agriculture

MDPI

Article

Arbuscular Mycorrhizal Fungi Inoculation Enhances Nutritional Quality of Prickly Pear (*Opuntia ficus-indica*) Fruits and Cladodes

Sonia Labidi [1], Adrien Servent [2,3], Ghofran Bouzoumita [1], Tina Julien [2,3], Guillaume Cazals [4], Manel Ibrahim [1], Sofiène B. M. Hammami [1] and Nawel Achir [2,*]

1 Laboratoire des Sciences Horticoles, Institut National Agronomique de Tunisie, LR13AGR01, Université de Carthage, 43 Ave Charles Nicolle, Tunis 1082, Mahrajène, Tunisia
2 UMR Qualisud, Univ Montpellier, Avignon Université, CIRAD, Institut Agro, IRD, Université de La Réunion, F-34398 Montpellier, France
3 CIRAD, UMR Qualisud, F-34398 Montpellier, France
4 IBMM, Univ Montpellier, F-34398 Montpellier, France
* Correspondence: nawel.achir@institut-agro.fr

Abstract

The effects of arbuscular mycorrhizal fungi (AMF) inoculation on the chemical composition of the fruits and cladodes of two *Opuntia ficus-indica* cultivars, characterized by their red and yellow fruit color, were investigated under field conditions. AMF treatment was found to significantly influence the concentration of phytonutrients in the fruits. The concentrations of betacyanin and betaxanthin increased by 1.2- and 1.9-fold in red and yellow fruits, respectively. The polyphenol content increased by 50%, with piscidic acid being the most abundant polyphenol in the red fruits. A similar increase in ascorbic acid was observed in the yellow fruits. Regarding the cladodes, AMF treatment was found to significantly affect macronutrient levels, with glucose and fructose contents being 90% and 34% higher, respectively. Additionally, cladodes from plants grown with AMF inoculation showed a 20% increase in ascorbic acid and phosphorus. These results demonstrate cultivar- and part-of-plant-dependent effects of AMF inoculation and confirm the nutritional and sustainable potential of *Opuntia ficus-indica*, particularly when coupled with mycorrhizal biofertilization practices.

Keywords: betaxanthin; betacyanin; piscidic acid; food dye; fodder

check for updates

Academic Editor: Soon-Jae Lee

Received: 25 June 2025
Revised: 28 August 2025
Accepted: 29 August 2025
Published: 8 September 2025

Citation: Labidi, S.; Servent, A.; Bouzoumita, G.; Julien, T.; Cazals, G.; Ibrahim, M.; Hammami, S.B.M.; Achir, N. Arbuscular Mycorrhizal Fungi Inoculation Enhances Nutritional Quality of Prickly Pear (*Opuntia ficus-indica*) Fruits and Cladodes. *Agriculture* **2025**, *15*, 1902. https://doi.org/10.3390/agriculture15171902

1. Introduction

The Mediterranean basin has been identified as a climate change "hot spot" and is particularly vulnerable, facing several climatic constraints [1]. Among emerging crops that require little water and generate a reasonable profit, prickly pear (*Opuntia ficus-indica*) stands out due to its many advantages [2,3]. Environmentally, it plays a key role in controlling soil erosion through its root system, which enhances soil properties [4]. In addition, it is a honey plant, and its fruits are edible. The global market for prickly pear fruit is expanding, with annual production exceeding 400,000 tonnes in countries such as Mexico and Italy. This growth is driven by increasing consumer interest in functional and exotic fruits [5,6]. Although it is currently only commercially viable in a few countries, such as Mexico, Italy, and South Africa, *Opuntia ficus-indica* has the potential to generate both income and employment when supported by structured value chains and effective marketing. In

Mexico, for example, cactus pear cultivation provides a primary or supplementary source of livelihood for around 20,000 families [7]. In Tunisia's semi-arid zones, recent studies have reported yields of 10–40 t of cladodes and 1–5 t of fruit per hectare. This level of production corresponds to an estimated net economic return of approximately USD 800 per hectare for farmers. Nearly 50% of the cladodes are used as livestock feed, reducing feed costs and highlighting the plant's role in improving farm resilience and household income [3]. Prickly pear fruits have a sweet flavor and mild aroma, with a soluble solids content typically ranging from 12 to 16° Brix, making them attractive for both fresh consumption and industrial processing. The economic relevance of both the fruit and the cladodes continues to grow due to their nutritional properties, drought tolerance, and their applications in the food, cosmetic, and pharmaceutical industries [8–10]. The two most commercially important *Opuntia ficus-indica* varieties for fruit consumption are those with orange skin and yellow pulp ("Gialla", meaning "yellow" in Italian) and those with red peel and pulp ("Rossa", meaning "red"), widely cultivated in Mediterranean countries such as Italy [8,11]. The traits arise from the various combinations of betaxanthins, which are yellow-orange pigments, and betacyanins, which range from red to purple [12]. Prickly pear fruits are recognized for their interesting phytonutrient content, particularly antioxidants such as flavonols (e.g., quercetin derivatives) and phenolic acids (e.g., piscidic and ferulic acids), which contribute to their potential as functional foods [13–16]. The cladodes (pads) of *Opuntia ficus-indica* also offer significant nutritional value for humans and animals. Young cladodes, known as 'nopalitos', are traditionally eaten as vegetables in Latin America [17,18]. In livestock feeding systems, particularly in arid and semi-arid regions, mature cladodes are used as a valuable source of forage, providing animals with water, carbohydrates, and minerals while improving the resilience of agriculture in areas prone to drought [19]. These multifaceted uses highlight the importance of *Opuntia ficus-indica* as a sustainable crop with significant agronomic and nutraceutical relevance.

More sustainable cultivation practices, such as mycorrhizal biofertilization, are emerging. This practice consists of using biofertilizers formulated with arbuscular mycorrhizal fungi (AMF), which are known to play a key role in improving water regulation in plants through several mechanisms [20]. These fungi stimulate the production of phytohormones that enhance stress tolerance and improve plant water status. For example, they increase the synthesis of abscisic acid, a key hormone in water stress management that regulates stomatal closure [21]. In addition, they stimulate the production of osmolytes, regulators of osmotic pressure, and the regulation of aquaporins, which promote hydraulic conductivity [22]. The effects of AMF inoculation on plant growth and stress responses can vary both in their nature and intensity, depending on the host plant. Different plant species—and even different cultivars within a species—may therefore exhibit variable responses to AMF colonization [23]. Some studies have shown that this practice can modulate the physiological performance of plants but also change the chemical composition of their various parts, especially the fruits [24,25]. Indeed, it was demonstrated that mycorrhizal inoculation of tomato plants subjected to water stress mitigates the negative effects of drought. This resulted in increased sugar levels in fruits and stimulation of vegetative growth, as well as improved accumulation of osmolytes and mineral nutrients, contributing to increased yield [26]. As a hardy crop traditionally grown without fertilizers, cactus represents a particularly suitable candidate for biofertilization. However, investigations into their interactions with AMF remain scarce. Previous research has primarily focused on physiological response to AMF inoculation and cladode biomass production under drought stress, but no studies have investigated the effect of this treatment on fruit chemical composition. Moreover, most studies have concentrated on nutrient supply and mineral absorption, reporting reductions in drought-induced stress markers, but exclusively in cladodes [27,28].

The present study therefore aims to investigate the effect of AMF inoculation on both cladode and fruit chemical composition, providing a more comprehensive understanding of the effect of AMF inoculation on the whole plant. Specifically, this study seeks to assess the impact of AMF inoculation on key nutrients and phytonutrients that are relevant to human and animal nutrition, as well as their potential applications in the food industry.

2. Material and Methods

2.1. Chemicals

All products, including the solvents (ethanol, methanol, acetonitrile), acids (sulfuric, formic, hydrochloric, nitric), hydrogen peroxide, sodium lauryl sulfate, α-amylase, protease, ethylenedinitrilotetraacetic acid (EDTA), sodium borate, sodium hydrogen phosphate, triethylene glycol, cetyltrimethylammonium bromide, sodium hydroxide, and standards (glucose, fructose, sucrose, piscidic, and ascorbic acids), were bought from Sigma-Aldrich (Saint-Louis, MA, USA).

