

Desert Plant for Saline and Drought Stricken Farmland: Assessment of *Opuntia cactus* Nutritional Characteristics

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Abstract

Cactus pear is remarkable for its ability to tolerate arid saline environments that are recognized as stressful for most plant species. In addition, cactus pear can be cultivated with minimum agriculture inputs and thus has great potential for cultivation and production on degraded lands. In this three-year study, we assessed the physiological responses relative to nutraceutical quality in fruit juice extracted from fruit of *Opuntia ficus-indica* (USDA no. 248 and no. 255) that has been cultivated in saline, Se and B rich soils in the west side of the San Joaquin Valley in Central California.

Results indicate that the two selected accessions i.e., no.248 and no. 255, of *Opuntia ficus-indica* can well tolerate saline, Se and B impacted soils. Despite growing under high saline conditions the nutritional characteristics in fruit juice (as analyzed in this study, e.g., nutrients, total phenolics, ascorbic acid, pigments and flavonoids) of both accessions, were not affected by long-term (3 years) exposure to excessive salinity and B. In addition, juice extracted from fruits collected from plants grown on the saline, Se and B rich soil and drainage sediment showed significantly higher concentrations of Se relative to juice from fruits collected from plants grown on non-saline (control) soil.

Keywords: Cactus Prickly Pear, Salinity, Selenium, Ascorbic Acid, Boron, Drought

Introduction

Cactus prickly pear (*Opuntia ficus-indica* (L.) Mill.) is native to the American Southwest and has been introduced in many other countries in the Mediterranean and western Pacific regions. The plant typically grows wild in desert and arid regions. The renewed interest in cultivation of cactus prickly pear can be ascribed to its multi-functionality as food, feed and medicinal and therapeutic uses [1]. Cactus prickly pear fruit is known to contain several useful chemical compounds that have desirable nutritional and medicinal properties, e.g., antioxidants, phenolics, and vitamins [2].

Various commercial *Opuntia* clones have phenological, physiological and structural adaptations favoring survival in arid environments, in which water is the main factor limiting the development of most plant species [3]. Cactus pear is remarkable for its ability to tolerate arid saline environments that are recognized as stressful for most plant species [4]. In addition, cactus pear can be cultivated with minimum agriculture inputs and thus holds great potential for cultivation and production on degraded lands. In the west side of San Joaquin Valley (SJV) in Central California, soil salinization is a problem that threatens crop production [5]. Soils in the west side of the SJV were derived from Cretaceous shale rock and contain high levels of naturally occurring selenium (Se) oxyanions,

sulfate, and boron (B) salts. In addition, Central California has been subject to recurrent severe drought from 2011 to 2015. Consequently, farmers in Central California are drilling more and deeper wells than ever before to pump water for the irrigation of their crops, fruit and nut orchards. Extensive drought causes economic losses for agriculture-based communities and is a threat to world food security. Hence, it is important that alternative solutions are identified and offered to farmers in the west side of the SJV to prevent thousands of acres of farmland from going fallow due to excessive soil salinization and water scarcity. Agricultural strategies, such as identifying new seed varieties, irrigation technologies, and innovative agronomic practices, as well as accepting greater diversity of drought-tolerant crops, are needed to increase survival of farming in this part of California.

Opuntia ficus-indica may become an alternative crop that is well suited for cultivation in the arid growing conditions found in the west side of the SJV. In this regard, [4] We have selected accessions of *Opuntia ficus-indica* that were able to tolerate high salinity, Se and B levels present in drainage sediment collected from the San Luis Drain, near Mendota (CA). These authors documented the potential of *Opuntia ficus-indica* to tolerate growing in poor quality drainage sediment and to accumulate and gently volatilize Se and thereby reduce Se concentration in these sediments [6,7].

In this study, we assessed the physiological responses, relative to fruit nutraceutical quality extracted from fruit juice of *Opuntia ficus-indica* (USDA no. 248 and no. 255) that have been cultivated in saline, Se and B rich soils and drainage sediment for at least three years.

Opuntia ficus-indica (USDA no. 248) is potentially an attractive crop for farmers in the west side of the SJV because its dark purple fruit may have medicinal benefits, and importantly this accession (among many tested) tolerates drought, salt, and B, while accumulating and volatilizing Se [4].

Opuntia ficus-indica (USDA no. 255) produces red fruit but the accession appears to be most salt and B tolerant and produces fruit later than other accessions.

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We measured the total phenolic concentration and composition of polyphenol compounds, color, pH, soluble solids, and concentration of mineral nutrients in whole fruit juice produced from fruit grown at three different field locations during 2012-2014, respectively.

Materials and Methods

Field sites

Three field sites (considered as treatments in the text) were used in this study containing the following: 1) a saline drainage sediment with high Se and B concentrations; 2) a saline, Se and B rich soil located at Red Rock Ranch, Five Points, CA; and 3) a non-saline sandy loam soil (control). The three sites were described as follows: 1) the saline rich drainage sediment with high Se and B concentrations was collected from the top 25-cm layer of residual sediment in the San Luis Drain, near Mendota, CA. The collected sediment was spread to a depth of 40 cm in an excavated field plot at the USDA Research Facility in Parlier, CA [8]. The drainage sediment was covered with a 4-cm layer of non-saline sandy loam soil to enhance biological activity, plant growth and survival; 2) the saline field site at Red Rock Ranch was located at Five Points, CA, in the west side of the SJV. The soil is classified as an Oxalis silty clay loam (fine montmorillonitic, thermic PachicHaploxeral with a well-developed salinity profile). The soil contains naturally high concentrations of salts, e.g., Na_2SO_4 , NaCl , CaCl_2 , Na_2SeO_4 , CaSO_4 , $\text{Na}_2\text{B}_4\text{O}_5(\text{OH})_4$, and $\text{CaB}_3\text{O}_4(\text{OH})_3$; and 3) the non-saline sandy loam soil (control) located at the USDA Research Facility in Parlier CA is classified as a Hanford sandy loam (coarse-loamy, mixed, superactive, non-acid, thermic Typic Xerothents) field soil with a sand/silt/clay distribution of 55%, 40%, and 5%, respectively. The non-saline control plots were prepared adjacent to the drainage sediment plots (field and plot description described later). See Table 1 for general soil chemical parameters at the respective growing sites. Normal agronomic management practices were applied on all test plots at all three field locations throughout each growing season, including applying a non-sulphur containing ammonium nitrate fertilizer at an application rate of 50 kg/ha per year. All plots were drip-irrigated using a surface-drip irrigation system consisting of one in-line turbulent flow emitter per bed with an emitter spacing of 0.45 m and a flow rate of 4L/h. The plants were irrigated with good quality water (electrical conductivity $\text{EC} < 0.8 \text{ dSm}^{-1}$) at the saline drainage sediment and the non-saline (control) plots. Irrigation was based on the rate of evapotranspiration (ET) losses recorded at the CIMIS weather station located 2 km away at the University of California (UC) Kearney Research Station in Parlier, CA. At the RRR field site, poor quality water (e.g., EC ranging 4-7 dS m^{-1} , B and Se concentrations ranging 3-6 mg L^{-1} and 0.100-0.125 mg L^{-1} , respectively) was applied with drip irrigation based on the rate of ET losses recorded at the CIMIS weather station at the UC west side Research Station located at Five Points, CA.

