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Prickly Pear Seed Oil by Shelf-Grown Cactus Fruits: Waste or Maste?

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Abstract: The chemical composition and properties of seed oils have attracted researchers nowadays. By this meaning, the physicochemical and bioactivity profile of prickly pear seed oil (PPSO) (a product of prickly pear fruits waste) were investigated. Seeds of shelf-grown cactus fruits (Opuntia ficus indica L.) were subjected to analysis. Moisture content (gravimetric analysis), seed content (gravimetric analysis), oil yield (Soxhlet extraction/gravimetric analysis), volatile compounds (HS-SPME/GC-MS), fatty acids profile (GC-FID), in vitro antioxidant activity (DPPH assay), and total phenolic content (Folin-Ciocalteu assay) were determined. Results showed that prickly pear seeds had a moisture content of 6.0 ± 0.1 g/100 g, whereas the oil yield ranged between 5.4 ± 0.5 g/100 g. Furthermore, the PPSO had a rich aroma because of acids, alcohols, aldehydes, esters, hydrocarbons, ketones, and other compounds, with the major volatiles being 2-propenal, acetic acid, pentanal, 1-pentanol, hexanal, 2-hexenal, heptanal, 2-heptenal (Z), octanal, 2-octenal, nonanal, 2,4-decadienal (E,E), and trans-4,5-epoxy-(E)-2-decenal. Among the fatty acids, butyric, palmitic, stearic, and oleic acids were the dominant. Finally, the pure PPSO had a high in vitro antioxidant activity (84 ± 0.010%) and total phenolic content (551 ± 0.300 mg of gallic acid equivalents/L). PPSO may be then used as a beneficial by-product, in different food systems as a flavoring, antioxidant, and nutritional agent.

Keywords: PPSO; volatiles; FAs; antioxidant activity; phenolics

1. Introduction

The exploitation of the food waste and the use of new materials, nowadays, has become more than ever a potential demand for the humanity for the future welfare and existence. The by-products of processed fruits (i.e., peel and seeds) could comprise a new and effective source of oil and food given their nutrients including bio-flavonoids, proteins, minerals, fatty acids, etc., [1]. The fact that millions of pounds of seeds, originating from different fruits, are discarded every year with no strategic and programmed disposal, leads to environmental concern [2]. Opuntia ficus-indica (L.) Mill. or prickly pear, is a tropical or subtropical plant of the Cactaceae family and is commonly used for fruit production. Given that it is a good source of natural antioxidants, hence, it can be used in foods or nutritional supplements [1].

On the other hand, the seed oils of fruits are of great interest because these are edible oils (possessing a high degree of unsaturation) with antioxidant, antimicrobial, and biological activity [1–4]. Therefore, the oil from seeds can be potentially used by the food industry for the production of natural-based foods [5], with extended shelf-life [6,7]. More specifically, the oil from cactus pear seeds has been reported to have considerable amounts of unsaturated fatty acids [1], and antioxidant [8,9] or antimicrobial activity [10], as well as cardioprotective, anti-thrombotic, anti-inflammatory, anti-arrhythmic, hypolipidemic, and anti-hyperglycemic properties [11,12]. These properties are of interest for the pharmaceutical and food sector. However, the yield of prickly pear seed oil may vary among cultivars, crop environmental
factors/geographical origin (i.e., light, temperature, rainfall, and type of soil nutrients), or methods and solvents used for the extraction [2,13]. Based on the aforementioned, the purpose of the present study was to determine the moisture content, seed content, oil yield, volatile compounds, free fatty acids profile, in vitro antioxidant activity, and total phenolic content of prickly pear seed oil, extracted from prickly pear fruits of the wild cultivar, grown in the region of Messinia (Peloponnese). Different procedures were followed including the use of either pure prickly pear seed oil (PPSO) or its methanolic extract for the characterization of seed oil antioxidant activity and total phenolic content.

It is worth mentioning, that this is the first report in the literature on the different physicochemical properties of PPSO obtained from Greek wild prickly pear cultivars. Present findings support the relevant literature, and may contribute to comparative studies dealing with the characterization and beneficial use of PPSO from different regions, concerning primarily the food or pharmaceutical industry.

