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Cladodes Flour from Opuntia ficus-indica: A Sustainable Source of Dietary Fiber and a Promising Alternative for Gluten-Free Formulations

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Abstract

Feeding a growing global population requires environmentally responsible food production and healthier dietary choices. In Portugal, although *Opuntia ficus-indica* is mainly cultivated for its fruit and oil-rich seeds, its cladodes remain largely underutilized. This study aimed to valorize cladodes by producing flour and characterizing it for food applications. The flour was obtained following an established procedure and was chemically characterised using AOAC methods. Amino acids were analysed by RP-HPLC-DAD-FLD, and free sugars by HPLC-ELSD. The flour exhibited a low-fat content (1.24%), high ash (21.34%) and dietary fibre (38.66%), and moderate protein levels (4.47%), with glutamic acid and lysine as main amino acids. Fructose, glucose, and sucrose were the main free sugars identified. Overall, cladodes flour is a sustainable, gluten-free, and nutritionally valuable ingredient suitable for developing functional and bakery products, promoting circular economy and crop sustainability.

Keywords: sustainability; circular economy; dietary fibre; amino acid profile; protein content; ash content;

1. Introduction

Figure 1. Prickly pear cactus.

The prickly pear cactus (*Opuntia ficus-indica* (L.) Miller) (Figure 1), belonging to the Cactaceae family, is a species native to Mexico and widely cultivated in arid and semi-arid regions around the world. Its cultivation has been expanding in Mediterranean countries such as Portugal due to its high adaptability and economic potential [1,2].

In addition to the fruit (prickly pear), which is widely consumed and processed into juices, jams and fermented beverages, the plant also provides other organs of interest, namely the cladodes.

Opuntia ficus-indica stands out for its role in agricultural sustainability. It is a low-water-demand species with high carbon fixation efficiency, as it uses the Crassulacean Acid Metabolism (CAM) pathway, allowing stomata to remain closed during the day and thereby reducing water loss [3-5]. These characteristics make it a resilient crop under climate change, capable of growing in poor and degraded soils, promoting environmental restoration and combating desertification [6]. The introduction of this crop in marginal areas contributes to the economic valorization of rural territories, reducing pressure on water resources and encouraging sustainable agricultural practices [7].

From an environmental perspective, Opuntia has a positive impact on CO_2 sequestration, soil structure

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improvement, and erosion control. Its integral use - including fruit, seeds, peels and cladodes - is in line with the principles of the circular economy, reducing agro-industrial waste and generating high value-added products [8, 9].

The health benefits associated with the consumption of *Opuntia ficus-indica* and its cladodes are widely reported. They are rich in dietary fibre, vitamins (C, E and B group), minerals (calcium, magnesium, potassium), and bioactive compounds such as polyphenols, flavonoids and betalains, recognized for their antioxidant, anti-inflammatory and hypoglycemic properties [10, 11].

The inclusion of cladode flour in food products can contribute to lowering the glycemic index, improving intestinal transit and helping to regulate cholesterol levels [12].

Young cladodes are traditionally consumed as a vegetable in several regions of Latin America (known as nopalitos), while mature cladodes, often discarded due to lignification and fibrous texture, represent an agricultural by-product with high valorization potential. When processed into flour, they can be incorporated into bakery products, pasta, biscuits or snacks, acting as a functional ingredient and a source of dietary fibre [12, 13, 16].

Furthermore, the mucilage extracted from the cladodes has applications in the cosmetic, pharmaceutical and biotechnological industries and can also be used as a natural biopolymer for biodegradable packaging [14, 15].

Hence, the valorization of *Opuntia ficus-indica* cladodes represents a concrete opportunity to integrate sustainability, food innovation and circular economy principles, transforming an agricultural by-product into a valuable resource with environmental and nutritional benefits, aligned with the United Nations Sustainable Development Goals (SDGs) [3, 4, 7].