Selection, propagation, and plantation

Spineless prickly pear cactus (*Opuntia ficus-indica* (L.) Mill. accessions no. 248 and no. 255, were originally selected from individuals growing at the USDA-ARS National Arid Land Genetic Resources Unit in Parlier,

CA, that originated in Mexico and Chile, respectively. Accessions no. 248 and no. 255 were selected for both their salt and B tolerance after growing for multiple growing seasons in agricultural drainage sediment [4], and importantly for the fruits' red and purple colors [9]. Fruits of these colors have potentially higher economic value not only for their coloring potential as a source of natural colored compounds but also for their beneficial effects on consumers' health as excellent source of dietary antioxidants. Both accessions were then clonally propagated, as described below.

For plant establishment, cladodes of both accessions were collected and propagated by placing an uprooted cladode (a stem segment) in soil. A lime/copper fungicide mixture was placed on the cut ends to prevent infections. The cladodes were then transplanted into pots containing the control sandy loam soil (see Table 1 for description of soil chemical characteristics). The cladodes were grown under natural growing conditions (200-400 $\mu\text{mol s}^{-1}$ light intensity, temperature ranging from 17-24 C, and 16/8 h light/dark period with circulated air) for 2 months within the greenhouse. Initially, the planted cladodes in the pots were grown without irrigation until new sprouts appeared for several weeks and then they were lightly watered with 1/5 strength Hoagland's solution. After two months, when the new sprouts started appearing, the pots were placed outdoor at the USDA Research Facility in Parlier, CA, for 1 month. After 3 months of initial root establishment, the plants were transplanted into the saline drainage sediment, saline soil at RRR, and non-saline (control) sandy loam soil. Each 30m x 1m plot contained 10 plants spaced 1.2 m from each other and was replicated four times in different plots in the saline drainage sediment and three times in the non-saline soil.

Fruit harvest and sample preparation

After three years of growth, the first fruit were handpicked from all plants in each plot at their respective growing site in 2012, 2013, and 2014. Fruit samples were washed and rinsed using DI water, dried with a paper towel, Ziploc bagged, and frozen at -20 C. Whole fruit were juiced using an electrical juicer (Hamilton Beach, Virginia, USA) that separates the peel, pulp and seeds from the juice.

In contrast to other studies [4,7], only the fruit juice was used for this study because the fruit collected from the plants grown on saline, B and Se rich soils are suitable for juice processing sold as fresh products for the market. The juice was stored in the freezer (-80 C) until analyses were performed, as described below.

Soluble solids, pH and color measurements in juice

Soluble solids (SS) content (Brix°) in fruit juice was determined using a digital refractometer (3810 PAL-1, Atago, Tokyo, Japan) and expressed as %. The pH was determined in fruit juice at room temperature using an Orion 420A pH meter (Thermo scientific, Waltham, USA).

The color of fruit juice was determined using a Chroma Meter CR-400 optical sensor (Konica Minolta Sensing, Inc., Osaka, Japan) according to the CIE Lab [10]. The system provides the values of three color components; L^* , and the chromaticity coordinates, a^* and b^* [11].

Table 1: Average total and extractable Se and other chemical parameters from 0 to 25 cm in various soil treatments (RRR = saline, Se and B rich soil at Red Rock Ranch, Sediment = saline drainage sediment plots, and Control = non-saline control soil). Data represent average of five replicates and standard error in parenthesis

Soil treatment	Total Se ($\mu\text{g g}^{-1}$)	Water extractable Se	Water extractable B	Water extractable Na	EC (dS m^{-1})	pH
		($\mu\text{g ml}^{-1}$)	($\mu\text{g ml}^{-1}$)	($\mu\text{g ml}^{-1}$)		
RRR	4.0 (0.3)	0.2 (0.003)	10 (0.25)	1469 (0.2)	10 (0.6)	8.0 (0.06)
Sediment	1.8 (0.5)	0.9 (0.1)	5.9 (0.8)	525 (95)	4.8 (0.6)	7.9 (0.01)
Control	< 0.1 (0)	< 0.1 (0)	0.1 (0.01)	85 (17)	0.7 (0.1)	7.6 (0.01)

Results were recorded as CIELAB values: L^* (brightness), a^* (redness), b^* (yellowness), c^* (chroma), and h^* (hue). The instrument was calibrated using a standard white and a standard black reflective plate. Each color value reported is the mean of three determinations.

Water soluble mineral nutrients, electrical conductivity, and pH in soil

Water soluble nutrients concentrations (expressed as mg L^{-1}), chloride, salinity [EC as dS m^{-1}] and pH were determined in a 1:1 soil-water extract. Water soluble Se was analyzed using an Agilent ICP-MS (Santa Clara, CA) and the other soluble ions were measured using Varian Vista ICP-OES (Palo Alto, CA) after preparing the samples, as described by [12]. Soil EC was measured at room temperature using an Orion Model 150 Conductivity Meter (Thermo Scientific, Waltham, USA), and pH was determined at room temperature using an Orion 420A pH meter (Thermo Scientific, Waltham, USA).