2.2. Experimental Design

The experimental study was carried out in two commercial prickly pear orchards at the Governorate of Nabeul (northeastern Tunisia). The experimental sites are situated in the superior semi-arid bioclimatic stage according to Emberger's climate classification. The first orchard covers approximately 245.5 m^2 (36.302304° N, 10.3542° E) and is characterized by sandy soil. It included 12 prickly pear plants, evenly divided into six AMF-inoculated and six non-inoculated ones. In this orchard, the spacing between the plants was 3.5 m. The second orchard was larger, with an area of 1010.07 m^2 (36.3123° N, 10.3323° E) and characterized by silty clay soil. In this site, 12 inoculated and 12 non-inoculated plants were considered. In this orchard, the spacing between the plants was 5.5 m. Both sites followed a complete block experimental design with two treatments: inoculated and non-inoculated plants. Inoculation was performed with an artisanal mycorrhizal biofertilizer prepared in the horticultural science laboratory (LSH-INAT, Tunis, Tunisia). It is a granular formulation (20 propagules/g) composed of native *Glomus* spp. (*G. deserticola* and *G. constrictum*) mixed with sand and perlite. In November 2022, 250 g of inoculum were added in a hole at 25 cm of depth and 50 cm from the plant stem on both sides, so 500 g per plant were applied. Harvesting was conducted one year later, in November 2023. All plants were maintained under uniform cultural practices within each site, minimizing orchard variability.

2.3. Plant Material

All plant material was harvested from the two experimental fields. Sampling of all the prickly pear plants among the two treatments: inoculated (with AMF) and non-inoculated (controls). For each plant, five fruits were collected from all the canopies. The fruits were all collected from the external part of the canopy at a human-length level in order to guarantee the same sun exposure for all the collected fruits. The fruits were separated according to their color, and only the largest ones were kept (mass $\geq$ 50 g). Since the plants produced mostly red fruits, 18 red fruits with AMF inoculation were used for analysis, along with 18 red fruits (Rossa) for the control. The yellow fruits being less common, 9 fruits (Gialla) with AMF inoculation were used and 9 for the control. In addition, 12 samples of 100 g cladodes were taken from each treatment. Samples were put in polyethylene bags and directly frozen at −20 °C.

2.4. Sample Preparation

Fruits were peeled, crushed, and sieved through a kitchen colander to separate the juice and the seeds. For each sample, the peels, juices, and seeds were weighed to calculate

the Juice/Fruit (J/F) and Seed/Fruit (S/F) ratios. The cladodes were crushed into a puree using a Thermomix (GM6, Vorwerk, Wuppertal, Germany). Juice and puree were sealed in plastic bags under vacuum and stored in the dark at −20 °C until analysis. A portion of the juice and crushed cladodes was freeze-dried in a freeze-dryer SMH15 (Usifroid, Élancourt, France) to carry out some specific analyses.

2.5. Proximal Composition

Moisture content, complementary to dry matter, was obtained by gravimetry, according to the AOAC method 925-09. Total soluble solid was determined by the AOAC method 932-12 using a digital refractometer (PAL-1, ATAGO, Tokyo, Japan). The pH was determined according to the AOAC method 981-12. Finally, the titratable acidity was measured according to the AOAC method 942-15 by potentiometric titration with NaOH (0.1 mol·L^{-1}) up to pH 8.1 of a 5 mL (for prickly pear juice) homogenized sample mixed with 25 mL of deionized water. The results were reported in g of citric acid·100 mL^{-1} or in g·100 g^{-1} of the sample [29].

Ash, nitrogen, and fibers were determined on freeze-dried samples. The ash content was determined by igniting 10 g dried samples in a muffle furnace (P330, Nabertherm, Lilienthal, Germany) at 550 °C for 4 h according to the AOAC method 942-05.

Total nitrogen content of all samples was determined by the Dumas combustion method according to the AOAC 990.03 method using a nitrogen analyzer (TruMac, Leco, Joseph, MI, USA). A conversion factor of 6.25 was used to estimate the concentration of proteins.

Fiber contents were obtained by difference for the fruits because of the low content of fiber expected in the fruits, while cladode fiber contents were measured according to the method of Van Soest. [30,31]. A precise quantity of perfectly homogeneous freeze-dried cladode puree (between 0.7 and 1 g) was weighed into macro-porous bags (F57, Ankom Technology, Macedon, NY, USA). Thereafter, all the methods were carried out in a FiberSac Ankom200 equipment (FiberSac Ankom200, Ankom Technology, Macedon, NY, USA). The samples were first subjected to consecutive enzymatic macerations to purify the fibers. First, the bags were immersed in a 1 mL·L^{-1} FAA thermostable alpha-amylase solution for 25 min at 100 °C. After cooling and rinsing twice with ultrapure water, the samples were immersed in a protease solution > 1000 U·L^{-1} for 15 min at 38 °C. The samples were then washed twice with ultrapure water before Neutral Detergent Fiber (NDF) measurement. To do so, 60 g of sodium lauryl sulfate was weighed directly into 800 mL of ultrapure water and mixed gently until completely dissolved. Then, 37 g EDTA, 13.6 g sodium borate, and 9.1 g sodium hydrogen phosphate were added and mixed, and the total volume was increased with 800 mL more ultrapure water. Finally, once the reagents were perfectly dissolved, 20 mL of triethylene glycol was added, and the solution was filled to 2 L with ultrapure water and adjusted to a pH between 6.9 and 7.1. The samples were immersed in this solution for 1 h 15 min at 100 °C, cooled, and rinsed 5 times with ultrapure water. The humid samples were dried at 103 °C at night and weighed before Acid Detergent Fiber (ADF) analysis. To do so, 40 g of cetyltrimethylammonium bromide was dissolved in 1 L of 0.5 mol·L^{-1} sulfuric acid. Once dissolved, the volume was adjusted to 2 L with 0.5 mol·L^{-1} sulfuric acid. The residues of NDF were immersed in this solution for 30 min at ambient temperature to rehydrate the samples. Then, the temperature was increased to 100 °C, and the treatment lasted 1 h 15 min. Thereafter, the samples were cooled and washed 5 times with ultrapure water. The humid samples were dried at 103 °C at night and weighed before Acid Detergent Lignin (ADL) measurement. In this last step, the ADF residues were immersed in 72% sulfuric acid for 3 h. Samples were then rinsed 5 times with ultrapure water (until water was neutral), dried at 103 °C overnight, and weighed.

2.6. Sugar and Ascorbic Acid Analysis

Glucose, fructose, sucrose, and ascorbic acid contents were determined using an HPLC system (1100 HPLC, Agilent, Santa Clara, CA, USA) equipped with RI and UV detectors. A column 300 × 8 mm (SH1011, Shodex, Yokohama, Japan) with a mobile phase composed of H_2SO_4 (0.1%) in water was used at an isocratic flow of 0.7 mL·min^{-1} and at 40 °C. Injection volume was 10 µL, and spectrophotometric detection was set at 210 and 245 nm. The RI detector was set at 50 °C. Opuntia fruit juices were directly injected after filtration using a 0.45 µm syringe filter (Satorius, Göttingen, Germany). Cladode purees were subjected to extraction with ethanol before injection. To do so, 2 g of puree was mixed with 10 mL of 80% ethanol for 20 min at 70 °C. After centrifugation at 10,000× *g* for 10 min and at 20 °C (Avanti-JE centrifuge, Beckman coulter, Brea, CA, USA), the supernatant was collected. The extraction was performed twice, and the supernatants were pooled and filtered through a 0.45 µm syringe filter (Satorius, Göttingen, Germany) before injection. Sugar recovery was ≥98%.

For sugars, the LODs (in g/L) were 0.2 for glucose and 0.3 for both fructose and sucrose. LOQs were 0.6 g/L for glucose and 0.9 g/L for fructose and sucrose. For ascorbic acid, the LOD and LOQ (in mg/L) were 9 and 28, respectively.

