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Effect of Drying Methods on the Nutraceutical Potential of Cactus Cladodes (*Opuntia* spp.)

Gerardo Martínez-Soto[1], Fabiola Celis-Fabián[2,3], Talía Hernández-Pérez[2], Octavio Paredes-López[2*]

[1]Division of Life Sciences, University of Guanajuato, México
[2]Research Center for Advanced Studies, National Polytechnic Institute, México
[3]Graduate Program in Food of the Center of the Republic, Autonomous University of Queretaro, México

***Corresponding author:** Octavio Paredes-López, Research Center for Advanced Studies, National Polytechnic Institute, Km 9.6 Libramiento Norte Carretera Irapuato- León, Irapuato, CP 36821, México, Tel:+52 462 6239674; E-mail: oparedes@ira.cinvestav.mx

Abstract

Different drying methods were used to obtain flour from two Mexican cactus cladodes (nopal) (*Opuntia* spp.), one wild and one commercially cultivated (Verde Valtierrilla), in terms of nutraceutical compounds. Total dietary fiber, phenolic compounds and flavonoids, antioxidant capacity, *in vitro* fermentability and production of short-chain fatty acids were analyzed by fluid bed, tunnel, spray and freeze drying methods.

Our results indicate that nopal flour obtained under hot air and freeze drying is an excellent source of dietary fiber; wild material showed the highest values (54.2% dry weight). Phenolic compounds and flavonoids were also higher ($p < 0.05$) in the wild than in the commercial cladodes powder, as well as the production of short-chain fatty acids. Acetic, propionic and butyric acids were found in high amounts in both flours, being acetic the most abundant fatty acid. Antioxidant capacity was not significantly affected by the drying temperatures of the evaluated methods. Flour from wild nopal exhibited higher levels of health promoting substances than Verde Valtierrilla. In brief, this study demonstrates that flour from *Opuntia* cladodes could be considered an excellent food with nutraceutical potential for human nutrition and outstanding potential features for the industries of health, food and pharmaceuticals, due to its functional components.

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Keywords: Cladodes; Dehydration; Antioxidant capacity; Dietary fiber; Fatty acids

Introduction

Opuntia ficus- indica is a cactus endemic to America and is well-adapted to arid lands[1]. The genus Opuntia embraces about 1,500 species of cactus and many of them produce edible tender stems and fruits[2]. The use of cactus cladodes has been recorded in México since pre-Hispanic times given their important role in the agricultural economy of the Aztec Empire and are thus considered as one of the oldest cultivated plants in México. Its production and consumption is not limited to Mexico; this crop is gaining popularity in various other countries because of its effects on human health and the palatability of its fruits[3,4].

Nopal cladodes are the tender young part of the cactus stem and are frequently consumed as a vegetable, while the cactus pear fruit is consumed as a fresh fruit. This cactus is a common item of consumption in México (10–17 g/person/day)[5]. Cacti to produce nopal can grow in poor and infertile soil of semiarid zones, contributing to the food security of populations in agricultural poor areas. Its production and consumption is not restricted to mexican territory; currently, this crop is popular in few other countries due to its attractive taste, nutritional quality, and effects on human health[6]. Nopal tastes tart and is slimy in consistency, it is consumed in myriad forms *e.g.*, raw, grilled or cooked; even top restaurants offer nopal-based delicacies[7].

Some of the major benefits of nopal attributed to its dietary fiber content[3], are mainly the control of diabetes, treatment of high blood pressure and gastrointestinal disorders, as well as its antihyperlipidemic and antihypercholesterolemic effects[8]. Dietary fiber escapes enzymatic digestion and becomes a substrate for fermentation by bacterial flora in colon, producing gas, water, and short-chain fatty acids (SCFAs)[9]. These fatty acids positively affect serum cholesterol, human colonic crypts cells and are fuels for colonocytes. Thus, nopal dietary fiber can act as prebiotics, which are non-digestible food ingredients, promoting growth and activity of probiotics[10,11].