Total mineral nutrients

A standard procedure was used to determine the mineral element concentrations in juice samples [4]. Fruit juice samples were wet acid digested with $\text{HNO}_3\text{-H}_2\text{O}_2\text{-HCl}$, as described by [13]. NIST coal fly ash (SRM 1633; Se content of $10.3 \pm 0.6 \text{ mg kg}^{-1}$, with a recovery of 93%) and NIST wheat flour (SRM 1567; Se content of $1.1 \pm 0.2 \text{ mg kg}^{-1}$, with a recovery of 94%) were used as an external quality control standards for the soil and plant material, respectively. Selenium and other elements were analyzed by an inductively-coupled plasma optical emission spectrometer (Agilent 7500cx, Santa Clara, USA) according to Agilent manufacture protocol.

Ascorbic acid

Ascorbic acid was quantified using a Shimadzu high-performance liquid chromatography (HPLC) device equipped with a Supelco C-610H ion-exchange column for separation. An isocratic flow of 0.1 mL/min 0.1% (v/v) phosphoric acid (from Sigma-Aldrich, St. Louis, MO) was used. Detection and quantification was made at 210 nm using a Shimadzu UV/Vis detector, with ascorbic acid standards (from Sigma) used for peak identification and to convert peak areas to gram amounts.

Total phenolics

Total phenolic concentrations were measured according to [14] using the Folin-Ciocalteu reagent assay. Absorbance was measured at 756 nm using a Spectra Max plus 384 spectrophotometer (Molecular Devices, Sunnydale, CA). Total phenols concentration were standardized against gallic acid (GA) and expressed as milligram of gallic acid equivalents (GAE) per L of fruit juice. The linearity range for this assay was determined as 50-250 mg L^{-1} GA, giving absorbance range of 0.5-2.55 AU.

Composition of polyphenols by liquid chromatography

The analysis of single phenolics was carried out to identify variation in specific compounds (pigments and polyphenols) because analysis of total phenolic comprises the bulk of all antioxidants and does not discriminate for single compounds. The Folin-Ciocalteu reagent also reacts with free phenylalanine and tyrosine, as well as proteins containing these amino acids.

In the juice samples, phenolic compounds were analyzed using a Shimadzu (Columbia, MD) high-performance liquid chromatography (HPLC) system equipped with a Shimadzu XR-ODS C18 column and a photodiode array detector (PDA) and wave lengths were set at 280 nm (for non-anthocyanins) and 520 nm (for betalains). A binary gradient was used, proceeding from 95% water (with 0.2% acetic acid) to 100% methanol (with 0.2% acetic acid) and back again over a 40 minute run time. All solvent reagents were obtained from Sigma [15]. Compounds were identified as previously described using a similar gradient and running on a HPLC equipped with a PDA to match UV/V is spectra and

Shimadzu LCMS-2020 mass spectrometer to derive molecular weights of quantified peaks [16,17,15]. In addition, standards of betanin and myricetin (obtained from Sigma) further confirmed identities of those compounds. Peak areas were converted to gram amounts using standard curves of quercetin-glucoside (from Sigma) for all flavonoid glycosides, and betanin for all betalains.

Statistical Analysis

Results were examined by factorial analysis of variance (ANOVA) with year, accessions, and growing location as main factors influencing the biochemical and quality parameters evaluated. Statistically significant differences were assumed for $P \leq 0.05$ and calculated using Last Significance Differences comparison test. Statistical data analysis was performed using Gretl (Gnu Regression, Econometrics and Time-series Library) [18].

Results and Discussion

Fruit juice pH, soluble solids and color

Fruit produced from all growing locations were collected and evaluated to determine if any positive or negative responses were observed on nutritional quality in fruit juice when growing accessions no. 248 and no. 255 under poor growing soil conditions for at least three years. The following parameters were analyzed: fruit juice pH, soluble solids and color, mineral nutrients, ascorbic acid content, total phenolics, and polyphenols. Fruit juice pH ranged 4.7-5.7 (Table 2) and soluble solid values were significantly higher in 2013 for all treatments, and values were significantly lower in no. 248 and no. 255 in 2012 and in no. 248 in 2014 on saline soil at RRR. Juice pH measured in this study was similar to that reported by [19,20] in various clones of *Opuntia ficus-indica* from Mexico. Values of soluble solids ranged 9.3-14.0% (measure of maturity and related to sugar content) and were similar to the values reported by [21,20]. There were no significant differences in soluble solids concentrations among treatments and accessions.

Fruit of no. 248 are purple and fruit of no. 255 are red. Color values b^* and c^* were similar among treatments within the same accession indicating that the purity of the color were the same among treatments (Table 3). L^* , h^* , and a^* values of the same accession were similar among treatments (Table 3). Differences in color values between accessions are attributed to the different color (purple no. 248 vs. red no. 255). The fruit color is of importance for consumers and absence of effect of treatment on fruit color is important to farmers. Red and purple fruit are considered to be the most desirable colors for prickly pear fruit and are the colors used by the natural dye industry [22].

Mineral nutrients

Concentrations of mineral nutrients in fruit juice produced from all treatments are shown in Table 4 and Table 5. Irrespective of treatment, there were no significantly different concentrations of Ca, K, Cu, Mn, and Zn. Magnesium concentration in fruit juice was significantly higher in fruit juice collected in 2012 and 2014 from both accessions grown on the saline soil at RRR (Table 4). The concentration of P was significantly higher in fruit juice collected from plants grown on the non-saline (control) treatment (Table 4). Sulphur concentration was higher in fruit juice collected from both accessions grown on saline drainage sediment in 2013 and 2014 (Table 4). In this study, the highest concentrations of Ca and Mg were observed in fruit juice of plants grown on saline, Se and B rich soil at RRR (Table 4).

Except for no. 248 (in 2014), Na concentrations were always greater in saline treatments (Table 4). The soil at RRR and drainage sediment contain high concentrations of soluble Na that are readily taken up by the plants.