2.7. Polyphenols and Betalains

Polyphenols, betalains, and pigments were analyzed following the Tamba et al. (2019) method [32]. Separation was realized using a chromatography (1260 HPLC, Agilent, USA) equipped with a diode array detector. A volume of 20 µL was injected through an Uptisphere C18 250 mm × 4.6 mm × 5 µm column (Uptisphere, Interchim, Montluçon, France). The pump was controlled as follows: Phase A, formic acid 1%; Phase B, acetonitrile. Flow was set at 0.7 mL·min^{-1} and temperature at 30 °C. The gradient was fixed as follows: at the initial time, 98% of A and 2% of B; stabilized at 2% B for 10 min; increased at 20% of B from 10 to 30 min; to 40% B from 30 to 50 min; to 60% B from 50 to 70 min; to 80% B from 70 to 80 min; to 100% B from 80 to 90 min; and returned to the initial condition (2% B) in 5 min and maintained for 10 min (phase A is always complementary to B to reach 100%). The UV-vis detector was set at 280 nm for polyphenols, 480 nm for betaxanthins, and 535 for betacyanins. Calibration curves of piscidic acid and eucomic acid were calculated between 0.1 and 1 g.L^{-1} [32]. Betacyanin and betaxanthin calibration was performed by obtaining concentrations from spectrophotometry (Specord 600, Jena, Germany) at 535 and 480 nm, respectively. Betacyanin (MW = 550 g/mol) and Indicaxanthin contents (MW = 308 g/mol) were calculated according to the Beer-Lambert law using 60,000 L·mol^{-1}·cm^{-1} as the betacyanin molar extinction coefficient in water and 48,000 L·mol^{-1}·cm^{-1} as the indicaxanthin one, after diluting until absorbance was between 0.2 and 1. *Opuntia ficus-indica* fruit juices were directly injected after filtration using a 0.45 µm syringe filter (Satorius, Germany). Cladode purees were subjected to 2 extractions with methanol before injection. To do so, 2 g of puree was mixed with 10 mL of 70% methanol for 15 min. After centrifugation at 10,000× *g* for 10 min and at 20 °C using the Avanti-JE centrifuge (c, USA), the supernatant was collected. The extraction was performed twice, and the supernatants were pooled and filtered using a 0.45 µm syringe filter (Satorius, Göttingen, Germany) before injection. Polyphenol recovery was ≥96%.

Limits of detection (LOD) were as follows (in mg/L): 6 for both piscidic and eucomic acids, and 1.5 and 4 for betanin and betaxanthin, respectively. Limits of quantification (LOQ) were 20 mg/L for piscidic and eucomic acids and 4 and 13 mg/L for betanin and betaxanthin, respectively.

2.8. Mineral Composition

The mineral contents were analyzed on freeze-dried samples by inductively coupled plasma mass spectrometry (Thermo Elemental, X-Series, Baden-Baden, Germany) following the method described by [33]. Around 50 mg of the freeze-dried sample was digested with HNO_3:H_2O_2 (65:35, *v*/*v*) with a MARS Xpress microwave system (CEM Corporation, Mathews, NC, USA). The digestion conditions were as follows: up to 120 °C for 15 min and then constant for 10 min; up to 160 °C in 20 min and constant for 15 min; finally, samples were cooled to 22 °C for 30 min and diluted to 25 mL with deionized ultrapure water. The uncertainty of the measurement is about 10%. The limits of detection were in µg per g: 22.1 for calcium, 8.6 for potassium, 5.4 for sodium, 9.8 for phosphorus, and 0.5 for magnesium. The limits of quantification were in µg per g: 321.0 for calcium, 39.0 for potassium, 14.0 for magnesium, 35.0 for sodium, and 97 for phosphorus.

2.9. Statistical Analysis

Due to sample sizes being less than 50, a non-parametric Kruskal–Wallis test was used to compare group means. When significant differences were detected, post hoc pairwise comparisons were performed using the Bonferroni correction. To compare results between treated and untreated cladodes, a Mann–Whitney U test was used. All analyses were performed with Statistica software version 14.1.0. (Statsoft, Tulsa, OK, USA).

3. Results and Discussion

3.1. Fruits

3.1.1. Macroconstituents of Fruits from the Two Prickly Pear Phenotypes and Effect of Mycorrhizal Biofertilization

The dry matter (DM) content of prickly pear fruits ranged from 8.23 to 9.59% for the Gialla variety and from 9.02 to 10.69% for the Rossa variety (Table 1). These values closely aligned with the total soluble solids (TSS) content, which ranged from 8.64 to 9.80% for the yellow variety and from 9.08 to 10.27% for the red variety. This correlation is further supported by findings in previous studies [34]. This similarity indicates that the majority of the dry matter consists of soluble molecules. This composition was aligned with the observation of the low viscosity of the juice extracted from the fruits. The values obtained in this study fall within the lower range of DM and TSS reported in previous studies. For example, TSS values ranging from 11.8 to 15.6% were observed in 25 fruit accessions from Texas and Argentina [35]. Stintzing et al. (2003) also reported TSS values of 12.3% and 13.6% for the Gialla and Rossa varieties, respectively, indicating higher TSS and DM levels in the Rossa variety, which is consistent with our observations [9]. Our lower DM values could be attributed to variability in terroir or accessions or agricultural practices, as noted in previous studies [8,35]. However, the pulp content found by Felker et al. (2005) was approximately 45%, which aligns with our findings [35].

As expected, the main constituents of the dry matter of fruits were sugars and, more particularly, glucose and fructose in similar amounts. Stintzing et al. (2003) and Melgar et al. (2017) also found that the main sugars in cactus pears were glucose and fructose, with glucose being slightly more predominant [9]. They also found a glucose/fructose ratio of 1.3, identical for both Gialla and Rossa varieties. For the two cultivars, ash, proteins, and fibers each accounted for approximately 6% of the dry matter. The main significant differences between the cultivars, as presented in Table 1, were their acid profiles. The Rossa variety exhibited a lower pH (5.63–5.74) compared to Gialla (6.01–6.37) and a significantly higher titratable acidity, with values of 0.11–0.14%, versus 0.01–0.02% for Gialla. Additionally, sucrose levels were higher in the Rossa variety. The mean sugar-to-acid ratio, as shown in Table 1, was close to 80 for Rossa and exceeded 400 for Gialla. This result was similar to

other studies that have demonstrated higher acidity in Rossa cultivars. This higher sugar-to-acid ratio contributes to explaining the sweet taste of prickly pear fruit and highlights the need to blend them with more acidic fruits before consumption to achieve a sugar-to-acid ratio of around 10, which is often necessary for consumer preferences to achieve a balanced sweet-sour flavor [9]. According to literature, the main acids are citric acids, representing 90% of the organic acids, followed by malic and lactic acids [15,36,37]. Succinic acid was also found as a dominant acid along with citric acid in other studies, showing that the acid profile may be region-dependent [38].

Table 1. Proximal composition (wet basis) of red and yellow fruits from prickly pear plants inoculated with AMF or not (control). Data between brackets represent the standard deviation (for Rossa, n = 18, and for Gialla, n = 9). Values in bold and bearing different letters for the same parameter are significantly different ($p < 0.05$).

