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NUTRITIONAL COMPOSITION OF *OPUNTIA SULPHUREA* G. DON CLADODES

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Abstract: Several studies have shown the interesting properties of *Opuntia* spp. ("prickly pears"), although most of this knowledge is based on *O. ficus-indica*. *O. sulphurea* is a species that is largely distributed in the Monte region of Argentina, where it has been used as an edible resource, especially in periods of food shortage. This is the first report evaluating the chemical composition of *O. sulphurea* cladodes. Our results show that cladodes are composed primarily of water, as with most other prickly pears that have been studied, which is consistent with their expected role as water reservoir in desert communities. Ash and protein content in *O. sulphurea* are consistent with values found for other species of the genus, whereas carbohydrates are well below levels of other *Opuntia* spp. Finally, the percentage of lipids in *O. sulphurea* cladodes is larger than in other studied species and fatty acid composition is quite different from observations made in similar studies. These earlier studies showed that linoleic acid is the major constituent of fatty acid fractions, followed by palmitic and oleic acids. Our analyses showed that these fatty acids are also principal constituents of *O. sulphurea* cladodes, although linolenic acid proved to be the most abundant. Curiously, the previous works found relatively low quantities of this fatty acid. Other minor fatty acids were also detected in cladodes of *O. sulphurea*, although the percentages are larger than in other studies of prickly pears. We discuss our results in the context of the potential nutraceutical and economic utility of *O. sulphurea* cladodes as a new source of essential fatty acids, especially in semi-arid areas as the Monte region where this species represents an abundant edible resource which is available even in periods of scarcity.

Keywords: prickly pear, *Opuntia*, nopal, nutritional value, fatty acids

INTRODUCTION

Opuntia is the most common and widespread genus of *Cactaceae*, traditionally with more than three hundred species, although this number has been recently reduced with the recognition of many segregated genera (Majure et al. 2012). These commonly named "prickly pear" cacti, are native to the western hemisphere and have been extensively used by non-industrialized cultures in the arid regions of the Americas for thousands of years (Ervin 2012). In its native range, *Opuntia* species also provide food for innumerable herbivores (Mellink and Riojas-López 2002), which underscores the ecological importance of the genus. In other areas, *Opuntia* species have been introduced into wildlands and pastures with non-desirable consequences due to the extensive invasion of escaped plants (Cronk and Fuller

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1995, Zimmerman et al. 2000, Vilà et al. 2003). In this sense, prickly pears constitute a significant threat to conservation of native species and the integrity of ecosystems, as well as to agricultural production in many parts of the world (Freeman 1992, Padrón et al. 2011, Robertson et al. 2011).

The genus *Opuntia* s. str. is also culturally important. For example in Mexico, where species of *Opuntia* have been cultivated for at least the past 14,000 years (Casas and Barbera 2002), they are an iconic national symbol. Many prickly pear species are cultivated worldwide as fruit and vegetable crops (Inglese et al. 2002) and are increasingly used as forage and fodder for livestock in different arid areas of the world (Pimienta-Barrios 1994, Nefzaoui and Salem 2002). Medicinally, various *Opuntia* species have shown hypoglycemic effects in diabetic patients (Trejo-González et al. 1996; Laurenz et al. 2003). Also, it has been shown that the extracts of *O. ficus-indica* cladodes present anti-inflammatory and chondroprotective effects (Panico et al. 2001). These anti-diabetic and anti-inflammatory functions have been recognized for hundreds of years (Feugang et al. 2006). Finally, the polysaccharides of some *Opuntia* species have been shown to protect brain tissue from glucose and oxygen deprivation (Huang et al. 2008), and polysaccharides extracted from *O. ficus-indica* have been used to protect the liver from harmful organophosphorous pesticides (Ncibi et al. 2008).