2. Materials and Methods

2.1. Prickly Pear Fruits and Seeds

Prickly pear fruits from the shelf-grown cultivar (Opuntia ficus indica L.), locally termed as the “wild” cultivar from the region of Messinia (Peloponnese) were used in the study to estimate first the contribution of the seeds to the total fruit mass. Randomly chosen fruits of yellow to green color, were peeled and cut in four pieces. Then, these were left to lose all the water nutrients at room temperature for 24 h. The next day the seeds were removed from the fruit with a niger, and weighted in a Sartorius balance. For the isolation of prickly pear seed oil (PPSO), approximately 5 kg of prickly pear seeds (originating from 1 ton of seeds) were provided by a local agricultural cooperative in the region of Messinia during the harvesting season 2015. The monthly climatological summary for August 2017, during which fruits had the optimum maturity, included the consideration of average temperature (°C), wind speed (km/h), and rainfall (mm). The respective values were 28.1 °C, 4.8 (km/h), and 2.8 (mm). Data were provided by the National Observatory of Athens. The number of the independently obtained PPSOs was n = 3. Afterwards, the PPSOs were combined and subjected to analysis.

2.2. Chemicals and Reagents

The chemicals and reagents used in the study such as gallic acid (3,4,5-trihydrobenzoic acid), methanol (MeOH), acetate buffer (CH3COONa·3H2O), Folin-Ciocalteu phenol reagent, sodium chloride (NaCl), potassium hydroxide (KOH), and sodium carbonate (Na2CO3), were purchased from Merck (Darmstadt, Germany). The stable free radical 2,2-diphenyl-1-picrylhydrazyl (DPPH) was purchased from Sigma-Aldrich (Darmstadt, Germany).

2.3. Determination of Moisture Content of PPSO

About 25 ± 1 g of prickly pear seeds were introduced in a metallic plate of low bas (Brieger, Sclieren, Switzerland) and dried at 105 °C in a Memmert (Memmert GmbH + Co, KG, Schwabach, Germany) drying oven until constant weight. Results were expressed as g/100 g.

2.4. PPSO Isolation Using Soxhlet Extraction

The PPSO was extracted using Soxhlet extraction. In particular, 25 ± 1 g of dried seeds were blended for 20 min (AVEC blender, Jumbo S.A., Athens, Greece) and placed in an extraction thimble of 30 × 100 mm size (Filtres Fioroni, Ingré, France) and introduced in the Soxhlet apparatus. The organic solvent used for the extraction was n-hexane (Merck, Darmstadt, Germany). The extraction was completed in 4–6 h. The n-hexane was evaporated under vacuum rotation (Büchi waterbath B-480, Büchi rotavapor R-114, Flawil, Switzerland). The prickly pear seed oil was then re-suspended in n-hexane, dried at 105 ± 1 °C for 2 h, and collected in vials. The drying process was carried out to ensure that no solvent residues were present. The extraction was carried out in triplicate (n = 3).
2.5. Determination of the Volatile Compounds of Prickly Pear Seed Oil (PPSO)

The PPSO volatile compounds were determined using headspace solid phase micro-extraction coupled to gas chromatography/mass spectrometry (HS-SPME/GC-MS). The divinyl benzene/carboxen/polydimethylsiloxane (DVB/CAR/PDMS) fiber 50/30 µm (Supelco, Bellefonte, PA, USA) was used for the extraction of PPSO volatile compounds. The PPSO samples (ca. 2 g), were placed in screw-cap vials of 15 mL volume, equipped with PTFE/silicone septa. For the headspace extraction, the vials were maintained at 45 °C in a water bath under stirring at 600 rpm. A magnetic stirrer of 10 mm diameter, coated with polytetrafluoroethylene (PTFE) (Semadeni, Ostermundigen–Bern, Switzerland) was placed inside the vials. The analysis conditions were: 30 min equilibration time, 15 min sampling time, 2 g sample mass, and 45 °C water bath temperature. Each PPSO sample was run in duplicate (n = 2).