2. Materials and methods

2.1. Preparation of Flour

Cladodes flour was obtained according to the method described by Sapata et al. [17], with minor adaptations (Figure 2). Intact cladodes, free from visible defects or mechanical damage, were collected in Torres Novas, Portugal, from plants belonging to a cultivar producing orange-pulp fruits. After harvesting, the cladodes were manually cleaned with a stainless-steel knife, removing the spines and approximately 5-10 mm from the edges to ensure sample uniformity.

The cladodes were thoroughly washed with potable running water to remove surface impurities and subsequently disinfected by immersion in an 80 ppm sodium hypochlorite solution for 3 minutes under gentle agitation. They were then rinsed again with potable water to eliminate residual chlorine and drained. The cladodes were placed on mesh trays and air-dried at room temperature (20-25 °C) for approximately 20-30 minutes to remove superficial moisture. Partially dried cladodes were cut into thin strips with a maximum width of 1 cm, maintaining consistent thickness and length. The strips were placed on the trays of a Captain Jerky 110 dehydrator (Klarstein) without overlapping and dehydrated at 50 °C for 12 hours until reaching a moisture content below 10%. After drying, the dehydrated samples were cooled to room temperature and collected for further processing.

The dried material was ground using a Vorwerk Thermomix® TM5 robot (Vorwerk Thermomix®, Germany) until a homogeneous powder was obtained. The resulting flour was sieved through a set of stainless-steel sieves with mesh openings of 350, 250, 180, 125 and 90 µm to determine the particle-size distribution. It was observed that approximately 35% of the flour particles had a size below 90 µm.

Finally, the flour was packed in moisture-barrier polyethylene bags, hermetically sealed, and stored in a cool, dry, and dark environment at a temperature ≤ 25 °C until subsequent analyses were performed.

Figure 2. Process flow for the preparation of *Opuntia ficus-indica* cladodes flour.

2.2. Nutritional composition

Moisture content was determined using an infrared moisture analyser (Scaltec SMO01, Scaltec Instruments, Heiligenstadt, Germany). The remaining proximate analyses were carried out in accordance with official AOAC procedures [18]. In brief, ash content was obtained by sample

incineration at 500 °C in a muffle furnace; total lipids were determined by the Soxhlet extraction method, and crude protein was quantified according to the Kjeldahl procedure; total and insoluble dietary fibre were analysed using the enzymatic-gravimetric method. Soluble fibre, total carbohydrates, and remaining carbohydrates were calculated by difference, considering the dry matter and the sum of the other proximate components.

All results were performed in triplicate and expressed in grams per 100 grams of dry weight (g/100 g dw).

2.3. Quantification of Total Amino Acids by RP-HPLC-DAD-FLD

The quantification of total amino acids in cladode flour was performed using a reverse-phase high-performance liquid chromatography system equipped with diode array and fluorescence detectors (RP-HPLC-DAD-FLD). Amino acids were released through two independent hydrolysis procedures: alkaline hydrolysis with 4 M KOH for 4 hours, applied for tryptophan determination, and acid hydrolysis with 6 M HCl for 24 hours for the remaining amino acids.

After hydrolysis, the samples were neutralised and mixed with the internal standard norvaline (2 mg/mL), following the protocol adapted from Machado et al. [19]. Aliquots of the resulting mixtures were injected into an HPLC system (Jasco, Tokyo, Japan) consisting of an LC-NetII/ADC interface, two PU-980 pumps, and an AS-4150 RHPLC autosampler equipped for automated on-line derivatisation using OPA/3-MPA and FMOC reagents.

Chromatographic separation was carried out on a ZORBAX Eclipse Plus C18 column (4.6 × 250 mm, 5 µm; Agilent Technologies, Santa Clara, CA, USA) using a gradient solvent system. Detection was achieved with a multiwavelength detector (MD-2015 Plus) for compound identification and a fluorescence detector (FP-2020 Plus) for quantification, operated at λ_{ex} = 340 nm and λ_{em} = 450 nm for OPA-derivatives (0–26.2 min), and λ_{ex} = 266 nm and λ_{em} = 305 nm for FMOC-derivatives (26.2-40 min).