Opuntia ficus-indica (L.) Miller has a long history of human use, being cultivated for uses as diverse as erosion control (Le Houérou 1996), hedges and host plant for production of cochineal dye among others (Ervin 2012 and references therein). In some arid regions, older maturational stages are primarily used for animal forage when other fresh food is not available, and may even be included in human diet as cooked items (Feugang et al. 2006, Rodríguez-García et al. 2007, Hernández-Urbiola et al. 2010, 2011). Fruits ("tunas") are usually consumed by humans, as well as the cladodes of young maturational stages ("nopales" or "pencas"), which are eaten as salads (Feugang et al. 2006, Rodríguez-García et al. 2007, Hernández-Urbiola et al. 2010, 2011, Ervin 2012). The nopal is an important source of nutritional elements, including essential amino acids, minerals and vitamins (Hernández-Urbiola et al. 2010, 2011 and references therein). Besides, it is a rich source of soluble fiber, especially during the cladodes' younger stages, and a cladode's calcium content increases with development (Rodríguez-García et al. 2007, Hernández-Urbiola et al. 2010, 2011). Fiber content has been related to the amelioration of symptoms of diabetes through reduction of glycemia, as well as to the diminution of body weight and cholesterol through its anti-hyperlipidemic and hypercholesterolemic effects (Hegwood 1990, Muñoz-Chavez et al. 1995, Reid et al. 1995, Trejo-González et al. 1996, Palumbo et al. 2003, Gebremariam et al. 2006).

The interesting properties of prickly pears are not restricted to *O. ficus-indica*. In 2006, Feugang et al. (2006) reviewed the nutritional and pharmacological properties of fruits and cladodes of several species of the *Opuntia* genus looking to offer a scientific basis for future studies and to achieve a more widespread recognition of these valuable plants. These authors concluded that their many potentially active nutrients and multifunctional properties turn them into perfect candidates for the development of health-promoting food (Feugang et al. 2006). They stated that, even though these properties were traditionally appreciated by the Native Americans, nowadays this hidden knowledge needs to be reassessed (Feugang et al. 2006). It is worth mentioning that all species included in their study were native to North America, despite the importance of this genus in Central and South America, from where the genus was supposed to have originated (Ervin 2012, Majure et al. 2012). In Argentina there are approximately 20 *Opuntia* species (Lambert 1998, Kiesling 2003). *Opuntia sulphurea* G. Don is one of those species which is largely distributed in the dry Monte phytogeographic region, where it constitutes an important element of the floristic compositions of *Larrea* communities (Méndez et al. 2004, Ladio and Lozada 2009). *O. sulphurea* is especially associated with open spaces, which could be related to its agamic propagation via cladodes (Méndez et al. 2004). Further, the increase of individuals of this species in overgrazed areas is favored by asexual propagation facilitated by livestock (Méndez 2006). Finally, a study analyzed some ethno-ecological strategies undertaken by human populations inhabiting the Monte region and determined that their ethnobotanical knowledge includes the use of many wild plants, including *O. sulphurea* (Ladio and Lozada 2009). Moreover, they showed that this species along with other xeric plants represent an important part of the dietary components of these rural communities (Ladio and Lozada 2009).

The objective of this work was to evaluate the chemical composition of *O. sulphurea* cladodes, an overlooked species, in order to assess its potential nutritional value for human and animal consumption.

MATERIALS AND METHODS

Samples of aerial parts (cladodes) of fresh *O. sulphurea* were collected in the Natural Reserve of San Agustín del Valle Fértil, San Juan province (Western Argentina, 30° 38' 04"S, 67° 28' 06"W) in summer months (from February to March) of 2012.

Refrigerated plant specimens were transported to the laboratory, where spines were removed with pruning shears for safe handling. A sample weighing half a kilogram was used in the analyses in order to establish its contents of water, ashes, proteins, total fat and fiber as well as lipid profile. The sample was composed of a mix of three cladodes (each one corresponding to a mature individual) with the following dimensions: (1) 19.7 × 14.2 cm, (2) 19.3 × 13.5 cm and (3) 20.5 × 12.7 cm. Results regarding the composition of *O. sulphurea* cladodes are the average of two independent determinations made on the same sample (the aforementioned mix of cladodes). Vari-

ance associated to the technique was less than 1%. Variance among individuals was not determined.

Analytic assays were conducted following AOAC (Association of Official Analytical Chemists) standards (Horwitz 2000). Here is a brief description of the employed techniques:

Water content (moisture) was measured by sample dehydration by lyophilization, a method that has been used extensively for moisture determination in various vegetables (e.g. Makower and Nielsen 1948, Blanco et al. 2008, Šircelj and Batic 2012). In fact, this freeze-drying method allowed us to obtain better quality products compared with products dried with traditional methods (Ciurzyńska and Lenart 2011). Moisture was measured using a Labconco lyophilizer, model 77530. Lyophilization cycle was performed by setting the freeze-drying chamber at -40 °C until balanced with the sample internal temperature. Next, a vacuum was applied until 300×10^{-3} Mbar were reached. At that moment, the temperature of the freeze-drying chamber was increased by 10°C, until equilibrium with the internal temperature of the sample was reached. Then, the process started all over again, repeating the steps described above, although temperature was further increased to 40 °C until constant weight.