Studies on phytochemicals of cladodes reveal that they contain phenolic acids, flavonoids, carotenoids, and vitamins; they reduce the risk of cancer, cardiovascular and chronic degenerative diseases[12]. The functional properties of nopal include protection against H_2O_2-induced damage, immunoestimulatory and free radical-scavenging effect, antiinflammatory, antitumor, blood lipid-lowering, and wound-healing activity[13].

Nopal is prone to rapid microbiology decay due to its high water content and low acidity, thus limiting its fresh marketing potential. Therefore, drying and grinding provide important advantages for storage and transport of this crop[8], but not much information is available about variations on functional ingredients when producing *Opuntia* flour. The objective of this study was to assess, for the first time up to our knowledge, the effect of different drying methods on the nutraceutical quality of flour from wild and cultivated nopal.

Materials and Methods

Plant Material

Two *Opuntia* morphospecies were used for this study: Verde Valtierrilla, a cultivated crop, and a wild material with commercial potential. Samples were harvested in the morning from an orchard and an open area in Salamanca, Gto, México, according to size for commercialization (15 days of maturity). Nopal cladodes were of 15 cm length and 42 g fresh weight. Commercial nopal flour (7% moisture), available in the market, provided by a Cooperative of Nopal Growers (PRONOPVAL S.C.L., Salamanca, Gto, México) was used as control.

Drying Methods

Pretreatment: Fresh nopal cladodes were immersed in distilled water (1:2, w/v) at 70°C for 3 min and immediately cooled. This method was made to avoid enzymatic browning and killing pathogenic microorganisms.

Hot Air Drying

Fluid Bed Drying: Nopal pads were cut into 1 x 1 cm pieces and frozen with liquid nitrogen and stored at -80°C. 200 g of sample was dried in a fluid bed equipment with constant flow rate of hot air (70°C) at 13.58 m/s for 70 min. Dehydrated nopal was ground in a RETSCH mill (RETSCH Inc., USA) using a mesh of 20 mm and it was sieved with a mesh of 0.25 mm to obtain a fine powder and stored at -20°C for further analysis.

Tunnel Drying: Nopal was cut and frozen as above mentioned. Samples (200 g) were dehydrated in an experimental drying tunnel with a centrifugal fan comprising an air flow rate of 1.5 m/s and a dryer temperature of 80°C, during 180 min[14]. They were weighed every 10 min during the procedure until reaching equilibrium moisture. For producing the flour, dehydrated nopal was ground in a RETSCH mill (RETSCH Inc., USA) using a mesh of 20 mm and sieved through a 0.25 mm mesh, it was stored at -20°C.

Spray Drying: Nopal (200 g) was blended with 400 mL distilled water (1:2, w/v) and cloth filtered. The filtrate was collected in a flask and 500 mL were dehydrated in an Apex atomizer laboratory spray drying system (model SSE68, London) with an air compressor at 4 kg/m^2, centrifugal disc at 35,000 rpm, extract feed flow rate of 250 mL h^{-1}, inlet air drying temperature of 100°C, and atomizing air flow rate of 125 mL h^{-1}. Samples were passed through a sieve with a mesh opening of 0.595 mm to determine the particle size and stored at -20°C.

Freeze Drying Nopal pads were cut into 1 x 1 cm pieces and frozen with liquid nitrogen and stored at -80°C. Frozen nopal (200 g) was freeze-dried using a Labconco Freezone 4.5 freeze dry system (Labconco, Kansas City, MO, USA) with condenser temperature of -55°C and vacuum pressure of 7 Pa, and stored at -20°C.

Analysis of Sample

Water Activity (a_w): a_w was determined at 22°C, using an AquaLab water activity meter (Decagon), in accordance with the methodology described by AOAC[15].

