Exploration of the nutritional profile of *Trichocereus terscheckii* (Parmentier) Britton & Rose stems

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Abstract

Columnar cacti *Trichocereus terscheckii* is an important source of food and water for insects, birds and wild cattle in dry areas of North West Argentina. Their fruits have been suggested as a plausible source of food, but are not directly included in the human diet yet. Stem chemistry has long been of interest because of its hallucinogenic properties, but little is known about the nutritional value. In this work, basic nutritional composition of a mature stem was analyzed establishing moisture, total fat, ash, fiber, protein, lipidic and sugar profile. The main constituents fatty acid found in *T. terscheckii* were linolenic, linoleic, palmitic and oleic acids, but also a rare isomer of oleic acid was relatively abundant. This pattern did not agree with the profiles found in other species of columnar cacti. Current exploration of nutritional profiles of *T. terscheckii* stem provides novel information that contributes to a better understanding of this species whose slow development rate, high water storage capacity and hallucinogenic properties are features that make it especially vulnerable to an increased rate of extraction, habitat fragmentation and extensive cattle ranching.

Keywords: alkaloids, cardon, *Echinopsis*, fatty acids, nutrition.

Resumen

El cactus columnar *Trichocereus terscheckii* constituye una importante fuente de agua y alimento de insectos, aves y ganado salvaje en áreas áridas del noroeste argentino. Se ha sugerido la posibilidad de que sus frutos sean una fuente de alimento, pero hasta el momento no ha sido incluido en la dieta humana de manera generalizada. Por otro lado, la química de los tallos ha sido largamente de interés por sus propiedades alucinógenas y sin embargo poco se conoce de sus valores nutricionales. En el presente trabajo se ha analizado la composición nutricional básica de un tallo maduro estableciendo su humedad, contenido graso, de ceniza, fibra, proteína total y sus perfiles lipídicos y de azúcares. Los principales ácidos grasos constitutivos de *T. terscheckii* fueron el linolénico, linoleico, palmítico y oleico; sin embargo también se encontró en relativa abundancia un raro isómero del ácido oleico. Este patrón lipídico no coincide con los perfiles reportados en otras especies de cactus columnares. De esta manera, se presenta información novedosa sobre el perfil nutricional del tallo de *T. terscheckii* con el fin de contribuir a una mejor comprensión de esta especie, cuyas características (lento desarrollo, gran capacidad de almacenamiento de agua y propiedades alucinógenas) la hacen especialmente vulnerable a elevadas tasas de extracción, fragmentación del hábitat y la ganadería extensiva.

Palabras clave: alcaloides, ácidos grasos, ácido oleico, cardón, *Echinopsis*, nutrición.

Introduction

Cacti are a typical component of vegetation in tropical and subtropical America, ranging from southern Patagonia to southwest Canada. They occur in almost every habitat throughout grassland, rainforest, cold mountain tops, sea shores and deserts as the most common habitat (Mauseth *et al*., 2002; Nobel, 2002). The emergence of cacti (Cactaceae, subfamily Cactoidae) is hypothesized to take place during late Cretaceous (65-90 million years ago) following the breakup of Gondwana (Axelrod, 1979; Shmida, 1985; Mauseth, 1999). In the middle Andean geographical region between Chile, Bolivia and Peru, the ancestor radiated into four main lineages that migrate to the North, South and East, following further radiation and specialization in its morphology and habits (Fleming and Banuet, 2002). South American tribes are mainly composed by *Browningieae*, *Cereeae* and *Trichocereeae*, while North American is represented by *Leptocereeae* and *Pachycereeae*, including in total more than 25 genera and 170 species (Fleming and Baunet, 2002; Nobel 2002).

In fact, columnar cacti are the second largest plant family in the new world (Anderson 2001). They are the dominant elements of Neotropical deserts ecosystems given the capacity of support massive stems that act as water and forage reservoirs for animals when resources are scarce.