Treatment	**With AMF**		**Control**	
Phenotype	**Gialla**	**Rossa**	**Gialla**	**Rossa**
Juice/Fruit ratio	**0.44 (0.03) [a]**	**0.53 (0.08)[a]**	**0.37 (0.03) [b]**	**0.43 (0.11) [a]**
Seeds/Fruit ratio	0.08 (0.01)	0.08 (0.02)	0.08 (0.01)	0.10 (0.02)
pH	**6.01 (0.17) [b]**	**5.63 (0.16) [a]**	**6.37 (0.09) [b]**	**5.74 (0.16) [a]**
Total soluble solids (%)	9.80 (0.75)	10.27 (2.71)	8.64 (0.25)	9.08 (0.91)
Dry matter (%)	9.59 (0.95)	10.69 (1.64)	8.23 (0.09)	9.02 (0.98)
Glucose (%)	4.34 (0.52)	3.29 (1.06)	3.70 (0.08)	3.77 (0.89)
Sucrose (%)	**0.02 (0.01) [b]**	**0.10 (0.02) [a]**	**0.01 (0.01) [b]**	**0.08 (0.01) [a]**
Fructose (%)	4.19 (0.61)	4.76 (1.49)	3.38 (0.10)	3.71 (0.79)
Titratable Acidity (%)	**0.01 (0.01) [b]**	**0.14 (0.02) [a]**	**0.02 (0.01) [b]**	**0.11 (0.02) [a]**
Ash%	**0.81 (0.38) [a]**	**0.80 (0.08) [a]**	**0.44 (0.06) [b]**	**0.64 (0.06) [a]**
Protein (%)	0.70 (0.07)	0.80 (0.08)	0.61 (0.06)	0.57 (0.06)
Estimated Fibers (%)	0.45 (0.29)	1.22 (0.55)	0.33 (0.12)	0.86 (0.50)

The effect of mycorrhization on the macroconstituents of the fruits was minor and was only observed in terms of ash content and the juice-to-fruit ratio for the Gialla phenotype. The ash content of mycorrhized fruits was higher, at 0.8%, compared to 0.44% for Gialla fruits that were not treated. This suggests that mycorrhization enhances the mineral content of the yellow phenotype without causing a concentration effect, as the fruit's juiciness increases. This effect is likely due to improved root system development facilitated by filamentous fungi [39].

3.1.2. Effect of Mycorrhizal Biofertilization on the Micronutrient Contents

Figure 1 and Table 2 illustrate the effect of variety and mycorrhizal biofertilization on the main phyto-micronutrients. These phytonutrients include pigments (betaxanthins for the Gialla variety and betacyanins and betaxanthins for the Rossa variety), as well as ascorbic acid and polyphenols.

The mean of the total pigment content was 45 $mg \cdot L^{-1}$ for Rossa and 75 $mg \cdot L^{-1}$ for Gialla. The yellow phenotype exhibited a higher amount of betalains than the red ones. These values are close to that found previously of a total of 75 $mg \cdot kg^{-1}$ and 95 $mg \cdot kg^{-1}$ of edible pulp for the red and yellow varieties, respectively [36]. The main betaxanthin of prickly pears is indicaxantin. According to Butera (2002) [36], the ratio of betanin to indicaxanthin is around 1:8 (w:w) in the yellow fruit to 2:1 (w:w) in the red one. In our case,

the yellow variety did not contain any betacyanin, while the red cultivar contained around 15 mg·L^{-1} betaxanthin, which makes a betanin-to-indicaxanthin ratio of 2:1, as observed by Butera et al. (2002) [36].

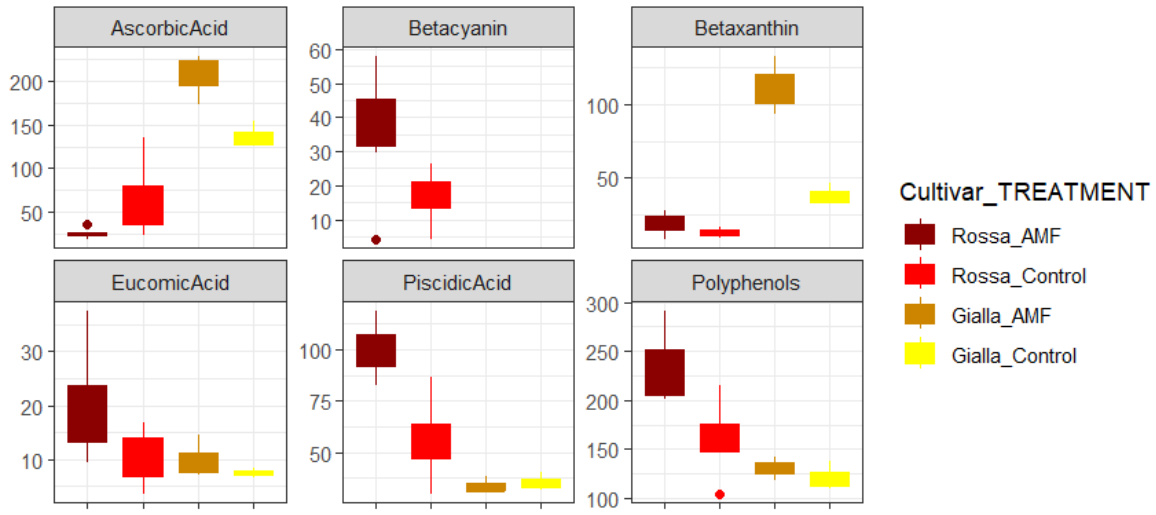

Figure 1. Phyto-micronutrient contents (mg·L^{-1}, wet basis) in red and yellow fruits from prickly pear plants inoculated with AMF or left untreated (Control).

Table 2. Phyto-micronutrient content (wet basis) of red and yellow fruits from prickly pear plants, inoculated with AMF or left untreated (Control). Data between brackets represent the standard deviation (for Rossa, n = 18, and for Gialla, n = 9). Values in bold and bearing different letters for the same parameter are significantly different ($p < 0.05$).

Treatment	**With AMF**		**Control**	
Phenotype	**Gialla**	**Rossa**	**Gialla**	**Rossa**
Ascorbic Acid mg·L^{-1}	**206 (30) ^{a}**	**26 (6) ^{b}**	**136 (15) ^{b}**	**64 (30) ^{b}**
Piscidic Acid mg·L^{-1}	**33 (4) ^{b}**	**99 (13) ^{a}**	**35 (4) ^{b}**	**55 (15) ^{b}**
Eucomic Acid mg·L^{-1}	10 (4)	21 (10)	7 (1)	10 (5)
Polyphenols mg·L^{-1}	**130 (12) ^{b}**	**232 (35) ^{a}**	**121 (14) ^{b}**	**159 (35) ^{b}**
Betacyanin mg·L^{-1}	**ND**	**46 (19) ^{a}**	**ND**	**21 (10) ^{b}**
Betaxanthin mg·L^{-1}	**111 (20) ^{a}**	**18 (7) ^{b}**	**38 (8) ^{b}**	**13 (3) ^{b}**
P (mg·100 mL^{-1})	23 (1)	22 (7)	22 (1)	21 (7)
K (mg·100 mL^{-1})	**237 (9) ^{b}**	**345 (49) ^{a}**	**250 (16) ^{a,b}**	**326 (74) ^{a}**
Ca (mg·100 mL^{-1})	**36 (2) ^{b}**	**84 (21) ^{a}**	**51 (3) ^{a,b}**	**80 (32) ^{a}**
Mg (mg·100 mL^{-1})	24 (4)	28 (8)	37 (2)	27 (7)
Na (mg·100 mL^{-1})	1.00 (0.01)	0.90 (0.01)	1.10 (0.01)	0.80 (0.01)

The effect of mycorrhization on pigment contents was significant. Indeed, for betacyanin in Rossa, the content was higher (46 mg·L^{-1}) in fruits of mycorrhized plants compared to non-mycorrhized ones (21 mg·L^{-1}). For indicaxanthin in Gialla, it was also higher in fruits from mycorrhized plants (111 mg·L^{-1}) compared to those from non-mycorrhized ones (38 mg·L^{-1}). The effect of increased pigment content due to mycorrhization has never been studied for prickly pear fruits. This increase in pigments due to mycorrhization has been proven on other types of pigments, i.e., anthocyanin in strawberries [25]. Therefore, mycorrhization could increase pigment content, whether directly or indirectly, by stimulat-

ing the metabolic pathways of pigment synthesis in the plant [25]. This effect of enhancing pigment accumulation is interesting. Indeed, prickly pears may be of interest for natural food dye applications, as they lack the earthy taste associated with beetroot, the main source of betacyanin used in the food industry to color low-acid foods [9,40].

A higher ascorbic acid content was observed in Gialla fruits. While Stinzing et al. (2003) reported similar levels in red and yellow varieties, Reis et al. (2017) found higher levels of ascorbic acid in white, yellow, and orange *O. ficus-indica* phenotypes [9,41]. AMF treatment increased the ascorbic acid content of the Gialla variety by 50%.