2.6. GC/MS Instrumentation and Conditions of Analysis

The SHIMADZU GC-2010 Plus series gas chromatograph was used for the analysis of the volatile compounds of PPSO. A DB-5MS (cross linked 5% PH ME siloxane) capillary column (60 m × 250 µm i.d., × 1 µm film thickness) was used, with helium as the carrier gas. The total flow rate was 6.7 mL min⁻¹, whereas that of the column was 3.69 mL min⁻¹ and the purge flow was 3.0 mL min⁻¹. The pressure during the analysis was 320 kPa. The injector temperature was 260 °C, respectively. For the SPME analysis, the oven temperature was held at 40 °C for 5 min, increased to 160 °C at 3 °C/min (0 min hold), and finally increased to 240 °C at 10 °C/min (17 min hold). The total program time was 70 min.

2.7. Identification of the Volatile Compounds of PPSO

The identification of compounds was achieved using the NIST 2011 (NIST-11) mass spectral library by considering the GC-MS spectra.

2.8. Determination of Free Fatty Acids (FAs) of PPSO

The analysis of FAs was carried out using gas chromatography coupled to flame ionization detector (GC-FID). The composition of FAs was determined after transesterification into fatty acid methyl esters (FAMEs). The PPSO was diluted in heptane (0.1 g in 2 mL) with 0.2 mL of 2 mol L⁻¹ potassium hydroxide in methanol, in a test tube with a screw cap and was vigorously shaken, to obtain the methyl esters. The mixture was then centrifuged and the supernatant layer containing the methyl esters was used for the GC analysis. Before the analysis, the mixture was filtered using PTFE membrane filters of 13 mm pore size (Millex-FG, Merck, Darmstadt, Germany). The FAMEs were analyzed on a GC-FID chromatograph (model 6890 N, Agilent Technologies, Wilmington, DE, USA), equipped with a 30 m × 320 µm i.d., Supelcowax column with a film thickness of 0.5 µm (Supelco, Sigma-Aldrich, Darmstadt, Germany). The carrier gas was helium, at a flow rate of 1.5 mL min⁻¹. The temperatures of the injector and detector were set at 250 and 260 °C, respectively. The initial oven temperature was 60 °C held for 5 min and increased from 60 to 250 °C at a rate of 7 °C min⁻¹. Finally, it was held at 250 °C for 2 min. The injection volume was 1 µL. A split ratio of 20:1 was used [14]. Each sample was analyzed in triplicate (n = 3).

2.9. Determination of In Vitro Antioxidant Activity (IVAA) and Total Phenolic Content (TPC) of PPSO

The antioxidant activity of either pure PPSO or its methanolic extracts was estimated in vitro using the DPPH assay [15]. A standard solution of DPPH (43 mg/L) was prepared by dissolving 0.0043 g of DPPH in 100 mL of methanol. For antioxidant activity test 1.8 mL DPPH plus 1 g (or 1 mL) of pure PPSO (or its methanolic extracts), plus 0.20 mL of the acetate buffer (pH = 6.9 ± 0.1) were mixed in a cuvette (final volume of 3 mL) and the absorbance of the reaction mixture was measured at 517 nm (Spectrophotometer Model UV-1280, Shimadzu, Kyoto, Japan). It is worth mentioning, that the extraction of PPSO with methanol was carried out after centrifugation of the sample for 20
min at 4000 rpm (model of centrifuge: Biofuge, primo R, Heraeus) (Kendro Laboratory Products, Osterode, Germany). The absorbance was measured every 60 min (regular time periods) until the value reached a plateau (steady state). The plateau was reached at 3 h. The IVAA was calculated using the following equation:

\[
\%\text{IVAA} = \left(\frac{A_0 - A_t}{A_0}\right) \times 100
\]  

where \(A_0\) is the initial absorbance of the DPPH free radical standard solution and \(A_t\) is the absorbance of the remaining DPPH free radical, after reaction with the PPSO pure samples or its methanolic extracts at the plateau. For the estimation of the effective concentration that causes inhibition of the DPPH radical by 50% (EC\(_{50}\)) a graphical plot of the percent inhibition of the DPPH radical versus different proportions of either the pure PPSO or its methanolic extracts was prepared (\(y = ax + b\)), and the EC\(_{50}\) (g or mg/mL) was determined by the obtained linear equation by inserting the y-value (%DPPH inhibition) = 50. The blank sample (final volume of 3 mL) consisted of methanol plus buffer (2:1 v/v). All samples were filtered using Whatman polyethersulfone (PES) membrane filters (Fisher Scientific, Loughborough, Leicestershire, UK) with a pore size of 0.45 μm before absorbance measurements.