Distribution of columnar cacti in Argentina showed highest species richness in the North West phytogeographic regions of Prepuna, Monte desert and Chaco dry forest, with a decreased distribution from west to east and north to south, declining above 34-36º S. Cacti distribution seems to be restricted mainly by low temperatures (specially frost-free days), probably due to the existence of a barrier at the south of the Chaco and Prepuna (Mourelle and Ezcurra 1996; 1997).The most important columnar cacti with studied distribution are from the genera *Trichocereus* and *Cereus* and in a less proportion *Cleistocactus, Echinopsis, Harrisia, Lobivia, Monvillea, Oreocereus, Stetsonia, Soehrensia* (Gibson and Nobel 1986; Mourelle and Ezcurra 1996). *Trichocereus* (Cactaceae: Cactoideae) genus is constituted by at least 45 species distributed along arid and semiarid areas in the Andean region of South America, ranging from Ecuador to central Argentina (Anderson 2001; Albesiano and Terrazas, 2012). The genus is characterized by its large size with cylindrical stems, shallow ribs and large flowers (up to 30 cm) with dense covering hairs (Kiesling 1978, Kiesling & Ferrari 2005). *Trichocereus terscheckii* (common name: "cardón") is a large columnar cactus endemic of high valleys of northwestern Argentina with a narrow corridor distribution from North to South across Puna, Prepuna, Chaco and Monte (Figure 1).

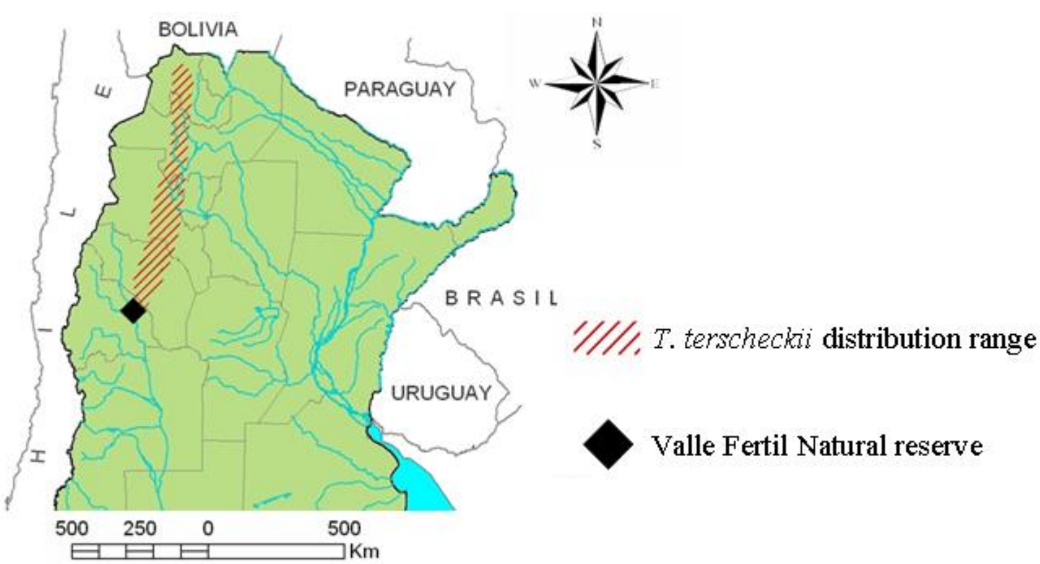

Figure 1. Distribution of *Trichocereus terscheckii* in Argentina (red oblique lines) and location of the collection site: Valle Fértil Natural Reserve (Modified from Argentine Biological information system, National Parks Administration).

Interestingly, the sample site (Valle Fértil provincial reserve, San Juan, Argentina) is located at the southern marginal distribution of this specie (with an average altitude of 1200 meters), where two phytogeographic regions converge. The Chaco Region with up to 400 mm precipitation per year, and the dryer Monte Region