Regarding polyphenols, the red variety exhibited a higher phenolic content, averaging 200 mg·L^{-1} compared to 120 mg·L^{-1} in the Gialla variety. These values are lower than that found in the literature (350 to 650 mg·L^{-1}) [42]. However, these last values were obtained by the Folin–Coicalteu method, which usually gives overestimated polyphenol contents in comparison to that obtained by HPLC [43]. Indeed, the spectrophotometric Folin–Ciocalteu method is not entirely specific to polyphenols; it also reacts with other reducing compounds such as ascorbic acid, sugars, and amino acids. Mycorrhization also had a significant impact on polyphenols, with a positive effect on the Rossa variety ($p < 0.05$). The increase in phenolic compounds due to biofertilization was already reported on tomatoes [44]. Notably, ascorbic acid is the primary antioxidant in yellow varieties, whereas polyphenols predominate in red varieties. Furthermore, it is worth noting that our AMF inoculation enhanced the concentrations of both. This trend may be due to these compounds acting as defense molecules following the establishment of mycorrhizal colonization in the plant. Regarding the polyphenol profile, the main compounds were phenolic acids, particularly piscidic acid, accounting for 50% of the total polyphenols, as can be seen in Figure 2. Mena et al. (2018) [15] conducted an exhaustive analysis of six Spanish cultivars, identifying 16 flavonols and 12 phenolic acids. García-Cayuela et al. (2019) identified 17 phenolic compounds, primarily flavonoid glycosides, with piscidic acid being the most abundant [45]. Similarly, Ramírez-Pérez et al. (2024) reported the presence of eucomic acid [37]. Logically, piscidic and eucomic acids were significantly higher for the mycorrhized red cultivar, with values of 99 and 21 mg·L^{-1}, respectively. This result is interesting, knowing that piscidic acid is an antioxidant and has shown a protective effect against UVA-induced oxidative stress [46].

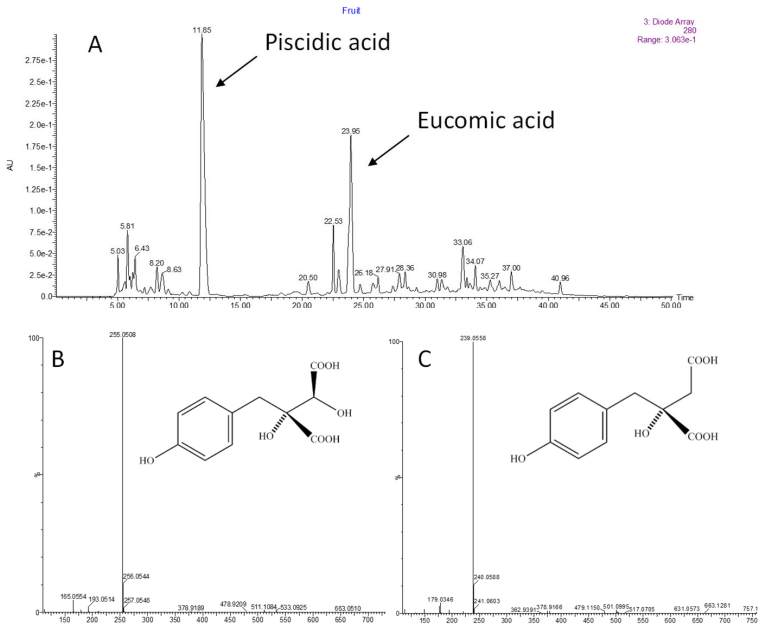

Figure 2. (**A**) Example of chromatogram of polyphenols in Rossa variety. Mass spectrum and structure of (**B**) piscidic and (**C**) eucomic acids.

Regarding minerals, a cultivar effect was observed for those with the highest concentrations in the fruits, namely potassium and calcium, with average contents of 210 and 50 mg·100 mL^{-1}, respectively. Indeed, the red fruits contained around 235 mg·L^{-1} of potassium and 60 mg·L^{-1} of calcium. Therefore, consuming 100 g of red fruits could cover approximately 7% of the daily requirements for these two minerals. Potassium plays a crucial role in various aspects of fruit growth and quality, particularly influencing the concentrations of total sugars, total soluble solids, and acidity [47,48]. This is consistent with the higher acidity due to organic and phenolic acids observed in the Rossa cultivar.

3.2. Cladodes

The composition of cladodes is given in Table 3. The dry matter content of the cladodes was approximately one percentage point higher than that of the fruits. However, the soluble solids were two percentage points lower. The main difference between the two was the content of simple sugars, which was notably low in the cladodes, representing only 5% of the dry matter. In contrast, the fiber content was much higher, accounting for 15–20% of the dry matter (vs. 7% in the fruits). Hemicellulose represented the majority of the fiber, contributing 55% of the total fiber content, followed by cellulose (36%) and lignin (4%). These values are consistent with those reported by Castellano et al. [49]. The protein content was similar to that found in the fruits, representing 8% of the total dry matter. Ash content in the cladodes was significantly higher, representing 14% of the dry matter, which is twice as high as the amount found in the fruits. This value aligns with the findings of Hadj Sadok et al. (2008) [18]. The remaining portion of the dry matter likely consisted of intermediate molecular weight carbohydrate compounds that were not measured here, constituting the mucilage of the cladodes. These compounds were identified by Di Lorenzo et al. (2017) [46] as xyloarabinan and a galactan polymer of different sizes. More precisely, Agostini-Costa et al. (2022) [50] reported galactose, arabinose, rhamnose, and xylose as the main constituents with average MW from 4.8×10^2 to 4.3×10^6 g·mol^{-1} [50]. These last authors also found low molecular weight compounds such as lactic acid and phenolic acids, including piscidic and eucomic acids [51]. The titratable acidity of the cladodes was approximately ten times higher than that of the fruit (1.2%), with a pH of 4 for the cladodes compared to 6 for the fruit. This is likely due to a higher content of organic acids. According to Di Lorenzo et al. (2017) [46], the mucilage associated with these organic and phenolic acids may contribute to the cicatrizing properties of cladode pulp.

Table 3. Composition (wet basis) of cladodes from prickly pear plants inoculated with AMF or left untreated (Control). Values in bold and bearing different letters for the same parameter are significantly different ($p < 0.05$). Data between brackets represent the standard deviation (n = 12).

Treatment	With AMF	Control
pH	4.21 (0.10)	4.34 (0.22)
Total Soluble Solids (%)	7.15 (0.42)	6.75 (0.49)
Dry Matter (%)	**11.35 (1.06) [a]**	**9.41 (0.92) [b]**
Titrable acidity (%)	1.21 (0.43)	1.24 (0.33)
Glucose (%)	**0.23 (0.04) [a]**	**0.12 (0.06) [b]**
Sucrose (%)	0.03 (0.02)	0.01 (0.01)
Fructose (%)	**0.31 (0.06) [a]**	**0.23 (0.04) [b]**
Hemicellulose (%)	1.15 (0.24)	1.07 (0.17)
Cellulose (%)	0.78 (0.06)	0.76 (0.05)

Table 3. *Cont.*

Treatment	With AMF	Control
Lignin (%)	0.07 (0.01)	0.15 (0.11)
Ash (%)	1.53 (0.19)	1.53 (0.23)
Proteins (%)	0.93 (0.06)	0.85 (0.04)
Ascorbic Acid mg·kg^{-1}	**263 (43)** [a]	**213 (25)** [b]
Piscidic Acid mg·kg^{-1}	132 (34)	111 (24)
Eucomic Acid mg·kg^{-1}	67 (30)	73 (12)
Polyphenols mg·kg^{-1}	450 (87)	406 (53)
P mg·100 g^{-1}	**29 (10)** [a]	**24 (1)** [b]
K mg·100 g^{-1}	260 (31)	272 (17)
Ca mg·100 g^{-1}	321 (37)	414 (80)
Mg mg·100 g^{-1}	85 (13)	121 (44)
Na mg·100 g^{-1}	0.80 (0.01)	0.90 (0.01)

Regarding micronutrients, the main minerals were calcium and potassium; however, the concentration of calcium was higher than that of potassium in the cladodes, contrary to the fruit. Additionally, magnesium content was notably higher than in the fruit, with values of 85–120 mg·100 g^{-1}. This trend was also observed by Hadj Sadok et al. (2008) [18], who noted that these values were comparable to those found in tomatoes and spinach.