Free sugars were determined by HPLC, using a Waters 6000A pump system, a Waters injector with a 50 µL sample loop and a refractive index detector (Waters R 401) and a Data Module Waters integrator. We used an Aminex HPX-87C (Bio-Rad) anion-exchange column for sugar analysis (250 × 4.0 mm) and a mobile phase with deionized water at 85°C at 0.6 ml/min flux rate. Standard solutions of mono- and disaccharides (1 g/100 ml) were employed in order to identify sugars commonly present.

Ash content was determined by method AOAC 942.05 via carbonization of the samples placed in porcelain capsules on heating plates until the disappearance of white fumes and the subsequent calcination in muffle at 550 °C until white ashes were formed. The resulting material was subsequently weighted.

Proteins were determined according to the Kjeldahl method (AOAC 984.13), which involves the digestion of the sample with concentrated sulfuric acid, employing a mixture of potassium sulfate and selenium as catalysts. Subsequently, the nitrogen formed was distilled in an alkaline medium with water vapor, and the ammonia was collected in a 4% solution of boric acid with methyl red/bromocresol green indicator. The assessment of released ammonia was performed with 0.1 N sulfuric acid. Protein composition was estimated using a nitrogen factor of 6.25.

Fiber content was determined by the enzymatic-gravimetric method AOAC 985.29 using a Megazyme kit (Ireland). Briefly, the sample was subjected to the successive action of three enzymes with proper pH and temperature for the optimal activity of all of them (α-amylase: pH 6.0 ± 0.2, 95–100 °C, 15–30 min; protease: pH 7.5 ± 0.2, 60 °C, 30 min; amyloglucosidase: pH 4.0–4.6, 60 °C, 30 min). After digestion, ethanol was added (final concentration 78%) to precipitate the soluble fiber that afterwards was filtered through celite (diatomite) in a tared crucible, brought to dryness and weighted.

Fat content was determined by the acid hydrolysis method (AOAC 954.02), which involves heating the sample in the presence of hydrochloric acid at 80 °C for 30–40 minutes and subsequently removing the fat with ethyl ether and petroleum ether. The ethereal extracts were collected in a tared ball, the solvent was recovered by rotary evaporation and the fatty residue was determined gravimetrically. Fatty acid profile was determined by gas chromatography. Fat extraction was performed by the Folch method and derivatization was done using sodium methoxide to form methyl esters according to IRAM 5650 Part II. Chromatographic conditions were as follows: Perkin Elmer Claurus 500 chromatograph, a fused silica capillary Supelco SP 2560 (Supelco Park, Bellefonte, PA, USA) 100 m × 0.25 mm × 0.20 um column. Column temperature was programmed at 150 °C for 1 min and a gradient ranging from 150 °C to 210 °C for 20 min at a rate of 5 °C per minute. The injection port and flame ionization detector were maintained at 240 °C and 280 °C, respectively. Nitrogen was employed as carrier gas in a linear speed of 1.3 ml/min.

Caloric value was calculated using the Atwater factor: the caloric value of protein and the carbohydrate contents (in grams) were multiplied by four and the lipid content was multiplied by nine. In the case of human diet, fiber is considered to provide no energy because of its low digestibility. However, as many organisms (e.g. ruminants) have the capability of digesting it, we used the value of four to estimate the potential caloric value of carbohydrates when considering fiber as an energy source.

RESULTS AND DISCUSSION

Several studies have shown interesting properties of prickly pear nutritional and medicinal utilization (see Feugang et al. 2006 and references therein). Even though *Opuntia* is a large genus with many important species in Latin America for centuries, most of this knowledge is based on work performed solely with *O. ficus-indica* (Ervin 2012, Majure et al. 2012). By contrast, *O. sulphurea* is a species that is largely distributed in the Monte region of Argentina where it has been used as an edible resource, especially, in periods of food shortage (Méndez et al. 2004, Ladio and Lozada 2009). However, there is no information regarding the nutritional properties of this species, this being the first report evaluating the chemical composition of *O. sulphurea* cladodes.