Similarly, the TPC of the pure PPSO or its methanolic extracts was determined using the Folin-Ciocalteu colorimetric method [16]. Briefly, in a 5 mL volumetric flask 0.20 g or 0.20 mL of the pure PPSO (or its methanolic extracts) followed by 2.50 mL of distilled water and 0.25 mL Folin-Ciocalteu reagent were added. After 3 min, 0.50 mL of saturated Na\(_2\)CO\(_3\) (30% w/v) were also added into the mixture. Finally, the obtained solution was brought to 5 mL with distilled water. This solution was left for 2 h in the dark at room temperature and the absorbance was measured at 760 nm, after filtration with Whatman PES membrane filters, in the aforementioned UV/VIS spectrophotometer. A calibration curve using gallic acid (GA) was prepared between 0–780 mg/L:

\[
y = 0.000700x + 0.0390, \quad R^2 = 0.9695,
\] 

The TPC was expressed as mg of gallic acid equivalents (mg GAE/mL). For the IVAA and TPC determinations, each sample was analyzed in triplicate (\(n = 3\)).

2.10. Statistical Analysis

Correlations were obtained using Pearson’s bivariate correlation coefficient (\(r\)), at the confidence level of \(p < 0.05\). The t-test was applied at the confidence level of \(p < 0.05\) for the comparison of average values. Statistical analysis was done using the SPSS (Statistical Package for the Social Sciences) version 20.0 statistics software (IBM Corp., Armonk, NY, USA, 2011). The average (±standard deviation) values of bioactivity parameter analyses were estimated using the Microsoft Office Excel spread sheets for Windows 2007 (Microsoft Corp., Redmond, WA, USA).

3. Results and Discussion

3.1. Contribution of Seeds to the Total Fruit Mass

The peeled fruits had an average weight of 47.3 g (ca. 47 g). The weight of the seeds was 3.82 g (ca. 3.8 g). Therefore, the seeds contributed by: 3.82/47.3 \times 100 = 7.44\%, to the total peeled fruit mass. There is scarce data available in the literature concerning the seed content of prickly pear fruits grown in Greece. Data on seed content of prickly pear fruits may involve the fruit processor in terms of the exploitation of seed extracts for the preparation of nutritional foodstuffs. It has been reported in the literature that seeds of prickly pear fruits are a good source of protein (ca. 11.8%) [16].

3.2. Moisture Content of Prickly Pear Fruits and Seeds

The moisture content of prickly pear fruits was 67.3 ± 2.29 g/100 g. In a previous study dealing with prickly pear fruits from Morocco the reported moisture content values were significantly \((p < 0.05)\) higher, in the range of 89.1 ± 8.09 to 91.2 ± 9.23 g/100 g [17]. Differences in the moisture content of the
aforementioned prickly pear fruits indicate the impact of the geographical origin on fruit composition, and especially, the maturity level of fruit. The higher the moisture content the less is the condensation of sugars, and therefore, different will be the maturity level of fruit. In addition, different moisture content may probably give information about the water absorption capacity of the cultivar and the soil conditions. On the other hand, prickly pear seeds had a significantly \((p < 0.05)\) lower moisture content than the fruit, in the range of \(6.0 \pm 0.1\) g/100 g.