In general, the antioxidant content in the cladodes was higher than in the fruits, with ascorbic acid being, on average, twice as abundant, and polyphenols three times more concentrated than in the fruits, with mean values of 425 mg·kg^{-1}. Indeed, in addition to a higher content of piscidic and eucomic acid (tr = 12 and 24 min, respectively), the chromatogram of cladodes in Figure 3 also revealed higher molecular weight (MW) compounds at tr = 33 to 34 min (m/z = 723 to 899). Agostini-Costa et al. (2022) [50] reported different isomers of mono-, di-, and triglycosides of flavonols like quercetin and kaempferol in cladodes, which can match with those MW.

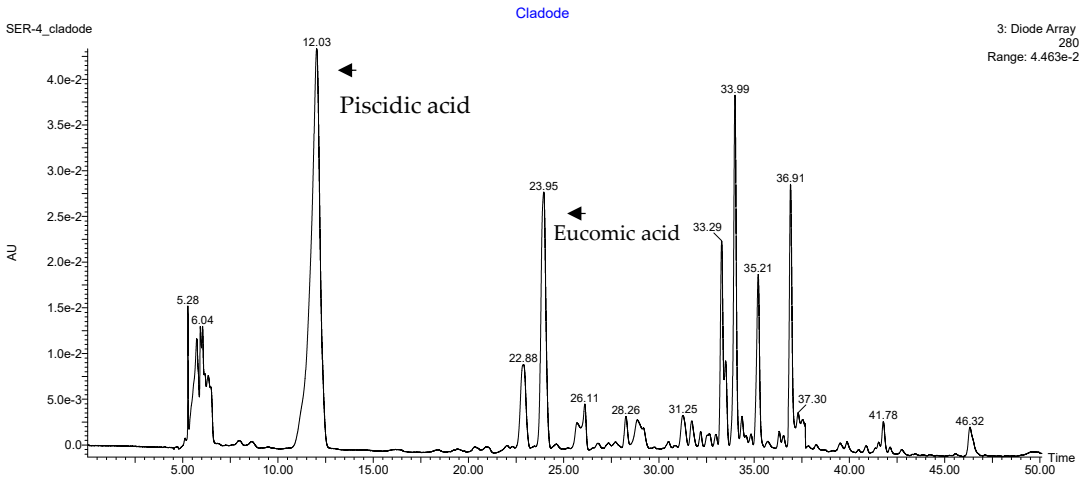

Figure 3. Example of chromatogram of polyphenols in cladodes.

Interestingly, the effect of mycorrhization on the cladodes differed from its effect on the fruits. Mycorrhization impacted the phyto-micronutrients of the fruits (such as pigments and polyphenols), but its effect on the cladodes was noticeable in terms of both macrocon-

stituents and micronutrients. Specifically, the dry matter content was significantly higher in the biofertilized plants, increasing by two percentage points compared to the control group. This increase could be attributed to significantly higher sugar content, whether simple or complex. The glucose and fructose content of cladodes from inoculated plants was higher than that of the control. Some studies have also observed an increase in monosaccharides in roots and leaves under AMF treatment, explaining that glucose and fructose may result from the cleavage of sucrose to supply carbon to arbuscular mycorrhizae [52]. No effect on polyphenols was observed regarding micronutrients. However, a notable effect was an increase in ascorbic acid content. Indeed, cladodes from plants grown with AMF treatment showed a 20% increase. This may be attributed to the well-known ability of AMF to enhance the antioxidant defense system of host plants under abiotic stress. As demonstrated in *Ephedra foliata*, inoculation with AMF led to significantly higher levels of ascorbic acid and increased antioxidant enzyme activities compared to non-inoculated plants [53]. In addition, those cladodes had a higher phosphorus content. This finding is consistent with previous research showing that AMF can enhance phosphorus uptake and metabolism under abiotic stress conditions. This effect is associated with increased phosphatase enzyme activity in the rhizosphere, improving phosphorus mobilization and availability [54,55].

4. Conclusions

In conclusion, arbuscular mycorrhizal fungi significantly influenced the chemical composition of the cladodes and fruits from red and yellow varieties. AMF inoculation modulated carbohydrate metabolism, increased phosphorus uptake, and enhanced ascorbic acid levels in cladodes. In fruits, the most notable effects were observed on phytonutrient composition—particularly pigments, polyphenols, and ascorbic acid—contributing to greater antioxidant capacity.

These findings are promising for fresh consumption as well as for the development of natural colorants and dietary supplements, given the increased pigment and polyphenol content, which may also improve pigment stability. While regulatory and biosafety considerations for AMF use vary by region, the observed benefits suggest biofertilization could enhance the nutritional value of cacti-derived products for both human and animal nutrition. Further research is warranted to explore these applications in more depth.

Author Contributions: Literature search, S.L., A.S., G.B., T.J., G.C., M.I., S.B.M.H. and N.A.; data collection, A.S., G.B., T.J., G.C., M.I. and S.B.M.H.; data interpretation, S.L., A.S., G.B., T.J., G.C., M.I., S.B.M.H. and N.A.; study design, S.L., A.S. and N.A.; writing—original draft preparation, S.L., A.S. and N.A.; visualization, N.A. All authors have read and agreed to the published version of the manuscript.

Funding: This research was supported by the ProsmallAgriMed PRIMA project "Promoting soil fertility, yield and income in smallholder agriculture of semiarid and arid Mediterranean regions by management of beneficial soil microbiota, conservation agriculture and intercropping" funded by the European Union.

Data Availability Statement: The data that support the findings of this study are available from the corresponding authors upon reasonable request.

Conflicts of Interest: The authors declare that they have no conflicts of interest related to this work.

References

1. Tuel, A.; Eltahir, E.A.B. Why Is the Mediterranean a Climate Change Hot Spot? *J. Clim.* **2020**, *33*, 5829–5843. [CrossRef]
2. Khatabi, O.; Hanine, H.; Elothmani, D.; Hasib, A. Extraction and determination of polyphenols and betalain pigments in the Moroccan Prickly pear fruits (*Opuntia ficus indica*). *Arab. J. Chem.* **2016**, *9*, S278–S281. [CrossRef]

