than those of blackberries (6.3 g fresh weight) (Milošević et al., 2016). The loengo fruits are dark blue and maroon or carmine drupes in the ripening stage ellipsoid (Figure 1), with the following average diameters: equatorial  $20.0 \text{ mm} \pm 2.7 \text{ mm}$  and polar of  $34.8 \text{ mm} \pm 1.8 \text{ mm}$ . These values are consistent with previous reports (Ruffo et al., 2002) for loengo fruits from Tanzania. These results are different from data published by Nkengurutse et al. (2021), who reported that the average length ranges from 33 to 40 mm, the width between 29 and 34 mm, and the average weight between 16 and 25 g. Variations in weight can be influenced by factors such as variety, soil, and environmental conditions (Skrovankova et al., 2015).

The loengo pulp has a sweet and sour taste, with a content of soluble solids (°Brix) of 19.5%, which is higher than that of the plum 10.7%; (Kitzberger et al., 2017), the blackberry (8.3%), the strawberry (10.4%), the blackcurrant (10.6%) the peach and the nectarine (Milošević et al., 2016; Pandey et al., 2021). Loengo titrateable acidity was determined as ( $1.2\% \pm 0.1\%$ ) expressed in citric acid, being more acidic than the strawberry (0.7%) and less acidic than the red currant (1.3%) (Milošević et al., 2016). Peach (0.8%) and nectarine (0.9%) have less acidity than the loengo fruit (Pandey et al., 2021). The presence of compounds of an acidic nature, such as amino acids, phenolic compounds, fatty acids, and other organic acids, is related to the titrateable acidity for a fruit tissue/organ (Castro et al., 2023). The composition and content of soluble solids in the fruit may vary depending on several factors, including the cultivar and variety, the location where it is grown, the environmental conditions, the plant nutrition, the stage of ripeness, and the time of harvest. Additionally, the length of post-harvest storage and the conditions or processing methods employed can also influence the composition of soluble solids in the berry crop (Kim et al., 2013; Skrovankova et al., 2015).

### Proximate composition of *Anisophyllea boehmii* fruits

As expected, the pulp of loengo fruit contained more water than the peel and seeds. The water content of loengo fruit components

TABLE 2 Proximate composition of peel, pulp, and seed for loengo fruits.

	Peel (epicarp)	Pulp (mesocarp)	Seeds (endocarp)
Weight (%) <sup>a</sup>	29.5 ± 0.3 <sup>b</sup>	43.3 ± 0.7 <sup>a</sup>	9.2 ± 0.1 <sup>c</sup>
Moisture (%)	64.3 ± 2.6 <sup>b</sup>	74.3 ± 1.5 <sup>a</sup>	39.1 ± 3.6 <sup>c</sup>
Ash (%)	5.2 ± 1.6 <sup>a</sup>	4.8 ± 0.3 <sup>a</sup>	3.4 ± 0.3 <sup>b</sup>
Lipids (%)	2.0 ± 0.4 <sup>b</sup>	0.12 ± 0.03 <sup>b</sup>	23.6 ± 1.2 <sup>a</sup>
Crude fiber (%)	11.7 ± 0.9 <sup>a</sup>	6.1 ± 0.8 <sup>b</sup>	7.2 ± 0.8 <sup>b</sup>
Total protein (%)	8.0 ± 0.1 <sup>b</sup>	7.4 ± 0.1 <sup>c</sup>	16.9 ± 0.2 <sup>a</sup>

The values represent the mean ± SD, obtained from different parts of the loengo fruit (peel, pulp, and seed) from three repetitions. <sup>a</sup>Percentage of the weight of the edible parts of the fruit. Moisture and ash parameters are expressed as a percentage of the total weight. Lipids, crude fiber, and total protein are expressed as a percentage of dry samples. Different letters on the same line mean statistically significant differences analyzed by Tukey's test ( $P < 0.05$ ).

was 74.3% (w/w) in the pulp, 64.3% (w/w) in the peel, and 39.1% (w/w) in the seed, with a significant difference among them, according to the approximate composition of *A. boehmii* fruits shown in Table 2. The loengo fruit exhibited a significantly lower moisture content when compared to other stone fruits, including apricots 83.0% w/w; (Hussain et al., 2021a), plums 87.2% w/w; (Hussain et al., 2021b), and peaches 89.0% w/w; (Hussain et al., 2021c).