(with up 200 mm), which is characterized by shrub steppe landscapes (Cabrera, 1994; Pereyra, 2000). High density of *T. terscheckii* was found in Valle Fértil reserve (predominantly silty to silt-clay soil and an average annual precipitation of 250 mm) where rivers descend to the eastern slope of the hills creating small "oasis" (Cabrera, 1994; Pereyra, 2000). However, scarce to none individuals were to be found in the dryer Monte side of the reserve, which is in line with Steenbergh & Lowe (1977) on hydrologic conditions as a key environmental factor for survival during the seedling stage, until the plant reach a bigger size with a lower surface/volume ratio. *Trichocereus terscheckii* is consider the largest member of the genus, with individuals easily reaching 10 to 12 meters high standing out from the shrubby landscape (Figure 2a) resembling the northern Saguaro (*Carnegiea gigantea*) native of the Sonora desert (Gibson and Nobel 1986). Flowers are white and nocturnal, up to 20 cm long (Figure 2b) and visited by moths, birds and bees, also their stems are a source of food for phytophagous insects adapted to xeric environments (Claps and Haro 2001; Ortega-Baes *et al*., 2011). Rotten arms and decaying pockets of this species are colonized by microbial flora (especially yeast) producing the "rotten milk" used by saprophytophagous insects as breeding substrate for their larvae (Fanara *et al.*, 1999; Soto *et al.*, 2008).
Historically many cacti have been exploited as food resource for human consumption since stems are cooked as vegetable, used as emergency water supply, or as forage to feed domestic animals when grass is scarce (Nobel 2002). In fact, Kiesling (2001) suggested the possible use of *T. terscheckii* fruits for human consumption. However, *T. terscheckii* stems still are an important source of consumption in those regions where this species is used as ceremonial plant due to their mescaline content (Records of the Valle Fértil natural reserve ranger office, personal communication).