3.3. Volatile Compounds of PPSO

Two hundred and twenty one volatile compounds were identified in PPSO using the GC-MS spectra as shown in Supplementary Table S1. The volatile compounds could be grouped in acids (2.70%), alcohols (9.13%), aldehydes (62.72%), esters (2.82%), hydrocarbons (5.06%), ketones (4.38%), and other compounds (12.71%). It is characteristic that substantial differences \((p < 0.05)\) were recorded among the classes of volatile compounds using t-test analysis (Figure 1). The most dominant volatile compounds were aldehydes. A typical gas chromatogram indicating the major volatile compounds of PPSO is shown in Figure 2. Among aldehydes, 2-propenal, pentanal, hexanal, 2-hexanal, heptanal, 2-heptenal, (Z), octanal, 2-octenal, nonanal, 2,4-decadienal (E,E), and trans-4,5-epoxy-(E)-2-decenal recorded the higher proportions (Table S1). There is limited data available in the literature, regarding the volatile composition of PPSO. Zito et al. [10] reported that the PPSO obtained from the Sanguigna cultivar grown in Sicily, contained mainly hydrocarbons (38.5%), fatty acids, and derivatives (31.9%) and terpenes (12.4%), whereas the PPSO obtained from the Sulfarina cultivar grown in the same region, contained the highest amounts of fatty acids and derivatives (68.9%), followed by terpenes (10.9%). In the present study, the volatile compound 2-propenal (or acrolein) is the simplest unsaturated aldehyde and has a piercing and acrid smell. For example, when cooking oil is heated to its smoke point, a burnt fat odor is caused by glycerol in the burning fat, breaking down then into acrolein [18]. The alkyl aldehydes pentanal, hexanal, heptanal, octanal, and nonanal possess a fruit-like odor with diverse senses where their concentration differs. In particular, it has been reported that pentanal has a fermented, bready, fruity, nutty, and berry smell. Similarly, hexanal contributes to a hay-like “off-note” flavor in green peas [19]. Heptanal has a strong fruity odor and naturally occurs in the essential oils of ylang-ylang (Cananga odorata), clary sage (Salvia sclarea), lemon (Citrus x limon), bitter orange (Citrus x aurantium), rose (Rosa), and hyacinth (Hyacinthus) [20].

The compound (E,E)-2,4-decadienal is a volatile substance found in butter, cooked beef, fish, potato chips, roasted peanut, buckwheat, and wheat bread crumb. The smell intensity is related to its concentration. For example, it smells of deep fat flavor, characteristic of chicken aroma (at 10 ppm), whereas at lower concentration, it has the odor of citrus, orange or grapefruit [21]. Likewise, trans-4,5-epoxy-(E)-2-decenal is an oxygenated \(\alpha,\beta\)-unsaturated aldehyde, and can be formed during the baking of fats that contain linoleic acid. The acids 13-hydroperoxy-9,11-octadecadienoic and 9-hydroperoxy-10,12-octadecadienoic are the intermediates in the biochemical process [22]. Aldehyde is also formed in cooked beef when it remains in the refrigerator for a long storage time, contributing to a fusty odor [23, 24]. It is also an important part of the smell of raw and cooked mutton [22]. Apart from the aldehydes, acetic acid was identified in considerable proportions. Acetic acid is the second simplest carboxylic acid with a pungent, sour, and overripe fruit-like odor. It is the product of ethanol oxidation or fermentation by acetic acid bacteria (Acetobacter and Clostridium acetobutylicum). These bacteria are commonly found in foodstuff, water, and soil, and acetic acid is produced naturally as fruits and other foods spoil. Acetic acid is traditionally considered as a mild antibacterial agent [25]. Finally, 1-pentanol has been reported to possess a fermented-like, yeasty, bready, and fusel odor. Numerous of the volatile compounds of PPSO, such as pentanal, hexanal, heptanal, octanal, nonanal, (E)-2-octenal, decanal, benzaldehyde, acetone, 1-pentanol, 1-hexanol, 2-pentylfuran, tridecane, acetone, 6-methyl-5-hepten-2-one, 3-octen-2-one, and others, have been previously reported in the seeds of Canadian faba bean from different genotypes (Vicia faba L.) [26]. What is also remarkable, is that numerous of the volatile compounds of PPSO were identified previously in prickly pear juice prepared
by the wild cactus fruits [27]. Based on the aforementioned, it is quite obvious that the volatile senses of PPSO are diverse, indicating thus, its potential multi-use in different materials and food matrices.

![Figure 1](image1.png)

**Figure 1.** Proportions of classes of volatile compounds identified in prickly pear seed oil (PPSO). Different letters in each bar indicate statistically significant differences (p < 0.05).

![Figure 2](image2.png)

**Figure 2.** A typical gas-chromatogram of PPSO indicating with numbers the most dominant volatile compounds. 1: 2-propenal, 2: acetic acid, 3: pentanal, 4: 1-pentanol, 5: hexanal, 6: 2-hexenal, 7: heptanal, 8: 2-heptenal (Z), 9: octanal, 10: 2-octenal, 11: nonanal, 12: 2,4-decadienal (E, E), 13: trans-4,5-epoxy-(E)-2-decenal.