Cladodes were chosen as the material of study because they are consumed by rural humans and livestock inhabiting this arid region (Ladio and Lozada 2009). Cladodes are composed primarily of water (89.8%, Table 1), as most of the prickly pears that have been studied (88–95% of fresh tissue; Stintzing and Carle 2005, Feugang et al. 2006), which is consistent with their expected role as water reservoir

	In 100 g of fresh sample	In 100 g of dry sample
Energetic value	35 Kcal (20 Kcal)*	339 Kcal (195 Kcal)*
Water content	89.800 g	0.000 g
Ash [a]	2.180 g	21.400 g
Carbohydrates	3.120 g	30.800 g
Proteins [b]	0.700 g	6.900 g
Fat content [c]	0.500 g	4.900 g
saturated	0.160 g	1.567 g
monounsaturated	0.067 g	0.650 g
polyunsaturated	0.266 g	2.610 g
trans	0.001 g	0.009 g
Sugar Profile [d]		
monosaccharides	1.600 g	15.800 g
disaccharides	1.140 g	11.200 g
Dietary fiber [e]	3.700 g	36.000 g

[a] **AOAC method 942.**05 -17 ed; [b] AOAC method 984.13 -17 ed; [c] AOAC method 954.02 -17 ed; [d] Determination by HPLC; [e] AOAC method 985.29 -17 ed. * Values in parenthesis are those excluding the caloric contribution of dietary fiber (see Materials and Methods).

Table 1. Primary compositional profile of *Opuntia sulphurea* stem.

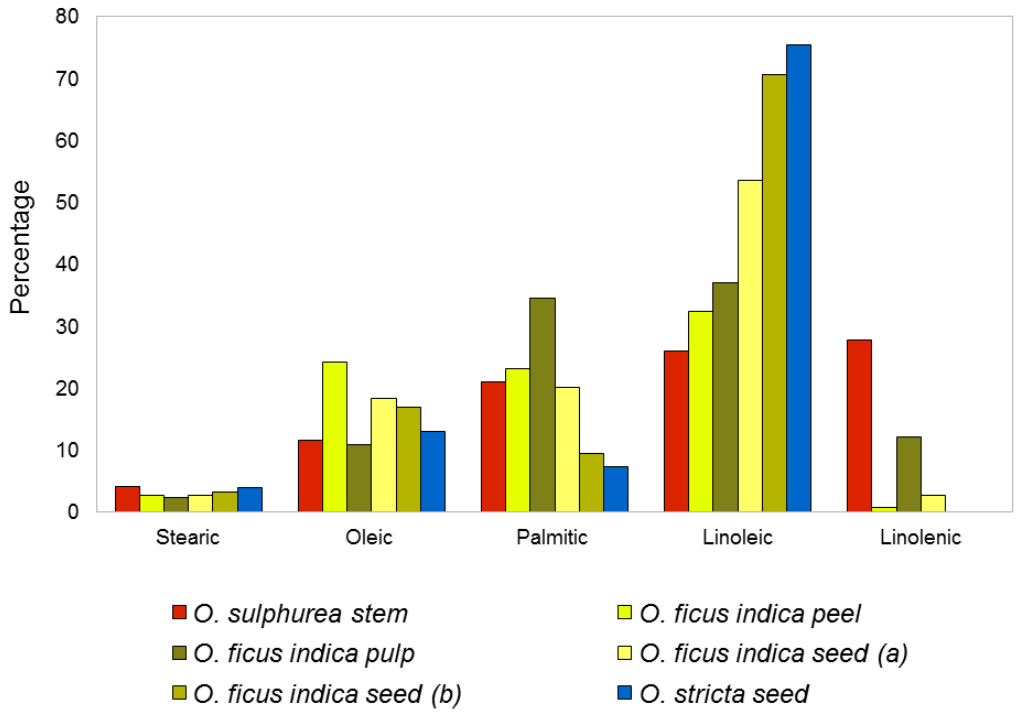

Figure 1. Percentage of the principal constituents of fatty acid fractions isolated from different tissues of *Opuntia* spp.: O. sulphurea stem, *O. ficus-indica* fruit peel (estimated from Ramadan and Mörsel 2003b), *O. ficus-indica* pulp and seed (a) (estimated from Ramadan and Mörsel 2003a), *O. ficus-indica* seed (b) and *O. stricta* seed (estimated from Ennouri et al. 2005).