Iron concentration was significantly lower irrespective of growing site

Table 2: pH and soluble solids (SS, % Brix) measured in fruit juice of prickly pear cactus (accessions no. 248 and no. 255) grown in various soil treatments (RRR = saline, Se and B rich soil at Red Rock Ranch, Sediment = saline drainage sediment plots, and Control = non-saline control soil). Fruit were collected in 2012, 2013, and 2014. Data represent average \pm standard error ($n=3$ or more than 3 depending on productivity (presence of fruit) for each replication in each year), respectively. Similar letters indicate no statistical ($P \leq 0.05$) difference among treatments and all year for each juice parameters

Juice parameters	Year	Treatment ¹					
		248 Control	248 Sediment	248 RRR	255 Control	255 Sediment	255 RRR
Juice pH	2012	5.47 \pm 0.36b	n.d.	5.00 \pm 0.11c	n.d.	5.55 \pm 0.12b	4.91 \pm 0.07c
	2013	5.60 \pm 0.10a	5.67 \pm 0.03a	5.68 \pm 0.03a	5.84 \pm 0.07a	5.77 \pm 0.04a	5.42 \pm 0.16b
	2014	5.32 \pm 0.06b	5.40 \pm 0.06b	4.78 \pm 0.07c	5.26 \pm 0.07b	5.43 \pm 0.03b	n.d.
Soluble solids (Brix, %)	2012	12.1 \pm 0.35a	n.d.	9.37 \pm 0.62ab	n.d.	9.33 \pm 0.77ab	12.1 \pm 0.35a
	2013	12.5 \pm 1.66a	13.5 \pm 0.32a	13.6 \pm 0.58a	14.0 \pm 0.25a	13.7 \pm 0.50a	12.9 \pm 1.31a
	2014	13.6 \pm 1.29a	12.2 \pm 0.48a	10.3 \pm 0.58a	11.0 \pm 0.33a	12.6 \pm 0.49a	n.d.

n.d. = no samples were collected

¹Treatment (growing locations: see materials and methods) includes Opuntia accession number

Table 3: Color parameters (L^* , a^* , and b^*) measured in fruit juice of prickly pear cactus (accessions no. 248 and no. 255) grown in various soil treatments (RRR = saline, Se and B rich soil at Red Rock Ranch, Sediment = saline drainage sediment plots, and Control = non-saline control soil). Fruit were collected in 2012, 2013, and 2014. Data represent average \pm standard error ($n=3$ or more than 3 depending on productivity (presence of fruit) for each replication in each year), respectively. Similar letters indicate no statistical ($P \leq 0.05$) difference among treatments and all years for each respective color

Color parameters	Year	Treatment ¹					
		248 Control	248 Sediment	248 RRR	255 Control	255 Sediment	255 RRR
L^*	2012	18.5 \pm 2.02a ²	n.d.	20.1 \pm 1.90a	n.d.	25.8 \pm 0.49a	28.0 \pm 1.45a
	2013	17.8 \pm 0.29ab	18.2 \pm 0.23a	21.7 \pm 4.49a	27.9 \pm 1.35a	28.9 \pm 0.79a	28.2 \pm 0.81a
	2014	29.6 \pm 11.7a	27.4 \pm 7.30a	17.3 \pm 0.49a	25.0 \pm 0.61	27.6 \pm 2.50a	n.d.
h	2012	13.9 \pm 1.24b	n.d.	26.3 \pm 10.2b	n.d.	68.3 \pm 2.42a	75.0 \pm 5.28a
	2013	15.8 \pm 1.67b	16.0 \pm 0.60b	20.8 \pm 5.85b	70.5 \pm 4.34a	67.5 \pm 1.29a	68.1 \pm 4.48a
	2014	28.9 \pm 18.1b	39.2 \pm 22.1b	9.0 \pm 0.68b	10.1 \pm 0.87b	13.9 \pm 5.25b	n.d.
a^*	2012	8.62 \pm 1.06a	n.d.	8.62 \pm 0.77a	n.d.	5.68 \pm 0.74a	4.85 \pm 1.68a
	2013	14.6 \pm 2.27a	15.0 \pm 1.38a	10.4 \pm 1.38a	6.14 \pm 1.23a	9.21 \pm 0.85a	7.22 \pm 1.41a
	2014	23.1 \pm 19.2a	32.2 \pm 22.3a	22.9 \pm 0.24a	15.1 \pm 0.75a	20.2 \pm 5.05a	n.d.
b^*	2012	2.16 \pm 0.46b	n.d.	6.01 \pm 3.19b	n.d.	14.2 \pm 1.28a	18.3 \pm 1.09a
	2013	4.32 \pm 1.03b	4.47 \pm 0.57b	4.05 \pm 1.07b	18.1 \pm 1.19a	22.1 \pm 1.51a	18.3 \pm 1.22a
	2014	11.3 \pm 1.20b	8.92 \pm 0.44b	9.26 \pm 0.71b	18.4 \pm 0.65a	17.5 \pm 0.57a	n.d.
c^*	2012	8.89 \pm 1.14c	n.d.	11.8 \pm 2.20c	n.d.	15.4 \pm 1.34b	19.1 \pm 0.89b
	2013	15.3 \pm 2.46c	15.6 \pm 1.48c	11.8 \pm 1.22c	19.4 \pm 0.85b	24.0 \pm 1.65b	20.1 \pm 1.07b
	2014	16.9 \pm 4.30c	13.4 \pm 0.56c	14.2 \pm 0.57c	56.2 \pm 3.00a	57.5 \pm 2.64a	n.d.

n.d.= no samples were collected

¹ Treatment (growing locations: see materials and methods) includes Opuntia accession number

² For this treatment/accession $n=2$

Table 4: Concentrations of macro-nutrients ($g L^{-1}$) detected in fruit juice of prickly pear cactus (accessions no. 248 and no. 255) grown in various soil treatments (RRR = saline, Se and B rich soil at Red Rock Ranch, Sediment = saline drainage sediment plots, and Control = non-saline control soil). Fruit were collected in 2012, 2013, and 2014. Data represent average \pm standard error ($n=3$ or more than 3 depending on productivity (presence of fruit) for each replication in each year), respectively. Similar letters indicate no statistical ($P \leq 0.05$) difference among treatments and all years for each mineral nutrient