2.4. Quantification of Free Sugars by HPLC-ELSD

For the extraction of free sugars, approximately 2 g of dehydrated sample was weighed into 50 ml Falcon tubes and 20 ml of ultrapure water was added. The obtained mixture was vortexed (Reax top, Heidolph, Germany) for 20 min at 4500 rpm, and then centrifuged for 15 min at 5000 rpm in a Megafuge 16 centrifuge (Heraeus, Hanau, Germany). The resulting supernatants were filtered through syringe filters and collected into HPLC vials for analysis. They were analysed in triplicate and the results expressed in g/100 g of dry weight (g/100 g dw)

Quantification of soluble sugars was performed on a high-performance liquid chromatography (HPLC) system (Jasco, Tokyo, Japan) coupled to an evaporative light scattering detector (ELSD), according to [20].

Chromatographic separation was achieved on a Shodex Asahipak NH_2P-50 4E column (4.6 mm I.D. × 250 mm) under isocratic conditions using a binary mobile phase composed of water (25%) and acetonitrile (75%). The elution was carried out at a flow rate of 0.5 mL/min for 20 minutes at a constant temperature of 30 °C. All analyses were performed in triplicate, and the results expressed in g/100 g of dry weight (g/100 g dw).

3. Results and Discussion

3.1. Particle Size Distribution

The granulometric analysis of the cladode flour (Table 1) revealed that approximately 35% of the particles were smaller than 90 µm, while the remaining fractions ranged between 11% and 21% for coarser sieves. This fine particle distribution indicates that the milling and dehydration procedures were effective in producing a homogeneous flour suitable for incorporation into bakery or functional food formulations. Particle size directly affects technological properties such as water retention, texture, and dough elasticity, influencing final product quality. Similar particle profiles have been reported in cactus-derived flours used for composite bread formulations, suggesting that a finer fraction (<100 µm) enhances product uniformity and functional performance [21].

Table 1. Cladodes flour granulometric analysis.

Sample	**Pore size** (µm)	**% Retention**
Cladodes flour (CF)	350 > CF > 250	21
	<250	18
	<180	14
	<125	11
	<90	35

3.2. Proximate composition

As shown in Figure 3, the cladode flour exhibited low moisture (7.6%), ensuring product stability and

extended shelf life. The ash content (21.3 g/100 g dw) was notably high, confirming its richness in mineral elements such as calcium, magnesium, and potassium, which are commonly abundant in Opuntia species [22]. This mineral contribution supports the use of cladode flour as a natural fortifying ingredient in functional foods.

The fat content (1.24 g/100 g dw) was low, consistent with the profile of cactus-derived ingredients, aligning with current consumer demand for low-fat products. The protein level (4.47 g/100 g dw) agrees with values reported for *O. ficus-indica* cladodes (4-10 g/100 g dw), highlighting its potential to complement cereal-based proteins in gluten-free formulations. The presence of all essential amino acids, especially lysine, often limited in cereals, further enhances its nutritional value [23].

A remarkable feature of the cladode flour is its high total dietary fibre content (38.66%), primarily insoluble fibre (37.40%), with a smaller soluble fraction (1.25%). Although slightly lower than values reported by Ayadi et al. [21], this composition confirms that cladodes are an excellent source of structural polysaccharides such as cellulose, lignin, and hemicellulose. Insoluble fibre promotes intestinal regularity, while the soluble portion contributes to cholesterol reduction and glycaemic control [24]. Such characteristics reinforce the potential of cladode flour for use in high-fibre and functional food products.

Regarding carbohydrates, the total simple sugar content (10.78 g/100 g dw) was mainly composed of fructose (5.66 g/100 g), glucose (3.75 g/100 g), and sucrose (1.38 g/100 g). These levels are consistent with other *Opuntia* studies [22] and contribute to a mild sweetness and pleasant flavour profile, advantageous for gluten-free bakery applications.