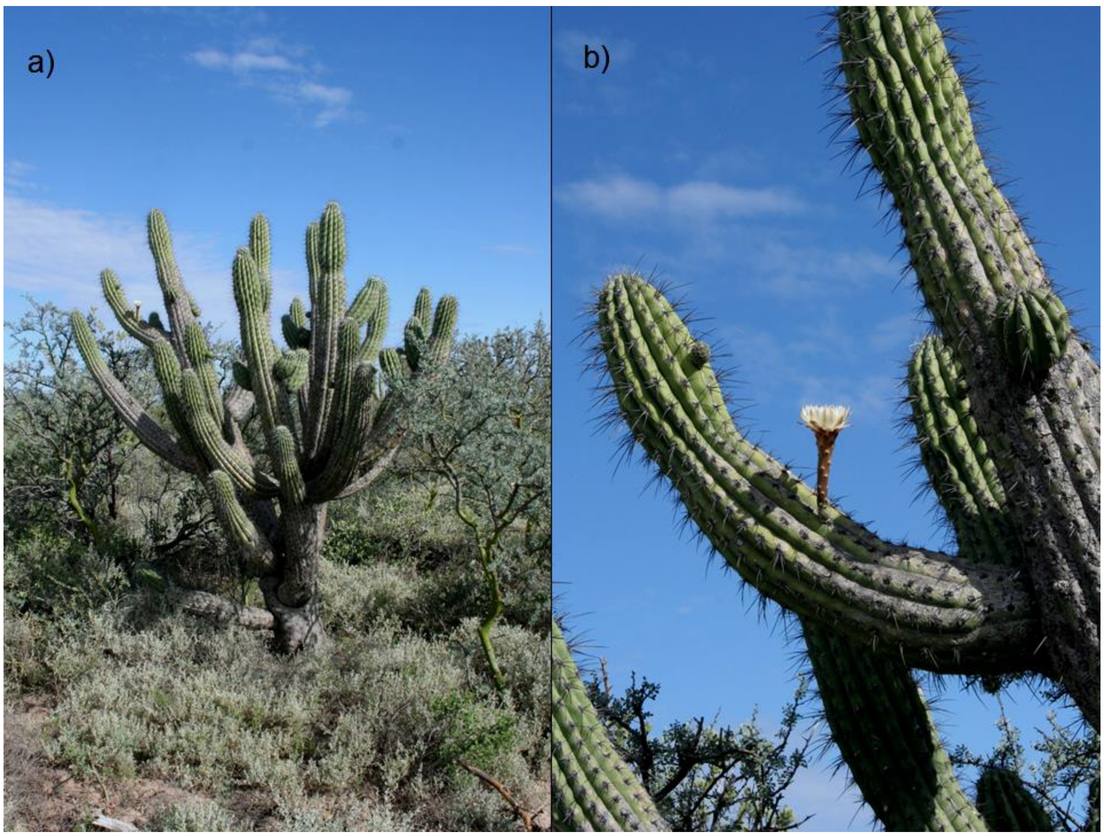

Figure 2. **a**) A 5-meter tall *Trichocereus terscheckii* specimen in the Natural Reserve of San Agustín del Valle Fértil, San Juan province (Western Argentina, 30°38′4″ S, 67°28′6″ W) in late summer. **b**) Detail of a stem showing the typical white long flower and an immature fruit. (Source: Ignacio M. Soto).

Stems chemistry of *Trichocereus* genus has long been of interest to phytochemists because are known to be highly alkaloidiferous (Reti and Castrillon 1951). Alkaloids primary function is to act as deterrents for insects, herbivores and microorganisms (Gibson 1982), However, psychomimetic compounds with hallucinogenic properties are widely found among the constituent alkaloids of the genus (e.g. mescaline). Since ancient times, hallucinogenic columnar cacti have been used for religious and therapeutic practices dating back to 1300 B.C. Tradition has been preserved and expanded their influence through time to present days (Schultes *et al*., 2001).

Although the most common cactus used for shamanic activities in South America is *Trichocereus pachanoi* ("San Pedro"; Ogunbodede *et al*., 2010), in Argentina is more frequent to find the similar *T. terscheckii,* also being called "San Pedro" and probably used indistinctly (Schultes *et al*., 2001). This species represented the first case where mescaline was found in a different species than *Lophophora williamsii* ("Peyote"; Reti and Castrillon 1951). However, content of alkaloids is higher in *Trichocereus pachanoi* (up to 6 % of dry weight) compare to the 0.25-1.5 % found in *T. terscheckii,* although alkaloids content is highly variable among *Trichocereus* species (Ogunbodede *et al*., 2010). Interestingly in this last species it was found a higher proportion of a new natural phenylethylamine alkaloid named trichocereine (N-dimethylmescaline) without any apparent hallucinogenic property (Reti and Castrillon 1951). However, recently was found to contain a potent mescaline analog (α-methylmescaline; Corio *et al.,* 2013) that impressed in a self-administration experiment because of the euphoria produced (Hardman *et al.,* 1973). Mescaline effects in man have been reviewed by many investigators (Kapadia and Fayez, 1970; Hardman *et al.,* 1973; Nichols, 2004) with doses ranging between 200-500 mg, observing the following general symptoms: Nausea, vomiting, hyperpnea, mydriasis, sweating, dizziness, headache, palpitation, feeling of hot or cold, abdominal cramps, mild tremor and incoordination, visual hallucination and synesthesia (effects can last up to 10-12 hr). Although seems to be no evidence that mescaline cause habituation or addiction (Kapadia and Fayez 1970). It is important to remark that studies of its serotonin binding receptor 5-HT2A is gaining increasing importance in the therapeutic field for various psychiatric disorders such as schizophrenia, depression and suicidal behavior (Pandey *et al.,* 1995; De Angelis, 2002; Abdolmaleky *et al.,* 2005).

On a conservational aspect, this specie is threatened by agricultural expansion, wood removal and cattle overgrazing mainly in its northern distribution (Peco *et al*., 2011), while in the south, its primarily affected by the herbivory activity of introduced species such as feral donkeys (Cortéz *et al.,* 2005). Cattle compete with native herbivores such as guanaco for the scarce resources leading to overgrazing in some places and, in consequence, to a loss of local wild plant species (Nates *et al.,* 2012). However, damage intensity on *T. terscheckii* trunks correlated with livestock but not with guanaco (*Lama guanicoe*, a native herbivore) abundance in the surroundings (Novillo and Ojeda, 2008; Malo *et al.,* 2011). Also and as previously stated, the exploitation of the *T. terscheckii* for recreational purposes seems to be an up growing threat according ranger stations in the north west of San Juan province. Despite the toxicological and other alkaloid-related studies, we had scarce information about other general chemical properties of these species. Therefore, we present the first primary compositional profile of *Trichocereus terscheckii* including basic nutritional facts. Our primary objective is to provide basic information for comparative purposes with other species, conservation or for assessments of potentialities of economic exploitation.