Fatty acid	Nomenclature	In 100 g of fat content
Lauric	(12:0)	1.920 g
Myristic	(14:0)	1.954 g
	(15:0)	0.460 g
	(15:1) cis	0.210 g
Palmitic	(16:0)	20.730 g
Palmitoleic	(16:1)	0.570 g
Margaric	(17:0)	1.010 g
Heptadecenoic	(17:1)	0.170 g
Stearic	(18:0)	4.040 g
Trans elaidic	(18:1) trans	0.000 g
Oleic	(18:1) n-9	11.400 g
Cis-octadecenoic	(18:1)	0.690 g
Other 18:1 cis		0.130 g
Trans linoelaidic	(18:2) trans	0.180 g
Linoleic	(18:2) n-6	25.580 g
Linolenic	(18:3) n-3	27.410 g
Gadoelic	(20:1)	0.180 g
Arachidic	(20:0)	0.850 g
Behenic	(22:0)	1.020 g
Gamma Linolenic	(18:3) n-6	0.210 g
Total saturated		31.980 g
Total monounsaturated		13.350 g
Total polyunsaturated		53.200 g
Total trans		0.180 g
Total unidentified minor components		1.290 g

Table 2. Fatty acids profile of *Opuntia sulphurea* stem.

in desert communities. Carbohydrates accounted for 30.8% of dry sample (Table 1), which is well below the levels of other *Opuntia* species (64–71 / 100 g of dry tissue; Stintzing and Carle 2005, Feugang et al. 2006). For *O. sulphurea*, 15.8% and 11.2% of dry weight were monosaccharides and disaccharides respectively, with no complex carbohydrates identified due to matrix effects (Table 1). Ash content was 21.4% of dry weight (Table 1), which is consistent with values for other species of the genus (19–23% of dry tissue; Stintzing and Carle 2005, Feugang et al. 2006).

Dietary fiber content was 36 g/100 g of dry sample, representing more than 42% of the putatively available calories of the sample (Table 1). This component generally refers to edible parts of plants (i.e. fruits, leafs, etc.) that are resistant to digestion and absorption in the human small intestine with complete or partial fermentation in the large intestine, including polysaccharides, oligosaccharides, lignin, and associated plants substances (AACC 2001). However, since many organisms have the capability of digesting these components, we used a conversion factor to estimate its potential caloric value (see Materials and methods). Results derived from this analysis showed that, including dietary fiber, the caloric value of *O. sulphurea* cladodes increases by more than 73% (Table 1).

Based on the previous results (Stintzing and Carle 2005, Feugang et al. 2006), our protein results (6.9% of dry sample, Table 1) are similar to estimates for other prickly pears (4–10% of dry weight). However, we found a larger percentage of lipids (4.9% of dry weight, Table 1), which more than other *Opuntia* species that have been studied (1–4% of dry weight; Stintzing and Carle 2005, Feugang et al. 2006). Furthermore, our results regarding fatty acid composition are quite different from observations made in previous studies. Unsaturated fatty acids in the cladodes made up 67.8% of the total (Table 1), which corresponds with an unsaturation ratio (ratio of unsaturated to saturated fatty acid) of 2.08. This value is well below those obtained from seed oil extracted from other species of the

genus (7.11 in *O. ficus-indica* and 7.61 in *O. stricta,* Ennouri et al. 2005; 3.38 in *O. ficus-indica*, Ramadan and Mörsel 2003a). It is more similar to those estimated for *O. ficus-indica* fruit peel (2.36, Ramadan and Mörsel 2003b) and pulp oil (1.63, Ramadan and Mörsel 2003a). Accordingly, the ratio of value of the polyunsaturated fatty acids to saturated fatty acids (P/S = 1.67) of O. *sulphurea* is lower than those of aforementioned seed oils (5.66 in *O. ficus-indica* and 6.48 in *O. stricta,* estimated from Ennouri et al. 2005; 2.46 in *O. ficus-indica*, estimated from Ramadan and Mörsel 2003a), but once again being more similar to those of *O. ficus-indica* fruit peel (1.03, estimated from Ramadan and Mörsel 2003b) and pulp oil (1.31, estimated from Ramadan and Mörsel 2003a). These results imply that the P/S ratio of the *O. sulphurea* cladoes and *O. ficus-indica* fruit peel and pulp oil are close to the 1.0 P/S ratio recommended for human consumption (Adam 1989).