Total Dietary Fiber Analysis: Total dietary fiber was determined using a commercial kit (Sigma-Aldrich, St. Louis, MO, USA). Nopal flour was gelatinized with α-amylase and enzymatically digested with protease and amyloglucosidase to remove the protein and starch present. The percentage (%) of total dietary fiber was estimated as indicated by Hernández-Pérez et al.[16].

NutraceuticalCompounds

Total Phenolics Assay: Nopal flour (1 g) was extracted with 20 mL of 80% methanol under 200 rpm shaking at 20°C for 24 h in dark conditions. It was centrifuged at 13,000 rpm for 10 min and the residue was mixed with 20 mL of 80% methanol (methanolic extract, ME) and stored at -20°C until use. In brief, 50 µL of nopal ME, 200 µL of water and 250 µL of Folin-Ciocalteu reagent (50%, v/v) were stirred. 500 µL of Na_2CO_3 (7.5%, w/v) were added to the mixture and allowed to stand at 45°C for 15 min. Absorbance was read at 760 nm with a Beckman DU 640 spectrophotometer (Beckman Instruments, Fullerton, CA, USA). Total phenolics were estimated using a standard curve with gallic acid and results were expressed as mg of gallic acid equivalents per gram of dry weight (GAE/g dw)[17].

Total Flavonoids Assay: Nopal flour (1 g) was added to 20 mL of 80% ethanol, sonicated for 30 min at 60°C and centrifuged at 13,000 rpm for 10 min. The supernatant was collected, allowed to stand for 40 min and absorbance was read at 415 nm in a Beckman DU 640 spectrophotometer (Beckman Instruments, Fullerton, CA, USA). A calibration curve was made of quercetin (Sigma-Aldrich) diluted with 80% ethanol (stock solution, SS). The SS was mixed with 1 M potassium acetate and 10% aluminum nitrate was added at different concentrations, 10 mL of 80%

ethanol were also added. The mixture was allowed to stand for 40 min in dark conditions and absorbance was read at 415 nm. Total flavonoids were expressed as mg of quercetin equivalents (QE) per gram of dry weight[18].

Antioxidant Capacity: This measure was carried out by two methods:

2,2-dipheny l-2-picrylhydrazyl (DPPH) Assay: According to Fukumoto and Mazza[19], a standard curve was prepared from a solution of 800 mM Trolox (Sigma-Aldrich), and at least five different concentrations were used (100 - 700 mM). Gallic acid and butylated hydroxytoluene (Sigma-Aldrich) were used as positive controls and 80% methanol as negative control. 20 µL of ME and 200 µL of DPPH solution were added to a 96-well plate and the absorbance read (515 nm) at 30, 60, 75, 90, and 120 min.

2,2'-azino-bis (3-ethylbenzothiazoline-6-sulphonic acid) (ABTS) Assay: Nine concentrations (0.5 - 0.8 mM) from a standard solution of 1 mM Trolox (Sigma-Aldrich) were used. A mixture of 230 µL $ABTS^{+\bullet}$ and 20 µL methanol was used as control and another of 230 µL ethanol with 20 µL methanol was the blank. The $ABTS^{+\bullet}$ solution was diluted with absolute ethanol to reach absorbance of 0.7 ± 0.02 at 734 nm. 250 µL of $ABTS^{+\bullet}$ solution was added to 20 µL of each methanolic extract and absorbance was recorded 20 s after the initial mixture. The antioxidant capacity was expressed as µmol Trolox equivalents per gram (Trolox Eq/g dw)[20].