Nutrient	Year	Treatment ¹					
		248 Control	248 Sediment	248 RRR	255 Control	255 Sediment	255 RRR
Ca	2012	0.79 \pm 0.48a ²	n.d.	1.28 \pm 0.33a	n.d.	0.82 \pm 0.14a	1.59 \pm 0.44a
	2013	0.53 \pm 0.07ab	0.59 \pm 0.10ab	0.65 \pm 0.13ab	0.69 \pm 0.12ab	0.48 \pm 0.09ab	0.58 \pm 0.06ab
	2014	0.51 \pm 0.05a	0.79 \pm 0.03a	1.08 \pm 0.15a	0.57 \pm 0.03a	0.67 \pm 0.02a	n.d.
K	2012	1.95 \pm 0.19a	n.d.	1.69 \pm 0.14a	n.d.	2.19 \pm 0.33a	2.33 \pm 0.16a
	2013	1.71 \pm 0.06a	2.04 \pm 0.25a	2.01 \pm 0.18a	1.99 \pm 0.10a	1.99 \pm 0.23a	2.36 \pm 0.19a
	2014	1.71 \pm 0.11a	1.90 \pm 0.09a	1.84 \pm 0.12a	1.61 \pm 0.10a	2.01 \pm 0.05a	n.d.
Mg	2012	0.45 \pm 0.04b	n.d.	0.52 \pm 0.07a	n.d.	0.29 \pm 0.04b	0.59 \pm 0.11a
	2013	0.35 \pm 0.04b	0.34 \pm 0.06b	0.37 \pm 0.05b	0.32 \pm 0.06b	0.25 \pm 0.03b	0.37 \pm 0.01b
	2014	0.25 \pm 0.01b	0.24 \pm 0.01b	0.52 \pm 0.05a	0.33 \pm 0.03b	0.22 \pm 0.07b	n.d.
P	2012	0.19 \pm 0.00a	n.d.	0.10 \pm 0.01b	n.d.	0.11 \pm 0.00b	0.13 \pm 0.04b
	2013	0.13 \pm 0.02a	0.09 \pm 0.01b	0.07 \pm 0.01b	0.17 \pm 0.02a	0.09 \pm 0.01b	0.08 \pm 0.01b
	2014	0.18 \pm 0.01a	0.11 \pm 0.01b	0.07 \pm 0.01b	0.15 \pm 0.01a	0.12 \pm 0.00b	n.d.
Na	2012	0.85 \pm 0.56c	n.d.	0.90 \pm 0.14b	n.d.	0.60 \pm 0.06b	0.93 \pm 0.30b
	2013	0.23 \pm 0.07c	0.60 \pm 0.17c	1.13 \pm 0.48b	0.59 \pm 0.16c	0.38 \pm 0.04b	1.18 \pm 0.22b
	2014	1.14 \pm 0.20b	0.83 \pm 0.12b	2.51 \pm 0.41a	1.18 \pm 0.29b	0.95 \pm 0.12b	n.d.
S	2012	0.06 \pm 0.00b	n.d.	0.12 \pm 0.00b	n.d.	0.12 \pm 0.01b	0.13 \pm 0.01b
	2013	0.06 \pm 0.00b	0.99 \pm 0.02a	0.09 \pm 0.01b	0.10 \pm 0.00b	0.79 \pm 0.01a	0.09 \pm 0.00b
	2014	0.07 \pm 0.00b	0.89 \pm 0.00a	0.11 \pm 0.01b	0.11 \pm 0.00b	0.10 \pm 0.00b	n.d.

n.d. = no samples were collected

¹ Treatment (growing locations: see materials and methods) includes Opuntia accession number

² For this treatment/accession $n=2$

and accession in 2013 and 2014 (Table 5). Boron concentration was lower in fruit juice produced from plants grown non-saline (control) relative to the saline treatments at RRR and drainage sediment treatment (Table 5). Boron concentration was insignificantly higher in fruit of plants grown in drainage sediment, even though the amount of water extractable B in drainage sediment is lower than in the soil at RRR (Table 5).

Results of mineral elements measured in fruit juice from this study were comparable to those reported by [23,24] in cactus prickly pear and *Myrtillocactus* fruit, respectively. Fruit from *Opuntia ficus-indica* is characterized by high amounts of Ca (up to 0.59 mg kg⁻¹) and Mg (0.98 mg kg⁻¹) [3]. Among all the elements analyzed, Se concentrations were significantly higher in the fruit juice collected from plants grown on saline drainage sediment relative to the saline soil at RRR except for no. 255 in 2013 (Figure 1). Even though irrigation water contained selenium and other salts at RRR, the water contains high sulphate concentrations, which inhibits Se uptake from the soil into the plant. In contrast, the drainage sediment plot received good quality water and there was little inhibition of selenium uptake. Selenium concentrations were always significantly lower in fruit juice produced from plants grown on non-saline (control) treatments (Figure 1), where very little Se was present in the soil.

The Se concentrations reported in this study for fruit juice were lower than those reported by [4] for fruit skin and flesh of accession no. 248 (≈2 mg Se kg⁻¹d.w.) grown on the same drainage sediment. [6] showed that most of the Se is stored in the seed and the pulp of the cactus fruit. Fruit of *Opuntia ficus-indica* have (been shown) to accumulate Se mostly as free seleno-cystathionine and Seleno-methionine [5]. Selenium is an essential micronutrient for humans and animals [6], and hence, drinking Se-enriched fruit juice may have extra health value for Se-deficient diets.

Ascorbic acid, total phenolic compounds, and polyphenols

Ascorbic acid in fruit juice ranged 0.8-1.7 mg ml⁻¹, irrespective of treatment and accession (Figure 2). The lowest concentration of ascorbic acid was reported in fruit juice of no. 248 grown in the saline, Se and B rich soil at RRR. The values of ascorbic acid reported in this study for plants grown on saline, Se and B rich soil at RRR were, however, comparable to those reported by [25] in *Opuntia ficus-indica* fruit from Argentina.

Concentrations of vitamin C in cactus pear fruit are higher than other common fruit such as apple, pear, grape, and banana [3].