3. Louhaichi, M.; Hamdeni, I.; Slim, S.; Sawsan, H.; Harbegue, L.; Gouhis, F. Economic valuation of cactus pear production in semi-arid regions of Tunisia. In *X International Congress on Cactus Pear and Cochineal: Cactus-the New Green Revolution in Drylands 1343*; International Society for Horticultural Science (ISHS): Leuven, Belgium, 2022; pp. 97–102. [CrossRef]
4. Neffar, S.; Beddiar, A.; Menasria, T.; Chenchouni, H. Planting prickly pears as a sustainable alternative and restoration tool for rehabilitating degraded soils in dry steppe rangelands. *Arab. J. Geosci.* **2022**, *15*, 287. [CrossRef]
5. Ávila-Gómez, E.S.; Meléndez-Ramírez, V.; Castellanos, I.; Zuria, I.; Moreno, C.E. Prickly pear crops as bee diversity reservoirs and the role of bees in Opuntia fruit production. *Agric. Ecosyst. Environ.* **2019**, *279*, 80–88. [CrossRef]
6. Inglese, P.; Saenz, C.; Mondragon, C.; Nefzaoui, A.; Louhaichi, M. *Crop Ecology, Cultivation and Uses of Cactus Pear*; FAO: Rome, Italy, 2017.
7. Andreu-Coll, L.; Cano-Lamadrid, M.; Noguera-Artiaga, L.; Lipan, L.; Carbonell-Barrachina, Á.A.; Rocamora-Montiel, B.; Legua, P.; Hernández, F.; López-Lluch, D. Economic estimation of cactus pear production and its feasibility in Spain. *Trends Food Sci. Technol.* **2020**, *103*, 379–385. [CrossRef]
8. Louppis, A.P.; Constantinou, M.S.; Kontominas, M.G.; Blando, F.; Stamatakos, G. Geographical and botanical differentiation of Mediterranean prickly pear using specific chemical markers. *J. Food Compos. Anal.* **2023**, *119*, 105219. [CrossRef]
9. Stintzing, F.C.; Schieber, A.; Carle, R. Evaluation of colour properties and chemical quality parameters of cactus juices. *Eur. Food Res. Technol.* **2003**, *216*, 303–311. [CrossRef]
10. Saenz, C. Processing technologies: An alternative for cactus pear (*Opuntia* spp.) fruits and cladodes. *J. Arid. Environ.* **2000**, *46*, 209–225. [CrossRef]
11. Barbera, G.; Inglese, P.; Mantia, T.L. Seed content and fruit characteristics in cactus pear (*Opuntia ficus-indica* Mill.). *Sci. Hortic.* **1994**, *58*, 161–165. [CrossRef]
12. Felker, P.; Stintzing, F.C.; Müssig, E.; Leitenberger, M.; Carle, R.; Vogt, T.; Bunch, R. Colour inheritance in cactus pear (*Opuntia ficus-indica*) fruits. *Ann. Appl. Biol.* **2008**, *152*, 307–318. [CrossRef]
13. Messina, C.M.; Arena, R.; Morghese, M.; Santulli, A.; Liguori, G.; Inglese, P. Seasonal characterization of nutritional and antioxidant properties of Opuntia ficus-indica [(L.) Mill.] mucilage. *Food Hydrocoll.* **2021**, *111*, 106398. [CrossRef]
14. Patel, S. Reviewing the prospects of Opuntia pears as low cost functional foods. *Rev. Environ. Sci. Bio/Technol.* **2013**, *12*, 223–234. [CrossRef]
15. Mena, P.; Tassotti, M.; Andreu, L.; Nuncio-Jáuregui, N.; Legua, P.; Del Rio, D.; Hernández, F. Phytochemical characterization of different prickly pear (*Opuntia ficus-indica* (L.) Mill.) cultivars and botanical parts: UHPLC-ESI-MS^n metabolomics profiles and their chemometric analysis. *Food Res. Int.* **2018**, *108*, 301–308. [CrossRef] [PubMed]
16. Gallegos-Infante, J.A.; Rocha-Guzman, N.E.; González-Laredo, R.F.; Reynoso-Camacho, R.; Medina-Torres, L.; Cervantes-Cardozo, V. Effect of air flow rate on the polyphenols content and antioxidant capacity of convective dried cactus pear cladodes (*Opuntia ficus indica*). *Int. J. Food Sci. Nutr.* **2009**, *60* (Suppl. S2), 80–87. [CrossRef] [PubMed]
17. De Santiago, E.; Domínguez-Fernández, M.; Cid, C.; De Peña, M.P. Impact of cooking process on nutritional composition and antioxidants of cactus cladodes (*Opuntia ficus-indica*). *Food Chem.* **2018**, *240*, 1055–1062. [CrossRef]
18. Hadj Sadok, T.; Aid, F.; Bellal, M.; Abdul Hussain, M.S. Composition chimique des jeunes cladodes d'Opuntia Ficus Indica et possibilités de valorisation alimentaire. *Agric.—ŞtiinŃă şi Pract.* **2008**, *1*, 65–66.
19. Dubeux, J.C.B.; dos Santos, M.V.F.; da Cunha, M.V.; dos Santos, D.C.; de Almeida Souza, R.T.; de Mello, A.C.L.; de Souza, T.C. Cactus (*Opuntia* and *Nopalea*) nutritive value: A review. *Anim. Feed. Sci. Technol.* **2021**, *275*, 114890. [CrossRef]
20. Khaliq, A.; Perveen, S.; Alamer, K.H.; Zia Ul Haq, M.; Rafique, Z.; Alsudays, I.M.; Althobaiti, A.T.; Saleh, M.A.; Hussain, S.; Attia, H. Arbuscular Mycorrhizal Fungi Symbiosis to Enhance Plant–Soil Interaction. *Sustainability* **2022**, *14*, 7840. [CrossRef]
21. Begum, N.; Qin, C.; Ahanger, M.A.; Raza, S.; Khan, M.I.; Ashraf, M.; Ahmed, N.; Zhang, L. Role of Arbuscular Mycorrhizal Fungi in Plant Growth Regulation: Implications in Abiotic Stress Tolerance. *Front. Plant Sci.* **2019**, *10*, 1068. Available online: https://www.frontiersin.org/journals/plant-science/articles/10.3389/fpls.2019.01068 (accessed on 1 January 2025). [CrossRef]
22. Ouallal, I.; Abbas, Y.; ElYacoubi, H.; Imtara, H.; Al Zain, M.N.; Ouajdi, M.; El Goumi, Y.; Alzamel, N.M. Effects of Arbuscular Mycorrhizal Inoculation by Indigenous Fungal Complexes on the Morpho-Physiological Behavior of Argania spinosa Subjected to Water Deficit Stress. *Horticulturae* **2022**, *8*, 280. [CrossRef]
23. Guo, X.; Wang, P.; Wang, X.; Li, Y.; Ji, B. Specific Plant Mycorrhizal Responses Are Linked to Mycorrhizal Fungal Species Interactions. *Front. Plant Sci.* **2022**, *13*, 930069. Available online: https://www.frontiersin.org/journals/plant-science/articles/10.3389/fpls.2022.930069 (accessed on 1 January 2025). [CrossRef]
24. Bona, E.; Lingua, G.; Manassero, P.; Cantamessa, S.; Marsano, F.; Todeschini, V.; Copetta, A.; D'Agostino, G.; Massa, N.; Avidano, L.; et al. AM fungi and PGP pseudomonads increase flowering, fruit production, and vitamin content in strawberry grown at low nitrogen and phosphorus levels. *Mycorrhiza* **2015**, *25*, 181–193. [CrossRef]
25. Lingua, G.; Bona, E.; Manassero, P.; Marsano, F.; Todeschini, V.; Cantamessa, S.; Copetta, A.; D'Agostino, G.; Gamalero, E.; Berta, G. Arbuscular mycorrhizal fungi and plant growth-promoting pseudomonads increases anthocyanin concentration in strawberry fruits (Fragaria x ananassa var. Selva) in conditions of reduced fertilization. *Int. J. Mol. Sci.* **2013**, *14*, 16207–16225. [CrossRef]