The loengo fruit is rich in crude fiber in all its components, with the peel containing a higher amount of fiber than the pulp and seeds (Table 2). The loengo seed was the richest in lipids and proteins, showing significant differences when compared to the pulp and peel. When working on the composition of seeds from *A. boehmii*, Nkengurutse et al. (2016) obtained results for ash (2.5% w/w), oil (24.0% w/w), and protein (12.0% w/w). The results obtained for ash and oil agree with the data obtained in present work. However, we found a higher percentage of protein in the seed (16.9% w/w) (Table 2).

The ash content (w/w) in fruit stones seeds, such as peach, apricot, and plum, ranged from 6.1% ± 0.1%, 4.8% ± 0.4%, and 2.16% ± 0.02%, respectively. The crude fiber in percentage for the same crops were peach (8.6% ± 0.0%), apricot (7.5% ± 0.0%) and plum (21.4% ± 0.2%), with only plum having a higher crude fiber content when compared to the seed of loengo (Rodríguez-Blázquez et al., 2023).

As described Rodríguez-Blázquez and colleagues (2023), a comparison of the loengo seed with that from other fruits stones, reveals a high degree of similarity, despite the loengo having a higher protein content (per g of fresh fruit) than the cherry and a lower amount than the peach, medlar. Additionally, the loengo seed has a higher percentage of water when compared to other seeds.

The results of our study indicate that the loengo fruit is richer in proteins, lipids and crude fibers than other stone fruits. Specifically, plums (Hussain et al., 2021b) exhibited 0.7% (w/w), 0.3% (w/w), and 2.2% (w/w) protein, lipid, and total fiber content, respectively. Similarly, peaches (Hussain et al., 2021c) exhibited 0.9% (w/w), 0.3% (w/w), and 1.5% (w/w) protein, lipid, and total fiber content, respectively. Finally, apricots (Hussain et al., 2021a) exhibited 0.7% (w/w), 0.4% (w/w), and 2.4% (w/w) protein, lipid, and total fiber content, respectively.

The loengo fruit approximate composition differs from the African wild fig (*Ficus capensis*) in what concerns moisture (33.2% w/w), dry matter (66.3% w/w), ash (7.7% w/w), protein

(1.4% w/w), fat (4.5% w/w) and fiber (3.2% w/w) (Okoroh et al., 2019).

## Mineral composition

The Loengo fruit have a nutritional interest due to its mineral content (Table 3). In what concerns *A. boehmii* seeds and as reported by Nkengurutse and collaborators (2016), the mineral composition expressed in mg.100 g<sup>-1</sup>, is as follows: K: 387.2, P: 267.01, Mg: 86.01, Ca: 43.8, and Fe: 2.3. These values are considerably lower than those observed in the present study. We suggest that this discrepancy is motivated by the different soil and climate conditions where the trees were grown (Onivogui et al., 2014). However, the same authors, Nkengurutse et al. (2021), compared the mineral composition of the same crop from different locations in Uganda and observed that in specific locations, the *Anisophyllea bohemii* fruit presented (mg.100 g<sup>-1</sup>) Ca: 87.2, Mg: 135.7 and Fe: 3.6 values that were close to those obtained in the present work. In the work reported by Onivogui and colleagues (2014) on *Anisophyllea laurina* fruit, K and Ca were the predominant minerals, with concentrations varying from 1,434.4 mg 100 g<sup>-1</sup> DW in the pulp and 411.3 mg 100 g<sup>-1</sup> DW in the seed for K, to 185.5 mg 100 g<sup>-1</sup> DW (pulp) and 64.3 mg 100 g<sup>-1</sup> DW (seed) for Ca, thus presenting similar concentrations to the loengo fruit.

A comparison of the mineral composition of loengo with those of hard stone fruits, including apricot (Hussain et al., 2021a), peach (Hussain et al., 2021b), and plum (Hussain et al., 2021c), revealed that loengo exhibited a higher concentration of essential minerals, including K, Mg, P, and Zn. Despite the fact that Ca and Fe were found to be present in high quantities in the peaches, these findings suggest that loengo may offer a unique nutritional profile, particularly in terms of its mineral content. Therefore, loengo fruit may be regarded as a good source of minerals, especially K, P, Ca, and Mg (Table 3).