In this study, the concentration of total phenolics ranged between 500 and 1000 mg GAE L⁻¹ (Figure 3) and there were no significant differences for both accessions among treatments. Similar total phenolic concentrations were reported by [19] in fruit juice extracts from *Opuntia ficus-indica*.

These results from the fruit juice are one order of magnitude lower than those reported by [7,26,27] for whole fruit extracts of *Opuntia ficus-indica*. One possible explanation why no significant differences were observed among treatments for ascorbic acid, total phenolics, and polyphenols (see below) could be that all plants in the San Joaquin Valley (CA) suffered a recurrent historic drought (starting in 2010). Precipitation was extremely low during the growth cycle (April to October, below 1mm) and temperatures were very high (between 30 and >40 C) during the summer months. These climatic conditions may have overwhelmingly affected the productivity and nutritional quality of all plants, irrespective of growing site. Another plausible explanation is that the high salinity and B levels in the soil at RRR and in drainage sediment were not high enough to negatively affect ascorbic acid, total phenolics and/or polyphenols production.

Concentrations of pigments and flavonoids analyzed in fruit juice (Table 6) were generally similar (with few exceptions) among saline and non-saline (control) treatments for both accessions. No clear pattern was observed among growing sites and years for both accessions for all the polyphenols analyzed as shown in Table 6. It would be necessary to test the plants under a controlled environment to isolate multi-environmental factors that may have contributed to the variation of polyphenols concentrations in fruit juice observed in this field experiment.

Betanin, a betalain pigment, had the highest concentration in both accessions among all compounds analyzed (Table 6). These results are consistent with previous studies of betalain pigments of prickly pear cactus fruit [28,29,30].

Our data confirm the potential of prickly pear fruit juice as a source of natural healthy colorants that are rich in antioxidants and may provide protection against oxidative damage. Betanin is an antioxidant apparently

Table 5: Concentrations of micro-nutrients (mg L⁻¹) detected in fruit juice of prickly pear cactus (accessions no. 248 and no. 255) grown in various soil treatments (RRR = saline, Se and B rich soil at Red Rock Ranch, Sediment = saline drainage sediment plots, and Control = non-saline control soil). Fruit were collected in 2012, 2013, and 2014. Data represent average ± standard error (n=3 or more than 3 depending on productivity (presence of fruit) for each replication in each year), respectively. Similar letters indicate no statistical (P ≤ 0.05) difference among treatments and all years for each nutrient

Nutrient	Year	Treatment ¹					
		248 Control	248 Sediment	248 RRR	255 Control	255 Sediment	255 RRR
B	2012	2.22 ± 0.11b ²	n.d.	8.05 ± 2.02a	n.d.	14.0 ± 2.34a	10.3 ± 2.03a
	2013	2.37 ± 0.35b	15.7 ± 2.7a	9.83 ± 1.96a	3.30 ± 1.00b	12.7 ± 1.58a	13.8 ± 2.71a
	2014	2.76 ± 0.20b	12.5 ± 1.08a	10.0 ± 1.01a	3.30 ± 0.89b	9.61 ± 0.50a	n.d.
Cu	2012	0.84 ± 0.00a	n.d.	0.46 ± 0.08a	n.d.	0.43 ± 0.05a	0.43 ± 0.05a
	2013	0.47 ± 0.05a	0.47 ± 0.05a	0.38 ± 0.07a	0.43 ± 0.02a	0.54 ± 0.15a	0.30 ± 0.03a
	2014	0.41 ± 0.03a	0.40 ± 0.02a	0.44 ± 0.04a	0.35 ± 0.03a	0.29 ± 0.01a	n.d.
Fe	2012	1.91 ± 0.26a	n.d.	2.13 ± 0.62a	n.d.	2.74 ± 0.06a	1.98 ± 0.13a
	2013	1.02 ± 0.12b	1.15 ± 0.10b	1.10 ± 0.25b	1.75 ± 0.34b	1.10 ± 0.14b	1.38 ± 0.22b
	2014	0.92 ± 0.09b	1.26 ± 0.07b	1.29 ± 0.32b	1.45 ± 0.23b	1.48 ± 0.10b	n.d.
Mn	2012	8.95 ± 1.47a	n.d.	6.08 ± 1.81b	n.d.	3.95 ± 0.22bc	5.28 ± 0.72b
	2013	3.73 ± 0.15c	2.91 ± 0.64c	3.09 ± 0.76c	6.42 ± 0.56b	3.18 ± 0.94c	3.25 ± 0.71c
	2014	5.46 ± 0.69b	4.27 ± 0.39bc	3.57 ± 0.76c	5.78 ± 0.79b	3.22 ± 0.31c	n.d.
Zn	2012	1.24 ± 0.22a	n.d.	1.14 ± 0.24a	n.d.	0.88 ± 0.05a	0.99 ± 0.10a
	2013	1.23 ± 0.15a	0.73 ± 0.06a	1.00 ± 0.19a	1.04 ± 0.04a	0.71 ± 0.06a	0.78 ± 0.04a
	2014	1.28 ± 0.09a	0.88 ± 0.04a	0.87 ± 0.08a	1.00 ± 0.06a	0.80 ± 0.02a	n.d.

n.d.= no samples were collected

¹ Treatment (growing locations: see materials and methods) includes *Opuntia* accession number

² For this treatment/accession n=2

Table 6: Concentration of polyphenols ($\mu\text{g g}^{-1}\text{f.w.}$) detected in fruit juice of prickly pear cactus (accessions no. 248 and no. 255) grown in various soil treatment (RRR = saline, Se and B rich soil at Red Rock Ranch, Sediment = saline drainage sediment plots, and Control = non-saline control soil). Fruit were collected in 2012, 2013, and 2014. Data represent average \pm standard error ($n=3$ or more than 3 depending on productivity (presence of fruit) for each replication in each year), respectively. Similar letters indicate no statistical ($P \leq 0.05$) difference among treatments and all years for each polyphenols