***In vitro* Fermentability and SCFAs Production**. The fermentation of flour from Verde Valtierrilla and wild nopal was carried out as described by Olano-Martin et al.[21] and Ferguson and Jones[22]. Briefly, the nopal substrate was placed in culture nutritive medium with human fecal inoculum under anaerobic conditions in a water bath at 37°C for 24 h, using raffinose (Sigma-Aldrich) as control. pH values of the products sample were registered at 6, 12 and 24 h of incubation and were frozen and stored at -70°C. The production of SCFAs after the fermentation of nopal flour was quantified by gas chromatography using a HP-INNOWAX 30 m x 0.25 mm x 0.25 µm column, with 108 mL/min flow rate, 120- 250°C oven temperature, it was increased every 5 min to reach 250°C. Pure acetic, propionic and butyric acids (Sigma-Aldrich) were used as standards.

Statistical Analysis: All measurements and chemical analyses were performed in triplicate. Data are presented as the mean ± standard deviation. Analyses of variance (ANOVA) and Turkey's comparisons were carried out for data analysis using the statistical software Statgraphics XVI. Significant differences were established for $p = 0.05$.

Results and Discussion

The flour from nopal evaluated in the four methods presented a_w values lower than those that could promote the development of bacteria, fungi and yeast, being spray drying the method with the lowest a_w. The initial moisture of the samples was 95% and the final moisture content of both flours were: 6.5% for tunnel, 5.2% for fluid bed, 5% for spray and freeze drying. These data suggest that the flours obtained are stable and there were no statistical differences ($p < 0.05$) between nopal variety.

Total Dietary Fiber Content: Nopal flour from Verde Valtierrilla and the wild material showed values of total dietary fiber in the range of 45.6 - 54.2% (Figure 1) through the heat drying methods. Tunnel drying yielded the highest amounts ($p < 0.05$) of dietary fiber in the flour from both samples, along the four dehydration methods. Fluid bed and spray drying had similar amounts of total dietary fiber in the nopal powder.

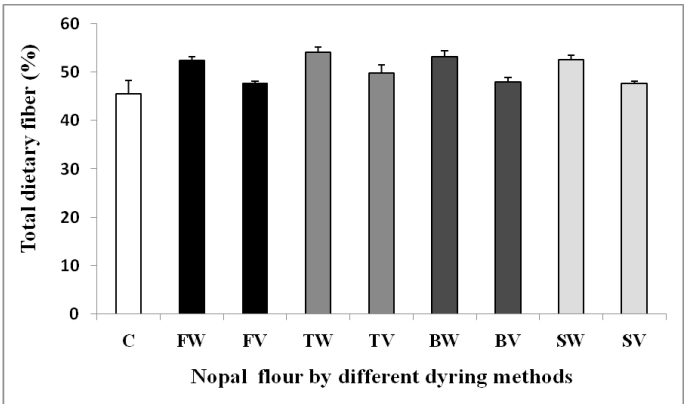

Figure 1: Total dietary fiber content (% dry weight) of nopal flour from two cactus cladodes dehydrated by freeze drying, tunnel, fluid bed and spray drying methods. Commercial product (C), freeze dried wild (FW), freeze dried Verde Vatierrilla (FV), tunnel dried wild (TW), tunnel dried Verde Valtierrilla (TV), fluid bed dried wild (BW), fluid bed dried Verde Valtierrilla (BV), spray dried wild (SW), spray dried Verde Valtierrilla (SV).

Our results showed that heat treatment may trigger a slight rise in total dietary fiber, which is in agreement with data from Santos-Zea et al[1]. On the other hand, the levels of total dietary fiber in Verde Valtierrilla and wild flour, generated by the four dehydration methods, were superior to those described by Guevara-Figueroa et al.[23] in *Opuntia* powder and also to those from Ayadi et al.[24] in *O. ficus inermis*. The amount of total dietary fiber in Verde Valtierrilla and wild nopal flours (33.7-38.3 g/100 g dry weight) appears to be higher than that reported by Nuñez-López et al.[25] in flour from cladodes at three maturity stages. Differences in fiber content could be attributed to *Opuntia* variety, climate, growing conditions, as well as precipitation and irrigation.