## Sugars and organic acids profiling

Sugars represent one of the main components of edible fruit quality by imparting sweetness as one of the attributes influencing

TABLE 3 Mineral composition of peel, pulp and seed of loengo fruits.

Mineral compounds	Peel	Pulp	Seed
K	933.2 ± 17.6 <sup>b</sup>	1,061.6 ± 30.2 <sup>a</sup>	885.9 ± 5.0 <sup>c</sup>
P	104.6 ± 5.1 <sup>bc</sup>	125.9 ± 1.5 <sup>b</sup>	566.7 ± 17.0 <sup>a</sup>
Ca	396.7 ± 168.2 <sup>a</sup>	159.9 ± 0.8 <sup>b</sup>	70.1 ± 6.3 <sup>c</sup>
S	252.1 ± 14.7 <sup>b</sup>	286.4 ± 6.7 <sup>a</sup>	30.4 ± 1.8 <sup>c</sup>
Mg	128.5 ± 4.2 <sup>ab</sup>	152.8 ± 4.3 <sup>a</sup>	132.8 ± 1.8 <sup>ab</sup>
Na	103.4 ± 13.8 <sup>c</sup>	126.5 ± 14.5 <sup>b</sup>	167.8 ± 7.5 <sup>a</sup>
Mn	32.2 ± 4.7 <sup>a</sup>	12.7 ± 1.3 <sup>b</sup>	1.0 ± 0.04 <sup>c</sup>
Zn	1.4 ± 0.1 <sup>bc</sup>	0.9 ± 0.03 <sup>b</sup>	6.7 ± 0.4 <sup>a</sup>
Fe	2.6 ± 0.3 <sup>ab</sup>	2.0 ± 0.07 <sup>ab</sup>	3.8 ± 0.01 <sup>a</sup>
B	1.5 ± 0.1 <sup>ab</sup>	1.8 ± 0.1 <sup>ab</sup>	4.7 ± 0.2 <sup>a</sup>
Cu	0.6 ± 0.01 <sup>ab</sup>	0.9 ± 0.01 <sup>ab</sup>	2.2 ± 0.1 <sup>a</sup>

Mineral composition of the different parts of the loengo fruit (mg. 100 g<sup>-1</sup> DW). Values are mean ± SD, for three replicates. Different letters in the same row mean statistically significant differences by the Tukey test ( $p < 0.05$ ).

TABLE 4 Carbohydrate content of loengo fruit.

Carbohydrate content	Peel	Pulp	Seed
Total carbohydrates	34.9 ± 0.2 <sup>b</sup>	70.3 ± 1.5 <sup>a</sup>	6.5 ± 1.4 <sup>c</sup>
Glucose	13.6 ± 0.9 <sup>aA</sup>	19.9 ± 1.1 <sup>aA</sup>	1.1 ± 0.3 <sup>bA</sup>
Fructose	12.6 ± 0.5 <sup>aA</sup>	18.1 ± 0.3 <sup>aA</sup>	0.7 ± 0.1 <sup>bB</sup>

Carbohydrates, glucose, and fructose contents in the different tissues of the loengo fruit (g.100 g<sup>-1</sup> DW). Each value is expressed as the mean ± SD. Values with different superscript letters in the same line significantly differ ( $P < 0.05$ ). Capital letters compare differences between sugars in the same column, lowercase letters compare differences between fruit components in the same row.

the degree of consumer satisfaction (Nowicka et al., 2019). Sugars and organic acids are important compounds contributing to the flavor of fruits and juices, promoting a strong impact on the sensory quality and on some chemical characteristics of these foods, such as pH, total acidity, sweetness, microbial stability, and general acceptability, and may also be used to predict the ripening fruit stage (Rodrigues et al., 2021; Oliveira et al., 2014). Loengo fruit total carbohydrates (including those derived from mono, oligosaccharides), quantified by the anthrone method, show a greater carbohydrate concentration (expressed in g.100 g<sup>-1</sup> DW) in the pulp (70.3 ± 1.5), followed by the peel (35.0 ± 0.2), with the seed (6.5 ± 1.4) being the fruit component presenting the lowest quantity of carbohydrate, as indicated (Table 4). Glucose and fructose were found in similar amounts in the peel and the pulp. However, these sugars exhibited differential expression in the seed, with glucose being the predominant sugar in this fruit component.