Materials and methods

Plant samples

Plants Samples of aerial parts (stem) of fresh *T. terscheckii* (Figure 2) were collected in the Natural Reserve of San Agustín del Valle Fértil, San Juan province (Western Argentina, 30°38′4″S, 67 28′6″ W) in summer months (February to March), 2012. The specimen studied was an adult cactus of three meters tall, collected right after the rainy season thus, the observed water content could be considered as an upper limit of the moisture variation. Specimen removal for this experiment was approved by the Environment and Sustainable Development Secretariat (SAYDS) of San Juan Province.

Plant specimen was transported refrigerated to the laboratory and spines were removed. A slice weighing half a kilogram was used in the analyses in order to establish moisture, fat, ash, fiber, protein and lipidic profile.

Results regarding the composition of *T. terscheckii* stems are the average of two independent determinations made using a single stem. Variance associated to the technique was less than 1 %.

Analytic essays were conducted following AOAC (Association of Official Analytical Chemists) standards. AOAC "Official Methods of Analysis" have been defined by regulations promulgated for enforcement of the Food, Drug, and Cosmetic Act (21 CFR 2.19), recognized in Title 9 of the USDA-FSIS Code of Federal Regulations, and in some cases by the U.S. Environmental Protection Agency.

Here is a brief description of the employed techniques:
Water content (Moisture) was measured by dehydration, using a Labconco lyophilizer, model 77530. Lyophilization cycle was performed by setting the freeze-drying chamber at -40 °C until balance with the samples internal temperature. Then started to apply vacuum until 300×10^{-3} Mbar and slowly increase the temperature of the freeze-drying chamber by 10 °C, until equilibrium with the internal temperature of the sample was reached. The cycle is repeated until -10 ºC was reached, and then the process starts all over again, repeating the steps described above, but the 10 °C increasing procedure was further increased to 40 °C until constant weight. Total run time was 150 h. Finally, the dried product was milled to a fine powder.

Ashes were obtained by carbonization of the samples placed in porcelain on a heating plate until the disappearance of white fumes and subsequent calcination in muffle at 550 °C until the formation of white ash. The resulting material was subsequently weighed.

Proteins: Determined according to the Kjeldahl method (Bradstreet, 1965) (digestion of the sample with concentrated sulfuric acid employing a mixture of potassium sulfate and selenium as catalyst). Subsequently, a distillation of the nitrogen formed was performed in an alkaline medium with water vapor and collection of the ammonia in a 4 % solution of boric acid with methyl red-bromocresol green indicator. The assessment of released ammonia was performed with sulfuric acid 0.1 N. Conversion factor protein/nitrogen: 6.25.

Fat: determined by acid hydrolysis method of heating the sample in the presence of hydrochloric acid at 80 °C and subsequent removal of the fat with ethyl ether and petroleum ether. The ethereal extracts are collected in a tared ball, the solvent is recovered by rotary evaporation and the oily residue determined gravimetrically.

Determination of fiber by enzymatic-gravimetric method AOAC 2009.01 using Megazyme kit, Irland (McCleary *et al.*, 2010): The sample is subjected to the successive action of three enzymes (alpha amylase, glucoamylase and protease) with proper pH and temperature for optimal activity of each of them. After digestion, ethanol is added (final concentration 78 %) to precipitate the soluble fiber that afterwards is filtered through celite (diatomite) in a tared crucible, brought to dryness and weighed. Ash and proteins determinations are performed on the residue performing the estimations final correction with the values obtained.

Calculation of the caloric value was performed using the Atwater factor: Multiplying the caloric value of protein content (in grams) by 4, the lipid content by 9 and the carbohydrate content by 4. In the case of fiber in the human diet is considered to provide no energy because of its low digestibility. However, many organisms (insects and vertebrate herbivores) are capable of digest it. For this reason and in order to include non-human consumption, we used the value 4, which applies to carbohydrates to estimate a potential caloric value when fiber is included as an energy source.