Overall, the proximate composition demonstrates that cladode flour is a low-fat, mineral-rich, and high-fibre ingredient, aligning with the principles of sustainable nutrition and functional food innovation.

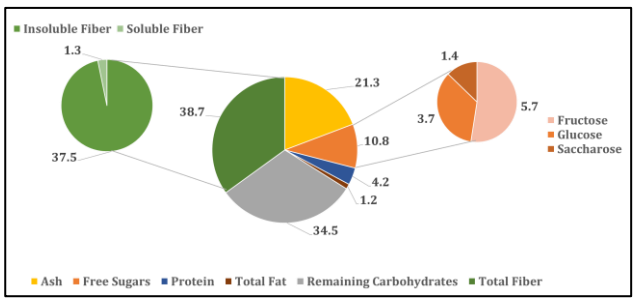

Figure 3. Nutritional composition of *Opuntia ficus-indica* cladodes flour.

3.3. Amino Acid profile

The amino acid composition of cladode flour (Table 2) confirms the presence of all essential amino acids, with glutamic acid (6.25 mg/g) and aspartic acid (2.69 mg/g) as the dominant ones, followed by proline, lysine, and leucine. This profile is consistent with findings from [2,22], who also identified glutamine, leucine, and lysine as major constituents. The high content of glutamic acid, an amino acid associated with energy metabolism and flavour enhancement, and lysine, essential for tissue repair and immune function, increases the nutritional relevance of cladode flour in cereal-based products where lysine is typically deficient.

Table 2. Amino acids profile of cladodes flour.

Amino acid	Cladodes flour
Aspartic acid	2.69 ± 0.13
Glutamic acid	6.25 ± 0.27
Serine	1.46 ± 0.08
Histidine	0.04 ± 0.01
Glycine	1.02 ± 0.06
Threonine	1.78 ± 0.06
Arginine	1.06 ± 0.08
Alanine	1.75 ± 0.08
Tyrosine	0.41 ± 0.03
Valine	1.47 ± 0.08
Methionine	0.01 ± 0.00
Tryptophane	0.29 ± 0.01
Phenylalanine	0.80 ± 0.05
Isoleucine	1.27 ± 0.05
Leucine	2.14 ± 0.10
Lysine	2.21 ± 0.08
Hydroxyproline	0.48 ± 0.02
Proline	2.34 ± 0.06
Total amino acids	27.47 ± 1.39

Results expressed in mg/g in dry weight as mean ± standard deviation (n = 3).

Although the overall protein content is modest, the balanced amino acid profile and the presence of both essential and non-essential amino acids suggest good biological quality. This makes the flour a valuable complementary protein source, especially for gluten-free and plant-based formulations aimed at health-conscious consumers.

3.4. Final remarks

The compositional profile of *Opuntia ficus-indica* cladodes flour confirms its nutritional, functional, and environmental value. Its high fibre and mineral contents, combined with a favourable amino acid composition and naturally gluten-free nature, make

it an excellent candidate for the development of sustainable, health-promoting food products. Furthermore, the utilisation of cladodes - a previously discarded by-product - supports circular economy principles, reducing agricultural waste while enhancing the economic viability of cactus cultivation in semi-arid regions.

4. Conclusion

In summary, *Opuntia ficus-indica* cladode flour represents a sustainable and nutritionally valuable ingredient derived from an underexploited agricultural resource. Its high dietary fibre and mineral content, together with moderate protein levels and a low fat fraction, make it a promising component for health-oriented formulations. The presence of essential amino acids, particularly lysine, further enhances its nutritional potential by complementing cereal-based proteins. Moreover, the natural absence of gluten positions cladode flour as an excellent ingredient for the development of gluten-free bakery and functional food products. Beyond its nutritional benefits, its use supports circular economy practices and promotes the sustainable valorisation of Opuntia crops, contributing to both environmental preservation and economic resilience in arid and semi-arid regions.

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