In their study, Nkengurutse and collaborators (2016) identified 48.5 g.100 g<sup>-1</sup> ± 0.4 g.100 g<sup>-1</sup> DW of carbohydrates present in the seed of *A. boehmii*. These values are similar to the sugar contents found in the pulp in the present study. The carbohydrate contents of the pulp and seeds of *A. laurina* were exceptionally high, at 89.8 g.100 g<sup>-1</sup> DW and 71.1 g.100 g<sup>-1</sup> DW, respectively (Onivogui et al., 2014).

In the present work, we used only low molecular weight carbohydrates or simple sugars found in the loengo fruit. These simple sugars play crucial roles in providing energy, facilitating

metabolic processes, enhancing nutrient absorption, and contributing to the flavor profile of foods (Berg et al., 2015). These sugars are integral to various biochemical and physiological processes in the human body. A primary energy source for cells, glucose is vital for brain function and physical activity (Nelson and Cox, 2017). Fructose is a naturally occurring sugar that is metabolized primarily in the liver (Vos et al., 2008). Galactose is essential for the synthesis of glycoproteins and glycolipids (Bailey, 2000). Low-weight sugars are a primary and immediate source of energy for the body. Glucose, in particular, is essential for cellular respiration and ATP production (Nelson and Cox, 2017). These sugars are pivotal in metabolic pathways, including glycolysis and the citric acid cycle, essential for energy production at the cellular level (Berg et al., 2015). The quality and composition of carbohydrates are primarily influenced by the variety of fruit, the weather conditions and the growing region (Lebaka et al., 2021) as well as the pre-harvest temperature, ripeness, variety of the crops under study and on some ecotypes (Trad et al., 2017). The carbohydrates present in the seeds of other stone fruits, such as peach (13 g.100 g<sup>-1</sup> ± 3 g.100 g<sup>-1</sup>), apricot (15 g.100 g<sup>-1</sup> ± 3 g.100 g<sup>-1</sup>), and plum (18 g.100 g<sup>-1</sup> ± 2 g.100 g<sup>-1</sup>), were found to be slightly higher than those found in the loengo seed in this study (Rodríguez-Blázquez et al., 2023).

Among other stone fruits, apricots are a relatively concentrated source of carbohydrates. The carbohydrate content of fresh apricots is 10%–13%, while the total sugar content is approximately 9 g per

TABLE 5 Organic acid content in the peel, pulp and seed of loengo fruit.

Organic acid	Peel	Pulp	Seed
Citric acid	10,282.9 ± 324.0 <sup>aA</sup>	5,259.8 ± 79.4 <sup>bca</sup>	2,090.8 ± 176.5 <sup>bca</sup>
Malic acid	771.4 ± 21.9 <sup>aB</sup>	442.1 ± 24.7 <sup>cB</sup>	221.9 ± 6.7 <sup>cB</sup>
Oxalic acid	453.7 ± 51.1 <sup>aB</sup>	50.7 ± 2.1 <sup>bB</sup>	207.5 ± 6.7 <sup>bB</sup>
Ascorbic acid	214.9 ± 19.9 <sup>aC</sup>	185.6 ± 10.2 <sup>cA</sup>	nd

Citric, malic, oxalic, and ascorbic acid (mg/100g DW) in edibles parts of the loengo fruit. Each value is expressed as the mean ± SD. Values with different superscript letters are significantly different and capital letters compare differences between organic acids in the same column, lowercase letters compare differences between fruit components in the same row ( $p < 0.05$ ). nd: not detected.