Nutraceutical Compounds in Nopal Flour

Total Phenolics Content: Nopal flour produced by heat drying methods showed a significant decrease in the concentration of total phenolics relative to freeze dried flour (Figure 2). Total phenolics in flour from the wild material were identified in higher concentrations (2.1 - 2.3 mg GAE/g dw) than in Verde Valtierrilla, in all the dehydration procedures. Flour from Verde Valtierrilla processed by spray drying showed the highest amounts of these bioactive compounds.

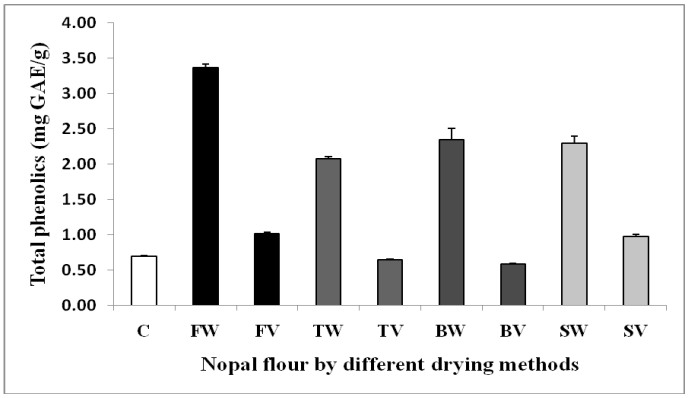

Figure 2: Total content of phenolic compounds (mg GAE/g dry weight) in nopal flour from two cactus cladodes obtained by freeze drying, tunnel, fluid bed, spray drying methods. Commercial product (C), freeze dried wild (FW), freeze dried Verde Vatierrilla (FV), tunnel dried wild (TW), tunnel dried Verde Valtierrilla (TV), fluid bed dried wild (BW), fluid bed dried Verde Valtierrilla (BV), spray dried wild (SW), spray dried Verde Valtierrilla (SV). GAE, gallic acid equivalents;

Values of total phenolics in flour from wild and commercial cladodes presented some differences that may be attributed to degrading effects of high temperature, time of exposure, type of heat, and oxidative process, as described by Jaramillo-Flores et al.[26]. The observed values of these compounds are above those for red onion, spinach, and beet[27]. It can be noticed that the heat drying methods we used to obtain nopal flour caused a decrease in the concentration of phenolic compounds. Furthermore, inappropriate air temperature during cladode drying results in the loss of total phenolic acids[28], thus, attention must be taken on the drying procedure.

Total Flavonoids Content: As indicated in Figure 3, the concentration of these compounds ranged from 0.19 to 0.26 mg QE/g dry weight in both Verde Valtierrilla and wild cladodes, but there were no statistical differences with respect to drying method. Moreover, freeze dried nopal samples exhibited a slightly superior content of total flavonoids. In this study, it has been demonstrated that the drying methods for producing nopal flour may affect total flavonoid content. This is in agreement with Guevara-Figueroa et al.[23], who found lower values of flavonoids in nopal powders than in fresh nopal. In addition, it has been reported that temperature tends to increase the degradation of flavonoids[29]. The concentration of phenolic compounds and flavonoids in nopal flour did not show significant difference in the four evaluated dehydration methods.