Sugar: Free sugars were determined by HPLC, Waters equipment with ion exchange column (Nucleogel sugar 810 Ca), with refractive index detector (Waters R 401).

Fatty acid profile was determined by gas chromatography. Fat extraction by Folch method (Folch *et al*., 1957) and derivatization using sodium methoxide to form methyl esters according to IRAM 5650 Part II. Chromatographic conditions: Chromatograph Perkin Elmer 500 Claurus. Column: Supelco SP 2560 100 mx 0.25 mm x 0.20 um. Carrier Gas: Nitrogen. Flame Ionization Detector (FID) at 280 °C.

Results and discussion

Probably due to the alkaloidiferous nature, *Trichocereus* species were not specially studied regarding their potential nutritional value. Thus, this is the first report of nutritional properties for the stem of a *Tricochereus* species. Stems of this species are readily consumed as water and food supply for many groups of animals in the deserts of North West Argentina including humans, but, in this case, it is mainly for recreational purposes. However, Kiesling (2001) suggested the possible use of *T. terscheckii* fruits for human consumption but little is known about its nutritional composition, nor the fruits neither the stems.

Consistent with Reti and Castrillon (1951), the stem proved to be composed almost entirely by water (95.4 %;), which together with the large size that these cactus can achieve, supports its key ecological role as water storages in dry environments. Ash content yielded 25.1 %, and for the dry sample nonstructural carbohydrates accounted for a quarter of its weight (25.2 %), making together half of the total weight. Sugars could not be properly separated because of the extract characteristics (i.e. effect of the matrix), although it was possible to identify a general composition of monosaccharide (66.1 %) and disaccharides (33 %) while not complex carbohydrates were detected.

Dietary fiber generally refers to parts of fruits, vegetables, grains, nuts and legumes that are resistant to human digestive enzymes (cellulose, hemicellulose, pectin, lignin, etc., Spiller, 1992; Periago *et al.*, 1993). Fiber determination value yielded a relative high percentage, estimated in 31 % (Primary compositional profile of *T. terscheckii* is presented in Table 1). However, since many organisms have the capability to digest fiber, a conversion factor was applied in order to estimate a potential caloric value (see material and methods). The results show that including fiber, the caloric value reaches a 60 % increment of the putatively available calories of the sample (Table 1).

Protein results (14.4 %) coincide with the total protein percentage found by Serrano and Guzman (1994) in the seeds of this species, with a slight 2.3 % difference. However, a greater difference in the fatty acid composition was found. Unsaturated fatty acid in the stem made up 67 % of the total value; while in the seeds it shows to represent almost 83 %. Ratio between polyunsaturated and saturated fatty acids (P/S ratio) for the stem was estimated at 1.6, while seeds show a ratio of 3.6, making the stem closer to the 1.0 ratio recommended for human's consumption (Adam, 1989).

Fatty acid profile for the analyzed stem (Table 2) was represented by α-linolenic acid (29.2 %), linoleic (23.1 %) and palmitic (19.6 %), jointly yielding almost 72 % of the total fatty acids present. It is worth to note that the first two major compounds are essential fatty acids (EFAs), making together more than half of the total amount found in the stem (Figure 3).

In both seeds, analyzed by Serrano and Guzman (1994), and stem, the major saturated and monounsaturated acids correspond to palmitic and oleic acids, while polyunsaturated acids quantities shows to vary according the organ. In the case of the seeds, fatty acid profile was explained by the presence of linoleic acid (60.3 %), oleic (22.6 %) and palmitic (14.6 %), making together 98 % of the total amount. However, seeds showed to contain a very low amount α-linolenic acid (0.42 %) which is in line with the observation that, in general, most seeds are high in linoleic but low in α-linolenic acids (Adam 1989; Milton 1999). α-linolenic acid has been found to be essential for normal growth in humans while linoleic acid has been found to be essential for almost every animal's species, with exception of some insects and protozoa that are able to synthesize by themselves (Gurr *et al.*, 2008).