26. Tahiri, A.I.; Meddich, A.; Raklami, A.; Alahmad, A.; Bechtaoui, N.; Anli, M.; Göttfert, M.; Heulin, T.; Achouak, W.; Oufdou, K. Assessing the Potential Role of Compost, PGPR, and AMF in Improving Tomato Plant Growth, Yield, Fruit Quality, and Water Stress Tolerance. *J. Soil Sci. Plant Nutr.* **2022**, *22*, 743–764. [CrossRef]
27. Lahbouki, S.; Fernando, A.L.; Rodrigues, C.; Ben-Laouane, R.; Ait-El-Mokhtar, M.; Outzourhit, A.; Meddich, A. Effects of Humic Substances and Mycorrhizal Fungi on Drought-Stressed Cactus: Focus on Growth, Physiology, and Biochemistry. *Plants* **2023**, *12*, 4156. [CrossRef] [PubMed]
28. Kebede, T.G.; Birhane, E.; Ayimut, K.M.; Egziabher, Y.G. Arbuscular mycorrhizal fungi increased biomass, nutritional value, and cochineal resistance of Opuntia ficus-indica plants. *BMC Plant Biol.* **2024**, *24*, 706. [CrossRef] [PubMed]
29. AOAC. Official Methods of Analysis of the AOAC. In *International*; Association of Official Analytical Chemist International: Washington, DC, USA, 1990; Available online: http://www.aoacofficialmethod.org/ (accessed on 1 January 2025).
30. Van Soest, P.V.; Robertson, J.B.; Lewis, B.A. Methods for dietary fiber, neutral detergent fiber, and nonstarch polysaccharides in relation to animal nutrition. *J. Dairy Sci.* **1991**, *74*, 3583–3597. [CrossRef] [PubMed]
31. Corrales, C.V.; Lebrun, M.; Vaillant, F.; Madec, M.N.; Lortal, S.; Pérez, A.M.; Fliedel, G. Key odor and physicochemical characteristics of raw and roasted jicaro seeds (*Crescentia alata* K.H.B.). *Food Res. Int.* **2017**, *96*, 113–120. [CrossRef]
32. Tamba, A.; Servent, A.; Mertz, C.; Cissé, M.; Dornier, M. Coupling of pressure-driven membrane technologies for concentrating, purifying and fractionizing betacyanins in cactus pear (*Opuntia dillenii* Haw.) juice. *Innov. Food Sci. Emerg. Technol.* **2019**, *52*, 244–255. [CrossRef]
33. Thanh, C.; Mith, H.; Peng, C.; Servent, A.; Poss, C.; Laillou, A.; Phal, S.; Avallone, S. Assessment of the nutritional profiles and potentially toxic elements of wild and farmed freshwater fish in Cambodia. *J. Food Compos. Anal.* **2024**, *133*, 106357. [CrossRef]
34. Reis, G.C.M.; Gazarini, C.L.; Ribeiro, M.M. Fruit production from Opuntia ficus-indica ecotypes in comparison to commercial Italian clones. *Hortic. Sci.* **2018**, *45*, 92–100. [CrossRef]
35. Felker, P.; Rodriguez, S.D.C.; Casoliba, R.M.; Filippini, R.; Medina, D.; Zapata, R. Comparison of Opuntia ficus indica varieties of Mexican and Argentine origin for fruit yield and quality in Argentina. *J. Arid. Environ.* **2005**, *60*, 405–422. [CrossRef]
36. Butera, D.; Tesoriere, L.; Di Gaudio, F.; Bongiorno, A.; Allegra, M.; Pintaudi, A.M.; Kohen, R.; Livrea, M.A. Antioxidant activities of sicilian prickly pear (*Opuntia ficus indica*) fruit extracts and reducing properties of its betalains: Betanin and indicaxanthin. *J. Agric. Food Chem.* **2002**, *50*, 6895–6901. [CrossRef]
37. Ramírez-Pérez, M.; Hidalgo-Martínez, D.; Reyes-López, C.A.; Pinedo-Espinoza, J.M.; Hernández-Fuentes, A.D.; Becerra-Martínez, E. Discrimination of different prickly pear (*Opuntia* spp.) accessions by NMR-based metabolomics. *J. Food Compos. Anal.* **2024**, *130*, 106158. [CrossRef]
38. Melgar, B.; Pereira, E.; Oliveira, M.B.P.; Garcia-Castello, E.M.; Rodriguez-Lopez, A.D.; Sokovic, M.; Barros, L.; Ferreira, I.C. Extensive profiling of three varieties of *Opuntia* spp. fruit for innovative food ingredients. *Food Res. Int.* **2017**, *101*, 259–265. [CrossRef]
39. Wu, Q.S.; Zou, Y.N. *Arbuscular Mycorrhizas and Stress Tolerance of Plants*; Springer: Singapore, 2017.
40. Sadowska-Bartosz, I.; Bartosz, G. Biological Properties and Applications of Betalains. *Molecules* **2021**, *26*, 2520. [CrossRef] [PubMed]
41. Reis, C.M.G.; Gouveia, C.; Vitorino, M.C.; Gazarini, L.C.; Ribeiro, M.M.; Peres, F. Bioactive Compounds and Morphology in Opuntia spp. Fruits from Portuguese Ecotypes. *Bulg. J. Agric. Sci.* **2017**, *23*, 929–938.
42. Dehbi, F.; Hasib, A.; Bouaziz, M.; Ouatmane, A.; Elbatal, H.; Jaouad, A.; Sayadi, S. Effect of phenolic compounds and betalain pigments on the antioxidant capacity of Moroccan prickly pear juices. *Nat. Technol.* **2013**, *5*, 2–7.
43. George, S.; Brat, P.; Alter, P.; Amiot, M.J. Rapid Determination of Polyphenols and Vitamin C in Plant-Derived Products. *J. Agric. Food Chem.* **2005**, *53*, 1370–1373. [CrossRef]
44. Pasković, I.; Soldo, B.; Goreta Ban, S.; Radić, T.; Lukić, M.; Urlić, B.; Mimica, M.; Bubola, K.B.; Colla, G.; Rouphael, Y.; et al. Fruit quality and volatile compound composition of processing tomato as affected by fertilisation practices and arbuscular mycorrhizal fungi application. *Food Chem.* **2021**, *359*, 129961. [CrossRef]
45. García-Cayuela, T.; Gómez-Maqueo, A.; Guajardo-Flores, D.; Welti-Chanes, J.; Cano, M.P. Characterization and quantification of individual betalain and phenolic compounds in Mexican and Spanish prickly pear (*Opuntia ficus-indica* L. Mill) tissues: A comparative study. *J. Food Compos. Anal.* **2019**, *76*, 1–13. [CrossRef]
46. Petruk, G.; Di Lorenzo, F.; Imbimbo, P.; Silipo, A.; Bonina, A.; Rizza, L.; Piccoli, R.; Monti, D.M.; Lanzetta, R. Protective effect of Opuntia ficus-indica, L. cladodes against UVA-induced oxidative stress in normal human keratinocytes. *Bioorganic Med. Chem. Lett.* **2017**, *27*, 5485–5489. [CrossRef]
47. Soliman, S.; Alebidi, A.; Al-Obeed, R.; Al-Saif, A. Effect of potassium fertilizer on fruit quality and mineral composition of fig (*Ficus carica* L. cv. brown turky). *Pak. J. Bot.* **2018**, *50*, 1753–1758.
48. Wang, J.; Lu, Y.; Zhang, X.; Hu, W.; Lin, L.; Deng, Q.; Xia, H.; Liang, D.; Lv, X. Effects of Potassium-Containing Fertilizers on Sugar and Organic Acid Metabolism in Grape Fruits. *Int. J. Mol. Sci.* **2024**, *25*, 2828. [CrossRef]

49. Castellano, J.; Marrero, M.D.; Ortega, Z.; Romero, F.; Benitez, A.N.; Ventura, M.R. *Opuntia* spp. Fibre Characterisation to Obtain Sustainable Materials in the Composites Field. *Polymers* **2021**, *13*, 2085. [CrossRef] [PubMed]
50. Da Silveira Agostini-Costa, T. Genetic and environment effects on bioactive compounds of Opuntia cacti—A review. *J. Food Compos. Anal.* **2022**, *109*, 104514. [CrossRef]
51. Di Lorenzo, F.; Silipo, A.; Molinaro, A.; Parrilli, M.; Schiraldi, C.; D'Agostino, A.; Izzo, E.; Rizza, L.; Bonina, A.; Bonina, F.; et al. The polysaccharide and low molecular weight components of Opuntia ficus indica cladodes: Structure and skin repairing properties. *Carbohydr. Polym.* **2017**, *157*, 128–136. [CrossRef]
52. Wu, Q.S.; Zou, Y.N.; Huang, Y.M.; Li, Y.; He, X.H. Arbuscular mycorrhizal fungi induce sucrose cleavage for carbon supply of arbuscular mycorrhizas in citrus genotypes. *Sci. Hortic.* **2013**, *160*, 320–325. [CrossRef]
53. Al-Arjani, A.B.F.; Hashem, A.; Abd_Allah, E.F. Arbuscular mycorrhizal fungi modulates dynamics tolerance expression to mitigate drought stress in *Ephedra foliata* Boiss. *Saudi J. Biol. Sci.* **2020**, *27*, 380–394. [CrossRef]
54. Han, Y.; Zhang, W.; Xu, T.; Tang, M. Effect of arbuscular mycorrhizal fungi and phosphorus on drought-induced oxidative stress and 14-3-3 proteins gene expression of *Populus cathayana*. *Front. Microbiol.* **2022**, *13*, 934964. Available online: https://www.frontiersin.org/journals/microbiology/articles/10.3389/fmicb.2022.934964 (accessed on 1 January 2025). [CrossRef] [PubMed]
55. Wang, G.; Jin, Z.; George, T.S.; Feng, G.; Zhang, L. Arbuscular mycorrhizal fungi enhance plant phosphorus uptake through stimulating hyphosphere soil microbiome functional profiles for phosphorus turnover. *New Phytol.* **2023**, *238*, 2578–2593. [CrossRef] [PubMed]