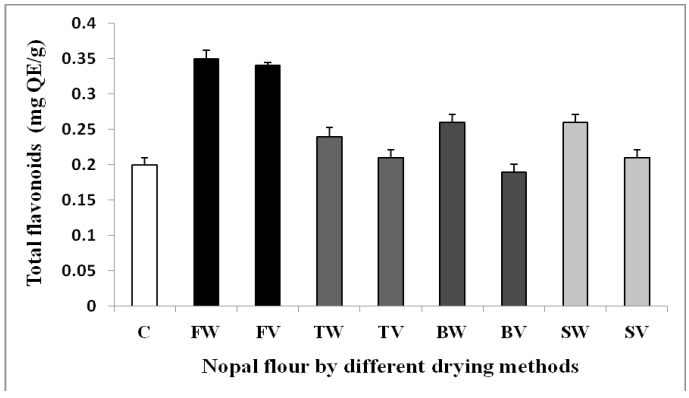

Figure 3: Total flavonoids content (mg QE/g dry weight) in nopal flour from wild and Verde Valtierrilla morphoespecies processed by freeze drying, tunnel, fluid bed and spray drying methods. Commercial product (C), freeze dried wild (FW), freeze dried Verde Vatierrilla (FV), tunnel dried wild (TW), tunnel dried Verde Valtierrilla (TV), fluid bed dried wild (BW), fluid bed dried Verde Valtierrilla (BV), spray dried wild (SW), spray dried Verde Valtierrilla (SV). QE, quercetin equivalents;

Antioxidant Capacity: The potential antioxidant capacity of flour from wild and cultivated nopal was evaluated by $ABTS^{+}$ and DPPH assays. Table 1 shows that the antioxidant capacity in heat dried nopal flour presented slightly higher values using the $ABTS^{+}$ assay than the DPPH method, but there were no significant differences regarding nopal morphoespecie and drying process ($p > 0.05$). The antioxidant capacity of freeze dried samples was always superior to that from heat drying procedures in both samples.

Table 1: Antioxidant capacity of flour from cultivated and wild nopal by DPPH and ABTS assays.

Method	Sample	DPPH	ABTS
		μM Trolox Eq/g dw	
Freeze drying	Wild	5.48 ± 0.0001^{Ab}	6.11 ± 0.049^{Aa}
	Verde Valtierrilla	5.21 ± 0.193^{Ab}	5.43 ± 0.138^{Aa}
Tunnel drying	Wild	4.52 ± 0.0001^{Aa}	4.87 ± 0.028^{Aa}
	Verde Valtierrilla	4.29 ± 0.131^{Aa}	4.81 ± 0.085^{Aa}
Fluid bed drying	Wild	4.79 ± 0.484^{Aa}	5.89 ± 0.006^{Aa}
	Verde Vatltierrilla	4.52 ± 0.290^{Aa}	4.99 ± 0.135^{Aa}
Spray drying	Wild	4.98 ± 0.352^{Acb}	5.43 ± 0.076^{Aa}
	Verde Valtierrilla	4.71 ± 0.158^{Acb}	4.98 ± 0.035^{Aa}

Upper case letters mean statistical difference respect to variety and different low case denotes statistical difference for drying process ($p > 0.05$). DPPH, 2,2-diphenyl-2-picrylhydrazyl; ABTS, 2,2'-azino-bis(3-ethylbenzothiazoline-6-sulphonic cid; dw, dry weight.

Our data for antioxidant capacity in nopal flour is in agreement with Serpen et al.[30]. They stated that ABTS assay could be more sensitive for food rich in phenolic compounds, while DPPH radical scavenging assay is more sensitive for Maillard reaction products. Thus, health beneficial effects of cactus polyphenols might be conditioned by their antioxidant and radical scavenging activities.

Table 2: Production of short-chain fatty acids from cultivated and wild nopal flour processed by tunnel, fluid bed and spray drying methods