Another remarkable finding of the present study was the observation of 5.6 % of cis-vaccenic acid, a rare isomer of oleic acid (represented in a 6.5 %) showing an equal balance between them (a ratio of 0.86). Cis-vaccenic acid is a natural constituent of plants, microorganisms, animals and dietary fats for humans, but little is known on the effects of dietary with high levels of this naturally occurring positional isomer (Reichwald-hacker *et al.*, 1979). It was reported as pheromone precursor in insects (Pennanec´h *et al.*, 1997; Ray *et al.*, 2011), involved in ethanol tolerance in yeasts (You *et al.*, 2003) and thermal regulation in bacteria (Russell, 1984; Yamanaka 1999).

Table 1. Primary compositional profile of *Trichocereus terscheckii* stems. Concentrations are given for the fresh and dry samples.

	In 100 g of fresh sample	**In 100 g of dry sample**
Energetic Value	15 Kcal (9 Kcal)	321 Kcal (197 Kcal)
Water content	95.4 g	0 g
Ash[a]	1.15 g	25.1 g
Carbohydrates	1.17 g	25.2 g
Proteins[b]	0.66 g	14.4 g
Fat content[c]	0.20 g	4.3 g
saturated	0.063 g	1.36 g
monounsaturated	0.027 g	0.59 g
polyunsaturated	0.10 g	2.20 g
trans	0.001 g	0.02 g
Sugar Profile[d]		
monosaccharides	0.76 g	16.6 g
disaccharides	0.38 g	8.3 g
Dietary fiber[e]	1.42 g	31 g

Values in parenthesis are excluding the caloric contribution of dietary fiber non-digestible by humans.
Note: [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 gas chromatography; [e]AOAC method 985.29-17 ed.

In sum, the main constituents fatty acids found in *Trichocereus terscheckii* stem are among the most abundant forms in higher plants (linolenic, linoleic, palmitic and oleic acids). However, it is worth to note that this pattern did not agree with the profiles found in other species of columnar cacti. For instance, the main constituent of agria (*Stenocereus gummosus*) and organpipe (*S. thurberi*), the widely studied columnars cacti of the Sonoran desert in North America, contains predominantly medium chain fatty acids (capric and lauric) (Fogleman and Kircher 1986). Nevertheless, and in line with the observed similarity regarding morphology and habits between the North American *Carnegiea gigantea* and the South American *T. terscheckii* (Gibson and Nobel 1986), fatty acid profile seems to be similar since both are rich in the more typical chain lengths C_{16} -C_{18}, with a predominance in oleic, linoleic, linolenic and palmitic acids in the former (Chuan and Nobel 1985).

Conclusions

This first primary compositional profile of *T. terscheckii* provides basic novel information for this species, an important element of southern Neotropical deserts. So far the exploration of the stem nutritional profile presented in this work show to be composed mainly by water while dry sample shows a high proportion of fiber no digestible by humans (see Materials and Methods). Fat content is rich in EFAs but its total content is very low. Highest values are for total sugars, protein and ashes; however, more detail analysis is required for qualitative aspects of these nutrients, also alkaloidiferous nature of stems suggest a toxicological risk factor for human consumption. Nevertheless nutritional data will help to make comparison with other cacti in order to glimpse ecological relationships between different groups of taxa that use this and other cacti as breeding and/or feeding resource in xeric environments (e.g. Manfrin and Sene, 2006; Corio *et al.*, 2013). It is important to carry out more detailed analyses in the future, on individual, temporal and spatial variance and its relation with environmental variables. This approach will help to understand environmental factors that modulate the expression of nutritional values and thus the best conditions for cultivating this species (e.g. for possible commercial endeavors in the fruit production). Although it's key role as water, food and shelter resource in xeric environments presuppose it as a particularly important species to conserve ecologically fragile environments like deserts. Furthermore its narrow distribution range along with its slow development rate, high water storage capacity and hallucinogenic properties are features that make this species especially vulnerable to an increased rate of extraction, habitat fragmentation and extensive cattle ranching. Elucidation of the favorable thriving conditions for this species will be paramount for its conservation in the future.