100 g of fruit weight. The sugar composition of the fruit includes glucose, fructose, sucrose, and sorbitol (Hussain et al., 2021a). Plums provide a relatively high proportion of carbohydrates in the diet, with approximately 15 g of carbohydrates per 100 g of fruit. Monosaccharides constitute a significant portion of the carbohydrates in plums, with glucose and sucrose being the primary contributors. Disaccharides, such as sucrose, are responsible for the sweetness of plums (Hussain et al., 2021c). The total carbohydrate content of peaches is 9.5 g per 100 g of fruit weight. Sucrose is the predominant sugar in the fruit, followed by glucose and fructose (Hussain et al., 2021b). In peach cultivars, the fructose content was higher than that of glucose (Nowicka et al., 2019). It can be concluded that these fruit stones, including loengo, are excellent sources of sugars, although the composition of these carbohydrates may vary depending on several factors.

Both organic acids and sugar compounds play a crucial role in shaping fruits' flavor and degree of sweetness. By analyzing these compounds in detail, we can accurately describe the sensory properties of these fruits (Nowicka et al., 2019). Citric acid was the most abundant organic acid present in the different components of the loengo fruit (Table 5), presenting itself as a potential source for this compound, with the peel containing the highest concentration. Other organic acids, such as oxalic acid, ascorbic acid and malic acid, were also present. The organic acids identified in the loengo fruit (citric, malic, oxalic, and ascorbic) exhibited a higher concentration in the peel, whereas the pulp exhibited a higher concentration of ascorbic acid than the seed. Onivogui and collaborators (2014) identified the presence of citric, fumaric, malic, tartaric, and oxalic acids in the fruit of *A. laurina*. Citric and malic acid were found to be the most abundant in the pulp, while tartaric acid was the most abundant in the seed of *A. laurina* fruit. However, the presence of ascorbic acid, which was identified in the present work, was not reported. In different mulberry genotypes (*Morus* spp.) studied by Eyduan and colleagues (2015a), malic acid was the main organic acid identified. However, grapevine (*Vitis vinifera* L.) citric and tartaric acid were the most predominant (Eyduan et al., 2015b).

There is a similarity between the main organic acids present in loengo fruit and the organic acids identified in peach fruits. Malic acid, quinic acid, and citric acid are the predominant organic acids in peach fruits (Nowicka et al., 2019).

## Total polyphenols and antioxidant activity

Polyphenolic compounds (e.g., phenolic acids, flavonoids and anthocyanins) are regarded as one of the most crucial bioactive

components of plants and fruits, offering a multitude of biological health benefits derived from their biological properties (Kim et al., 2013; Corrêa et al., 2023). In this context, the total polyphenol compounds (TPC) were evaluated in the loengo fruit. The results presented in Table 6 are comparable to those of *A. laurina* fruits, where the seeds exhibited a greater total polyphenolic content (TPC) (Onivogui et al., 2014), while the pulp exhibited a lower TPC level. There is a paucity of studies evaluating the total phenol content of loengo fruit. Nkengurutse et al. (2019) posited that the TPC of *A. boehmii* almond seed oils may be responsible for some bioactivity. Berries, particularly members of several families, such as Rosaceae (strawberry, raspberry, and blackberry) and Ericaceae (blueberries), are notable dietary sources of bioactive compounds, including ascorbic acid and phenolic compounds, such as phenolic acids and flavonoids. These compounds, including also anthocyanins, tannins, and ascorbic acid, act as potent antioxidants, helping to prevent inflammatory disorders, cardiovascular diseases, and various types of cancer (Batista et al., 2017; Ramos et al., 2023). Loengo presented noteworthy levels of total polyphenols (Table 6), although lower than those reported by other red fruits, such as blackberry and strawberry (Milošević et al., 2016). When we compare to other stone fruits, such as peach, apricot and plum (Hong et al., 2021), our results indicate that the loengo seed exhibited a higher TPC and antioxidant activity. The considerable presence of total polyphenols in loengo fruits is consistent with the findings of Genskowski et al. (2016), which indicate that dark blue fruits are rich in these compounds and can be utilized as a source of antioxidants (Eyduan et al., 2015a) even when compared to vegetable (Singh et al., 2016).