Method	Sample	Short chain fatty acids (mM)			
		Time (h)	Acetic	Propionic	Butyric
Tunnel drying	Wild	6	14.81 ± 0.11^{Ab}	5.19 ± 0.06^{Aa}	0.53 ± 0.02^{Aba}
		12	32.74 ± 0.10^{Bb}	2.41 ± 0.02^{Ba}	6.87 ± 0.15^{Bba}
		24	29.42 ± 0.45^{Bb}	3.14 ± 0.02^{Ba}	6.50 ± 0.22^{Bba}
	Verde Valtierrilla	6	4.77 ± 0.29^{Aa}	2.52 ± 0.06^{Aba}	0.22 ± 0.01^{Aba}
		12	9.48 ± 0.45^{Ba}	7.35 ± 0.01^{Bba}	1.44 ± 0.05^{Bba}
		24	24.84 ± 0.67^{Ba}	12.39 ± 0.08^{Bba}	6.82 ± 0.16^{Bba}
Fluid bed drying	Wild	6	13.19 ± 0.06^{Ab}	3.10 ± 0.02^{Ab}	3.28 ± 0.11^{Ab}
		12	30.31 ± 0.02^{Bd}	17.45 ± 0.19^{Bb}	7.41 ± 0.19^{Bb}
		24	36.44 ± 0.38^{Bd}	17.63 ± 0.24^{Bb}	9.54 ± 0.44^{Bb}
	Verde Valtierrilla	6	5.50 ± 0.24^{Aa}	2.31 ± 0.38^{Aba}	2.38 ± 0.05^{Aba}
		12	23.84 ± 0.21^{Ba}	12.53 ± 0.07^{Bba}	11.25 ± 0.06^{Bba}
		24	11.57 ± 0.17^{Ba}	5.21 ± 0.04^{Bba}	4.41 ± 0.13^{Bba}
Spray drying	Wild	6	9.53 ± 0.07^{Aa}	1.12 ± 0.10^{Aa}	1.68 ± 0.06^{Aa}
		12	9.42 ± 0.11^{Ba}	2.42 ± 0.05^{Ba}	2.55 ± 0.02^{Ba}
		24	17.59 ± 0.20^{Ba}	2.04 ± 0.05^{Ba}	2.04 ± 0.05^{Ba}
	Verde Valtierrilla	6	6.84 ± 0.07^{Aa}	1.50 ± 0.11^{Aa}	1.74 ± 0.07^{Aa}
		12	8.57 ± 0.07^{Ba}	0.58 ± 0.02^{Ba}	2.23 ± 0.27^{Ba}
		24	16.66 ± 0.14^{Ba}	2.70 ± 0.27^{Ba}	2.43 ± 0.27^{Ba}

Upper case letters mean significant differences in time and low case means difference in the carbohydrate source ($p < 0.05$)

***In vitro* Fermentability and SCFAs Production:** The effect of nopal flour on the production of SCFAs is described in Table 2. We found that the concentration of acetic acid comprised more than 50% of the total SCFAs evaluated. The production of these beneficial fatty acids resulting from the fermentation of flour from Verde Valtierrilla and wild nopal at 24 h was in the proportion of 63:21:16 (acetic:propionic:butyric). Fluid bed and tunnel drying methods to produce nopal flour yielded higher levels of propionic acid than those from spray drying, and 20% more than those from the control (raffinose).The *in vitro* fermentability of heat-dried nopal flour was always superior in wild than in Verde Valtierrilla materials in the three methods, but fluid bed drying showed better results than tunnel and spray drying.

The wild material produced the major concentration of total SCFAs in each drying process and the best capacity of fermentation was identified in the samples dehydrated by tunnel and fluid bed drying. We found that Verde Valtierrilla and wild flour produced high concentrations of SCFAs, even higher than those from staple food crops like oat, soy, pea, apple, corn, wheat, and pear[31]. The concentration of SCFAs in flour from wild nopal processed by fluid bed drying was comparable to that from wheat arabinoxylan[32]. This prebiotic effect of *O. ficus-indica* cladode has been demonstrated by Guevara-Arauza et al.[33], which also found increased production of SCFAs. These findings indicate that nopal could be used as a prebiotic source.

In conclusion, flour from wild and cultivated nopal can be considered an excellent source of bioactive compounds. Besides, *Opuntia* spp. powder may become an outstanding form to consume cladodes in many countries around the world. Our results suggest that nopal flour can help to improve the overall oxidative status in healthy humans by reducing the risk of some chronic degenerative diseases.

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