### 3.4. Oil Yield and Fatty Acid Profile of PPSO

The oil yield based on the grounding process with the household blender was $5.4 \pm 0.5\%$ (g/100 g). For example, the cold press method resulted in a significantly lower oil yield compared to maceration-percolation method in the study of Regalado-Renteria et al. [4], concerning the isolation...
of PPSO from eight different prickly pear fruit varieties. The cold press method resulted in oil yield ranging from 0.51 ± 0.01 to 6.1 ± 0.6 g/100 g, whereas that of the maceration-percolation method ranged between 6.2 ± 0.3 to 16 ± 0.48 g/100 g. At this point it should be stressed that the oil yield of seeds depends primarily on the prickly pear variety and second on the process of isolation that is followed. In the present study, the use of n-hexane for the extraction of prickly pear seed oil resulted in comparable results, especially with the oil yield obtained through the cold press method as reported in the study of Regalado-Renteria et al. [4]. In addition, no hexane residues were observed during the HS-SPME/GC/MS analysis, indicating the absence of any contamination. In the study of Ramirez-Moreno et al. [2] the oil extraction with hexane was higher for both fruit varieties of Opuntia (green and red cultivar) (11.83% and 6.89%, respectively), compared to ethanol or ethyl acetate. The use of maceration-percolation method, however, cannot be considered as the most effective procedure for the increase in seed oil yield, given the fact that varietal differentiation is the dominant parameter for the isolation of PPSO. Indeed, in the study of Morales et al. [3] the oil yield of Xoconostle seeds in Mexico ranged between 2.45 and 3.52%. In another study concerning the PPSO obtained from Algerian prickly pear cultivars (Opuntia ficus indica) the PPSO yield ranged between 7.3–9.3% [1]. The oil yield in Tynisian and Turkish PPSOs ranged between 9.88–11.75% and 5.00–14.4%, respectively [9,28–30], whereas the oil yield in prickly pear seeds of different South African varieties ranged between 2.24–5.69% [13]. The next step was to characterize the fatty acid profile of PPSO. The most dominant fatty acids were butyric acid (C4:0), palmitic acid (C16:0), stearic acid (C18:0), and oleic acid (C18:1) (Figure 3).

![A typical gas chromatography-flame ionization detector (GC-FID) chromatogram indicating the fatty acids of PPSO.](image)

The respective amounts were 0.48 ± 0.14%, 0.62 ± 0.02%, 0.68 ± 0.06%, and 1.56 ± 0.84%. The higher proportions were found for oleic acid. Therefore, PPSO could comprise an alternative source of oleic acid, among the other fatty acids. Previous results are in accordance with those of Regalado-Renteria et al. [4] in a study concerning the fatty acid content of PPSO obtained from different Mexican prickly pear fruit cultivars. South African PPSO showed higher proportions of palmitic, stearic, and oleic acids [13]. Italian PPSO obtained from the Opuntia ficus indica, Sanguigna and Surfarina varieties grown in Sicily, also showed higher proportions of palmitic, stearic, and oleic acids [31]. The same trend was also reported for Moroccan PPSO obtained from Opuntia ficus indica and Opuntia dilleni prickly pear fruits [32]. However, butyric acid was not reported in the above studies [13,31,32]. Butyric acid in combination with the (E,E)-2,4-decadienal identified in the volatile fraction, enhance further, the butter-like flavor of PPSO.
3.5. IVAA and TPC of PPSO