Even though others have studied *Opuntia* spp. lipids, suggesting prickly pears as rich sources of oils (see Feugang et al. 2006 and references therein), apparently they have not investigated the fatty acid composition of the cladodes of these species (Stintzing and Carle 2005). These studies analyzed different parts of the fruit (i.e. peel, seed and pulp), mostly in *O. ficus-indica,* and all of them showed that linoleic acid is the major constituent of fatty acid fractions (32%–75%), followed by palmitic (7%–34%) and oleic (11%–24%) acids (Figure 1). Our analyses showed that the mentioned fatty acids are also principal constituents of *O. sulphurea* cladodes (26%, 21% and 12% for linoleic, palmitic and oleic acids, respectively (Figure 1). Curiously, the aforementioned works found relatively low quantities of this fatty acid (0%–12%). Other fatty acids that have been detected in these studies are stearic (2%–4%, Figure 1), myristic (0%–2%), lauric (0%–2%), behenic (0%–1%), palmitoleic (0%–2%) and gamma linolenic acid (0%–9%; Table 2; Ramadan and Mörsel 2003a, b; Ennouri et al. 2005). Most of these fatty acids showed larger percentages in *O. sulphurea* than in other studies of prickly pears (Table 2; Ramadan and Mörsel 2003a, b; Ennouri et al. 2005). Finally, we have found small amounts of other long chain fatty acids that apparently have not been detected in other *Opuntia* spp. tissues (e.g. margaric, arachidic, cis-octadecenoic, trans elaidic, gadoelic, trans linoelaidic, heptadecenoic, etc.; Table 2; Ramadan and Mörsel 2003a, b; Ennouri et al. 2005).

Linolenic acid (or alpha-linolenic acid, ALA) and linoleic acid (LA) are polyunsaturated fatty acids (PUFAs), each one belonging to a family of essential fatty acids. ALA and LA are the principal omega-3 (n–3) and omega-6 (n–6) fatty acids, which must be included in the diet because they cannot be synthesized within the human body. Gamma linolenic acid (GLA) is another omega-6 PUFA that has been detected in *O. sulphurea* cladodes and must be obtained from foods. While the omega-6 essential PUFAs are abundant in all kinds of vegetable oils and meats, the omega-3 essential PUFAs are much harder to incorporate with meals. Seed oils are the richest sources of ALA, particularly those of chia, perilla, flaxseed, walnut, canola and soybean (Harper and Jacobson 2001). ALA is similar to other omega-3 PUFAs that are available from fish and algae oils, for example eicosapentaenoic acid (EPA) and docosahexaenoic acid (DHA), which play a vital role in many metabolic processes and can be synthesized by humans from dietary ALA. Preliminary studies have related ALA to a lower risk of cardiovascular disease (Connor 2000, Kris-Etherton et al. 2002), as well as to reduced levels of anxiety and stress (Yehuda et al. 2005). It has been also suggested that ALA may display major neuroprotective effects (Lauritzen et al. 2000). Finally, a recent study found that a higher intake of ALA (combined with a lower intake of LA) was associated with a significant reduction in depression, while the intake of EPA and DHA did not have the same beneficial effect (Lucas et al. 2011). In this sense, our results suggest that *O. sulphurea* cladodes may represent a valuable source of ALA for human consumption.

Our work highlights the nutritional value of *O. sulphurea* cladodes, especially in semi-arid areas such as the Monte region of Argentina, where this species is abundant and represents an edible resource which probably acts as a buffer in periods of food shortage. Further studies will help to improve our knowledge regarding the composition and properties of *O. sulphurea* cladodes. In particular, these studies should take into account a larger sample representing different populations as the composition of cladodes may vary with factors related to the geographical origin of the plants. Also, they should analyze the abundance of other components (e.g. minerals and vitamins) in different maturity stages, as has been recently done in *O. ficus-indica* (Rodríguez-Garcia et al. 2007, Hernández-Urbiola et al. 2010, 2011, Contreras-Padilla et al. 2011). These studies will help to confirm the potential nutraceutical and economic utility of *O. sulphurea* cladodes as a new source of essential fatty acids, including ALA.

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