Table 2. Fatty acid profile of *Trichocereus terscheckii* stems. Common names are given along with the nomenclature in the form C: D, where C is the number of carbon atoms and D is the number of double bonds in the fatty acid. N-X refers to a double bond is located on the *x*th carbon–carbon bond (counting from terminal methyl to the carbonyl carbon).

Fatty acid	Nomenclature	g/100 g fat content
Capric	(10:0)	0.09 g
Lauric	(12:0)	2.58 g
Myristic	(14:0)	1.54 g
Pentadecanoic	(15:0)	0.29 g
Pentadecenoic	(15:1) cis	0.49 g
Palmitic	(16:0)	19.18 g
Palmitoleic	(16:1)	0.62 g
Margaric	(17:0)	1.72 g
Heptadecenoic	(17:1)	0.10 g
Stearic	(18:0)	2.96 g
Trans elaidic	(18:1) trans	0.31 g
Oleic	(18:1) n-9	6.42 g
Cis-vaccenic	(18:1) n-7	5.51 g
Other cis		0.25 g
Trans linoelaidic	(18:2) trans	0.18 g
Linoleic	(18:2) n-6	22.6 g
α-Linolenic	(18:3) n-3	28.58 g
Gadoelic	(20:1)	0.31 g
Arachidic	(20:0)	0.45 g
Behenic	(22:0)	1.18 g
Arachidonic	(20:4)	0.57 g
Lignoceric	(24:0)	1.63 g
Total saturated		31.62 g
Total monounsaturated fatty acids		13.70 g
Total polyunsaturated fatty acids		51.21 g
Total trans fatty acids		0.49 g
Total unidentified minor components		2.98 g

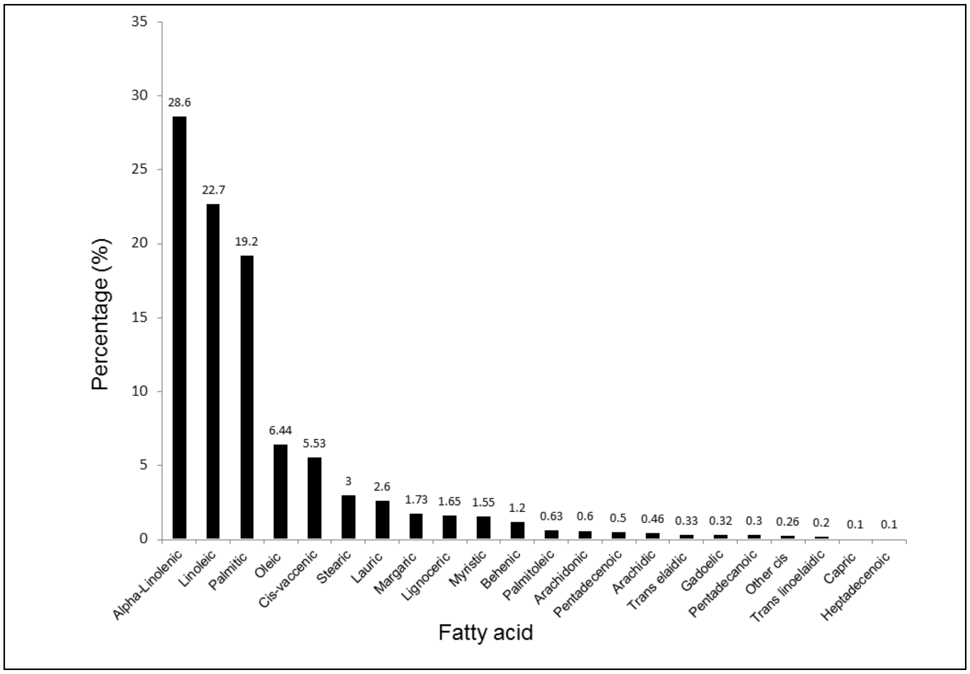

Figure 3. Relative abundance (percentage) of each type of identified fatty acid present in *Trichocereus terscheckii* stems. The first seven majoritarian fatty acids jointly represent over 90 % of the total mass.

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