The DPPH assay has been used to predict the oxidative stability of edible oils [33]. The IVAA of the pure PPSO (initial amount of 0.2286–0.23 g) which was directly inserted in the cuvette containing 1.8 mL of DPPH plus 1 mL of the acetate buffer was 84 ± 0.010%, whereas that of the PPSO methanolic extract (mother solution of 0.5086 g PPSO/50 mL MeOH) was 54.21 ± 0.01% and that of the same amount of PPSO (~0.23 g) in 50 mL of methanol was 49.04 ± 0.02%. For the estimation of EC50 different proportions of either the pure PPSO or its methanolic extract were used to obtain a graph of DPPH inhibition versus amounts of pure PPSO or its methanolic extracts. In particular, for the pure PPSO the amounts used were: 0.2286 g, 0.1185 g, 0.056 g, 0.0331 g, and 0.0120 g. The obtained linear curve is shown in Figure 4a. The obtained EC50 value was estimated from the graph and was 0.096–0.1 g. In the case of PPSO methanolic extract, the amounts used were: 10.17 mg/mL, 5.09 mg/mL, 2.54 mg/mL, 1.02 mg/mL, and 0.51 mg/mL. Similarly, the EC50 value was 7.44 mg/mL (Figure 4b). As it can be observed from Figure 4a,b differences in the linearity (and intercept/slope) of the curves were obtained, depending on the use of either pure PPSO or its methanolic extract. In that sense, two major issues must be addressed: (i) the DPPH inhibition is dependent of the amount of the antioxidant used and (ii) the extraction of PPSO with methanol reduced substantially its IVAA compared to the direct use of pure PPSO. In a previous study dealing with the in vitro antioxidant capacity of Algerian prickly pear seeds [34], the authors reported that the best results of antioxidant capacity were obtained when the seeds were extracted with 75% acetone (among ethanol, methanol, and water 50%, v/v) using 0.2 g/10 mL of the extract. The obtained in vitro antioxidant capacity was 95%, in general agreement, with present results concerning the direct use of PPSO for the estimation of antioxidant activity. Prickly pear seed oil of Sicilian cultivars (Sanguigna and Surfarina) [29] obtained with Soxhlet extraction, showed the highest inhibitory concentration (IC50) against the DPPH free radical, in agreement with present results. In addition, present results are in accordance with those of Ramirez-Moreno et al. [2], who reported a considerable antioxidant activity concerning the seed oil obtained from two Mexican prickly pear cultivars.

The same trend was also observed in the TPC of pure PPSO and those of its methanolic extracts. More specifically, the TPC of pure PPSO was 551 ± 0.300, whereas that of the methanolic extracts was significantly lower, ranging between 93.3 ± 0.140 mg/L. There was a perfect Pearson’s correlation (r = 1.000, p = 0.01) between the IVAA and TPC content of pure and methanolic extracts of PPSO, indicating again the impact of solvent extraction. Even though solvent extraction is usually used for the isolation of antioxidants, the efficiency of the extraction depends on the selected solvent for the complete isolation/extraction of different antioxidant compounds with varying polarity [35]. The use of pure PPSO resulted in a much higher TPC compared to that of the methanolic extract. This is owed to the nature of the Folin-Ciocalteu assay given that it measures all the reducing agents (phytochemicals, fatty acids, minerals, etc.) being present in the pure PPSO, and not only the extracted antioxidants.
4. Conclusions

Results of the present study showed that prickly pear seed oil (PPSO) is a matrix of a rich aroma, considerable proportions of fatty acids, and high *in vitro* antioxidant activity in relation to the total phenolic content. What is worth mentioning, is that the PPSO obtained from the wild cultivars grown in Messinia (Peloponnese), showed the highest proportions of oleic acid, and considerable amounts of butyric acid, the latter reported for the first time in the relevant literature [13,31–33]. In addition, the rich fraction of over 200 volatile compounds of different class, gives a regional identity to the product. Apart from the cultivar impact [10], it should also be noted, that the chemical composition of PPSO may be affected by the harvesting season concerning the general weather conditions in a specific region [31]. Considering that the seeds of prickly pear are ignored at a global level during fruit consumption, and the PPSO is primarily used in cosmetics, the present study aims to reconsider the former use by proposing its application in food systems, either as flavoring and nutritious matrix, or as antimicrobial and antioxidant agent, in accordance with the cited literature. Therefore, the question...
in the title of this study has an answer: PPSO is a “maste.” Future work with direct applications in different food systems will approve further the present findings.

**Supplementary Materials:** The following are available online at http://www.mdpi.com/2227-9717/8/2/132/s1. Table S1: Volatile compounds of PPSO tentatively identified using HS-SPME/GC-MS and NIST MS 11 mass spectral library.

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