According to Mousavi et al. (2019), stone fruits seed almonds, including peach (*Prunus persica* L.), cherry (*Prunus avium* L.) and damson (*Prunus domestica* subsp. *insititia* L.) exhibited in mg GAE/g DW, 27.7; 38.9 and 25.6 respectively, higher total polyphenol content compared to loengo fruit. The TPC of the loengo seed was similar to that of the plum seed, therefore, the loengo seed may possess beneficial bioactivities for human health (Hong et al., 2021).

The peel exhibited a higher total phenolic content (TPC) than the pulp in mature fruit stones. The reduction in the phenolic content of the pulp can be attributed to a series of chemical and enzymatic changes of some phenols during the fruit ripening process. The seeds exhibited the highest total phenolic content, irrespective of the maturation stage (Redondo et al., 2021). The results obtained in the present study differ from those reported by Redondo and colleagues (2021), who analyzed the antioxidant activity by DPPH of various fruit stones, including apricot, plum and peach, and found differences between the pulp and skin of these fruits at commercial maturity stages. However, when using the FRAP methodology, the values obtained were similar to those reported in the present work.



TABLE 6 Total phenolic content and antioxidant activity of the loengo fruit.

	TPC	DPPH	FRAP
Peel (epicarp)	38.1 ± 10.1 <sup>b</sup>	27.9 ± 1.8 <sup>b</sup>	30.8 ± 1.7 <sup>b</sup>
Pulp (mesocarp)	25.5 ± 3.1 <sup>b</sup>	35.3 ± 1.8 <sup>b</sup>	13.9 ± 1.5 <sup>c</sup>
Seeds (endocarp)	117.1 ± 3.5 <sup>a</sup>	93.5 ± 7.9 <sup>a</sup>	35.9 ± 1.4 <sup>a</sup>

Total polyphenolic contents (TPC) are expressed in gallic acid equivalents (mg GAE .100 g<sup>-1</sup>DW); ferric reducing antioxidant power (FRAP) and 2,2-diphenyl-1-picryl-hydrazyl (DPPH) assays are expressed as mg Trolox equivalents per g DW., Each value is expressed as the mean ± SD. Values with different superscript letters in the same column are statistically significant ( $p < 0.05$ ).

Appropriate management during pre- and post-harvest handling, along with further characterization of phytochemicals, could help increase the levels of these compounds, leading to future improvement of the fruit not only to enhance organoleptic characteristics but also to benefit human health (Lara et al., 2020). Consequently, extracts derived from stone fruits, including loengo, can be a source of healthy beneficial compounds. The understanding of the nutritional and pharmaceutical properties of loengo fruits could enhance production systems and post-harvest conservation, as well as the potential for the development of nutraceutical foods (Chalchisa et al., 2022), improving nutrition and food security strategies. The high nutrient content of fruits suggests its future potential applications for the food and pharmaceutical industry (Dhatwalia et al., 2024).

# Conclusion

Loengo (*A. boehmii*) occurs as spontaneous trees that are not cultivated as a crop in Huambo, Angola. Their fruits can be a food of the future, being a valuable source of nutrients and bioactive molecules for local populations, and if cultivated it can be a promising marketable fruit. These fruits were subject to chemical and nutritional characterization. The findings of this study provide consistent evidence to recommend the consumption of this fruit due to its sweet flavor, chemical and nutritional composition, and, most importantly, because it is rich in beneficial minerals and antioxidant bioactive compounds, which are important in the control of degenerative diseases. The loengo fruit and its potential derivatives can be considered a nutraceutical food. This fruit can be a valuable resource to improve nutrition and food safety strategies in the near future, where foods may have nutritional and pharmaceutical properties.

# Data availability statement

The raw data supporting the conclusions of this article will be made available by the authors, without undue reservation.

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# Author contributions

AL: Writing–original draft, Writing–review and editing, Conceptualization, Data curation, Formal Analysis, Funding acquisition, Investigation, Methodology, Software, Validation, Visualization. MM: Writing–original draft, Writing–review and editing. CP: Writing–original draft, Writing–review and editing. IS: Writing–original draft, Writing–review and editing, Conceptualization, Data curation, Formal Analysis, Funding acquisition, Methodology, Project administration, Resources, Supervision, Validation, Visualization. RF: Writing–original draft, Writing–review and editing, Supervision.

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# Conflict of interest

The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

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