Polyphenols	Year	Treatment ¹					
		248 Control	248 Sediment	248 RRR	255 Control	255 Sediment	255 RRR
Indicaxanthin	2012	139 \pm 10.0b ²	n.d.	293 \pm 42.7a	n.d.	184 \pm 14.1ab	175 \pm 19.7ab
	2013	97.9 \pm 6.80bc	182 \pm 21.2ab	239 \pm 48.5a	138 \pm 8.65b	208 \pm 45.2ab	178 \pm 41.8ab
	2014	187 \pm 24.0ab	185 \pm 15.8ab	207 \pm 55.6ab	226 \pm 45.7ab	219 \pm 30.6a	n.d.
Betanin	2012	6559 \pm 229a	n.d.	5030 \pm 950ab	n.d.	4593 \pm 308ab	4110 \pm 44.6b
	2013	4617 \pm 809ab	5091 \pm 426ab	4060 \pm 753b	5200 \pm 477ab	5643 \pm 549ab	6385 \pm 1325a
	2014	4530 \pm 508ab	4970 \pm 494ab	5223 \pm 1088ab	4634 \pm 560ab	3853 \pm 455b	n.d.
Iso-betanin	2012	1718 \pm 108ab	n.d.	1013 \pm 125b	n.d.	1561 \pm 156b	1014 \pm 35.4b
	2013	1113 \pm 208b	1791 \pm 277ab	2631 \pm 517a	979 \pm 110c	762 \pm 44.6c	1031 \pm 155b
	2014	861 \pm 133c	1863 \pm 183ab	1951 \pm 366b	851 \pm 40.9c	798 \pm 36.1c	n.d.
Gomphrenin	2012	1283 \pm 31.2bc	n.d.	430 \pm 85.5d	n.d.	1561 \pm 156a	865 \pm 198c
	2013	776 \pm 129c	1791 \pm 277b	690 \pm 136d	1040 \pm 97.8bc	762 \pm 44.6c	1125 \pm 224bc
	2014	939 \pm 100c	1863 \pm 183b	828 \pm 146c	1122 \pm 170bc	3853 \pm 455a	n.d.
Diflavonoid glycoside	2012	237 \pm 8.35c	n.d.	239 \pm 73.6cd	n.d.	219 \pm 15.7cd	255 \pm 22.1c
	2013	156 \pm 10.2d	235 \pm 21.3c	189 \pm 38.7d	325 \pm 14.9b	352 \pm 22.3b	502 \pm 31.8a
	2014	267 \pm 29.3c	192 \pm 19.1d	320 \pm 42.1a	299 \pm 16.2b	287 \pm 27.5bc	n.d.
Myricetinglucoside	2012	1141 \pm 122b	n.d.	791 \pm 273bc	n.d.	219 \pm 15.7c	1210 \pm 452b
	2013	691 \pm 53.3bc	253 \pm 21.3c	594 \pm 116bc	1220 \pm 344b	352 \pm 22.3c	2709 \pm 382a
	2014	1508 \pm 216b	192 \pm 19.1c	706 \pm 162bc	2155 \pm 311a	287 \pm 27.5c	n.d.
Quercetage tintrimethyl glucoside	2012	182 \pm 11.6c	n.d.	180 \pm 78.9c	n.d.	234 \pm 19.9c	263 \pm 55.2c
	2013	162 \pm 18.8c	281 \pm 35.1c	175 \pm 33.8c	349 \pm 14.7bc	419 \pm 25.3b	656 \pm 113b
	2014	317 \pm 39.4bc	188 \pm 19.2c	233 \pm 28.5c	366 \pm 32.2bc	1280 \pm 130a	n.d.
Flavonol-3-O-methyl ether	2012	157 \pm 11.5bc	n.d.	96.4 \pm 53.7c	n.d.	162 \pm 16.0bc	102 \pm 45.0c
	2013	144 \pm 10.9bc	202 \pm 24.5b	152 \pm 32.7bc	257 \pm 21.2b	323 \pm 37.6a	328 \pm 38.6a
	2014	237 \pm 27.7b	138 \pm 13.7bc	216 \pm 32.1b	316 \pm 25.1a	195 \pm 21.1b	n.d.
Flavonoid glycoside 1	2012	133 \pm 12.3bc	n.d.	89.5 \pm 47.1c	n.d.	174 \pm 15.5bc	49.0 \pm 18.0c
	2013	63.3 \pm 6.35c	221 \pm 23.8b	122 \pm 26.8bc	211 \pm 40.7b	327 \pm 35.7a	332 \pm 70.5a
	2014	205 \pm 23.3bc	159 \pm 18.4bc	169 \pm 45.1bc	237 \pm 22.5b	164 \pm 21.4bc	n.d.
Flavonoid glycoside 2	2012	332 \pm 9.69b	n.d.	181 \pm 80.5c	n.d.	307 \pm 26.9b	457 \pm 20.1b
	2013	197 \pm 141c	369 \pm 54.5b	319 \pm 60.0b	358 \pm 93.1b	328 \pm 50.3b	857 \pm 176a
	2014	367 \pm 48.2b	254 \pm 29.2bc	349 \pm 61.1b	478 \pm 93.7b	403 \pm 32.8b	n.d.
Isorhamnetin-glucosyl-rhamnosyl-rhamnoside	2012	732 \pm 55.3b	n.d.	512 \pm 358c	n.d.	688 \pm 65.0c	867 \pm 248b
	2013	346 \pm 2.49c	692 \pm 100b	533 \pm 108c	836 \pm 89.3b	973 \pm 92.8b	1397 \pm 96.5a
	2014	915 \pm 124a	678 \pm 55.3b	1062 \pm 167ab	1368 \pm 166a	999 \pm 108b	n.d.
Isorhamnetin-rhamnosyl-rhamnoside	2012	143 \pm 21.5c	n.d.	237 \pm 178bc	n.d.	193 \pm 24.6bc	267 \pm 63.0b
	2013	49.8 \pm 1.64d	195 \pm 34.bc	150 \pm 32.9c	286 \pm 40.6b	341 \pm 44.9b	743 \pm 83.3a
	2014	307 \pm 44.6b	156 \pm 18.9c	291 \pm 53.8b	480 \pm 86.9b	316 \pm 47.9b	n.d.
Isorhamnetin glucosyl rhamnoside	2012	328 \pm 154b	n.d.	164 \pm 111c	n.d.	163 \pm 25.3c	53.6 \pm 0.39d
	2013	270 \pm 153b	174 \pm 25.7c	285 \pm 61.8b	380 \pm 98.2b	383 \pm 61.4b	760 \pm 105a
	2014	293 \pm 60.7b	125 \pm 9.24c	277 \pm 37.7b	379 \pm 237b	68.7 \pm 10.1d	n.d.

n.d.= no samples were collected

¹ Treatment (growing locations: see materials and methods) includes *Opuntia* accession number

² For this treatment/accession $n=2$

involved in a number of model systems of lipid oxidation. [31] found that very small concentrations of betanin, extracted from red beet, inhibited lipid peroxidation and heme composition. The authors concluded that inclusion of betanin-rich foods (i.e., red beet) in the diet might provide protection against certain oxidative stress-related disorders in humans.

Betalain is a widely used natural colorant in the food industry and betalains for food use are extracted from red beet (*Beta vulgaris* L.). However, the high concentrations of betalain found in fruit juice from *Opuntia ficus-indica* in this study make it an even better source of betalains than red beet [3].

Most flavonoid concentrations reported in this study (Table 6) are in the range 100- 600 mg g^{-1} and are comparable to data reported by [26] in fruit juice of *Opuntia ficus-indica*.

Conclusions

Overall, our study indicates that the two selected accessions no.248 and no. 255 of *Opuntia ficus-indica* can be grown under high saline, B, and drought conditions and their nutritional characteristics in fruit juice (as analyzed in this study e.g., nutrients, total phenolics, ascorbic acids and pigments, and flavonoids) are not affected by adverse growing conditions.

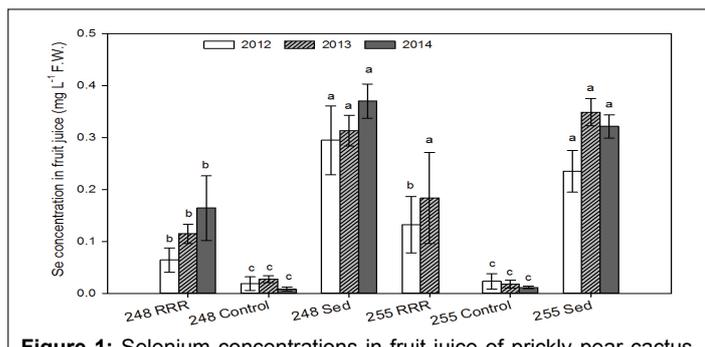


Figure 1: Selenium concentrations in fruit juice of prickly pear cactus (accessions no. 248 and no. 255) grown in various soils (RRR = saline, Se and B rich soil at Red Rock Ranch, Sed = saline drainage sediment plots, and control = non-saline control soil). Fruit were collected in 2012, 2013, and 2014. Bars and error bars represent average and standard error ($n=3$ or more than 3 depending on productivity (presence of fruit) for each replication in each year), respectively. Treatment 248 control consists of $n=2$. Similar letters indicate no statistical ($P \leq 0.05$) difference among treatments and all years

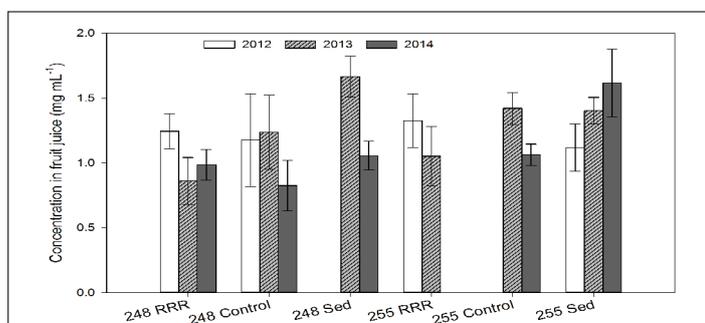


Figure 2: Ascorbic acid in fruit juice of prickly pear cactus (accessions no. 248 and no. 255) grown in various soils (RRR = saline, Se and B rich soil at Red Rock Ranch, Sediment = saline drainage sediment plots, and Control = non-saline control soil). Fruit were collected in 2012, 2013, and 2014. Bars and error bars represent average and standard error ($n=3$ or more than 3 depending on productivity (presence of fruit) for each replication in each year), respectively. Treatment 248 control consists of $n=2$. Similar letters indicate no statistical ($P \leq 0.05$) difference among treatments; when no letters are present then there were no significant differences among treatments and all years

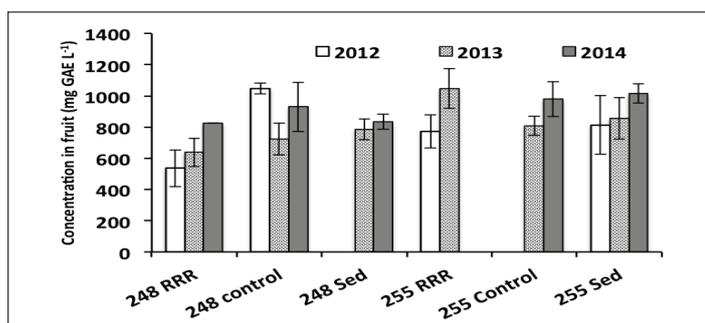


Figure 3: Concentration of total phenolics in fruit juice of prickly pear cactus (accessions no. 248 and no. 255) grown in various (RRR = saline, Se and B rich soil at Red Rock Ranch, Sediment = saline drainage sediment plots, and Control = non-saline control soil). Fruit were collected in 2012, 2013, and 2014. Bars and error bars represent average and standard error ($n=3$ or more than 3 depending on productivity (presence of fruit) for each replication in each year), respectively. Treatment 248 control consists of $n=2$. Similar letters indicate no statistical ($P \leq 0.05$) difference among treatments; when no letters are present then there were no significant differences among treatments and all years

In addition to no adverse effect observed on the nutritional quality of fruit juice produced on poor quality soil and drainage sediment, fruit juice produced from plants grown on the saline, Se and B rich soil and drainage sediment showed significantly higher concentrations of Se relative to fruit juice of plants collected on non-saline (control) soil.

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