



Nutritional and functional evaluation of three powder mixtures based on mexican quelites: alternative ingredients to formulate food supplements

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Abstract

The aim of this work was to evaluate the nutrients and antioxidant compounds of three mixtures based on leaves of *Portulaca oleracea* L. (P), *Amaranthus hybridus* L. (A) and *Chenopodium berlandieri* L. (C). A mixtures design was made of which 10 combinations were obtained and three of the best mixtures were selected to analysis: OP1 (P+C), OP2 (P+A) and OP3 (C+A). A macronutrient, micronutrient analysis and a HPLC profile of phenolic compounds and amino acids were performed; in addition, *in vitro* antioxidant activity was measured by DPPH, ABTS and FRAP assays. Results showed that OP1 has a greater content of phenolic compounds, as evidenced by increases in antioxidant activity. Proximal chemical analysis showed that OP3 has a higher protein content and dietary fiber. The main phenolic compound present was phloridzin in OP1 and OP3; on the other hand, the amino acids lysine and glutamic acid were present at high concentrations in the three mixtures. These results showed that the OP1 mixture has the best antioxidant and nutritional properties; therefore, it has the potential to be included as an ingredient in food systems or nutraceutical and biomedical applications.

Keywords: wild edible plants; amino acid content; phenolic compounds; alternative ingredients.

Practical Application: Quelites as alternative ingredients for food and dietary supplement industry.

1 Introduction

Nowadays it is known that the consumption of native species brings benefits to the health of consumers and may represent alternatives to human nutrition, due to the presence of natural compounds that contain (Nana et al., 2012; Barreira et al., 2019). Plants belonging to the genera *Portulaca* spp., *Amaranthus* spp., and *Chenopodium* spp., are considered viable food options due to their high nutritional value and economic advantages associated with the ability of these plants to develop under unfavorable climatic conditions (Fasuyi, 2007; Oliveira et al., 2009; Uddin et al., 2014; Slabbert & Krüger, 2014; Hsu et al., 2016). One further advantage of using these plants is that they are distributed around the world (Fasuyi, 2007; Slabbert & Krüger, 2014; Bhargava et al., 2006). Particularly in Mexico, we can find a vast variety of native vascular plants, belonging to the genera mentioned above and popularly known as “quelites” (Santiago-Saenz et al., 2019). Several studies have shown that some species of these genera are a good source of nutrients and bioactive compounds, as Ca, Mg, Zn and Fe, protein, amino acids such as alanin, leucine and glycine and important concentrations of phenols, flavonoids, carotenoids and omega 3 (Fasuyi, 2007; Santiago-Saenz et al., 2019; Slabbert & Krüger, 2014; Alam et al., 2014; Simopoulos, 2004).

According to the literature, the consumption of these wild plants brings multiple health benefits due to their antioxidant effects (Santiago-Saenz et al., 2019; Al-Quraishy et al., 2012).

On the other hand, consumers are more conscious of leading a healthy life, increasing interest in preventive measures such as a high consumption of fruits and vegetables, because they are rich in bioactive compounds. However, no food has a composition that meets all the nutrients necessary to meet the daily demands of the body (Bresciani et al., 2015; Rajakumari et al., 2017). In this context, the market for nutraceuticals based on plants and foods is being promoted to meet the demands of consumers (Goldfarb et al., 2011; Rajakumari et al., 2017; Bresciani et al., 2017). Several investigations have proposed the elaboration of ingredients or dietary supplements, containing combinations of herbs, extracts, vitamins, minerals and amino acids in various presentations, with the aim of increasing the contribution of nutrients necessary for a healthy life (Rajakumari et al., 2017). Therefore, some works have made mixtures based on wheat starch, whey protein concentrate and peanut oil to promote an adequate intake of macronutrients in diabetic patients (Pawar & Thompkinson, 2014). Mixtures of *Chenopodium quinoa* Willd and *Lupinus Albus* L. have also been made, to increase the protein content that the species alone would not have and thus meet the daily requirements in children aged 2-5 years,

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who are considered as a population at risk of nutritional deficit (Cerezal-Mezquita et al., 2007). On the other hand, a clinical trial (Bresciani et al., 2017) showed that the intake of supplements based on fruits and vegetables allowed the effective absorption of phenolic compounds in plasma.

In this context, quelites are a rich source of phytochemicals and nutrients that can be incorporated into the diet as supplements and/or food ingredients to meet daily requirements of essential macro and micronutrients (Santiago-Saenz et al., 2019). Therefore, the aim of this work was to evaluate the nutritional and antioxidant properties of three mixtures made with quelites (leaves) of species *Portulaca oleracea* L., *Amaranthus hybridus* L. and *Chenopodium berlandieri* L. and thus provide reference data for future applications as production of food human supplements.

2 Materials and methods

2.1 Plant materials

Purslane (*Portulaca oleracea* L.), quintonil (*Amaranthus hybridus* L.) and quelite cenizo (*Chenopodium berlandieri* L.), were collected on March 2018, at Acaxochitlan, Hidalgo, Mexico (Latitude: 20°04' and 20°16'N; longitude: 98°06' and 98°18'W). Young plants were selected and non-rot and non-infested samples were used. Then, leaves were detached from stems and used for the mixtures. Selected leaves were washed and disinfected with colloidal silver (0.35% w/v), and dried at room temperature (25°C). Samples were stored at -76 °C (Thermo-Scientific, 703, Outside, USA) for 72 h, and then lyophilized at 133×10⁻³ mBar at -40°C (LABCONCO, 79480, Missouri, USA). Once the samples were lyophilized, they were ground in a blade mill (Grindomix, Retsch GM 200, Germany) at 9000 rpm for 1 min until a 150 µm powder was obtained.

2.2 Mixtures preparation

Previously, a 3-component mixture design (q = 3) was created in the Minitab 14 statistical package (Minitab Inc., Pennsylvania) using a simplex centroid design based on the Scheffé model and including additional points. The design of mixtures provided 10 different experiments of which 7 resulted in combinations. In the experiments obtained, the antioxidant activity was evaluated by DPPH and ABTS methods and the concentration of total phenols and flavonoids, considered as the response variables to identify the best mixtures with antioxidant properties, were also evaluated. Afterward, the parameters of the model were investigated to predict the maximum value of f(x) and the ones that best adjusted the response variables were used with the help of the statistical package. The general expression of the model was calculated (Equation 1).

$$f(x) = \beta_1x_1 + \beta_2x_2 + \beta_3x_3 + \beta_{12}x_1x_2 + \beta_{13}x_1x_3 + \beta_{23}x_2x_3 + \beta_{123}x_1x_2x_3 \quad (1)$$

where f(x): depended variable; β: linear coefficients and x: independent variable

Later, an optimization process was carried out. A selection of the best mixtures derived from the process were the following: OP1 [*Portulaca oleracea* L. (50%) + *Chenopodium berlandieri* L. (50%)], OP2 [*Portulaca oleracea* L. (50%) + *Amaranthus*

hybridus L. (50%)] and OP3 [*Chenopodium berlandieri* L. (50%) + *Amaranthus hybridus* L. (50%)]. After determining the best combinations, these were stored in Stand-Up bags (Clifton Packaging brand) and were kept at 4 °C for one week. After, we proceeded to evaluate the nutritional properties and obtain a more detailed evaluation of the bioactive compounds present in the selected mixtures.

2.3 Nutrients content

Proximal chemical analysis

Carbohydrates, fats, proteins, ashes, fiber and humidity, were determined according to official methods of analysis of AOAC International (Association of Official Analytical Chemists International, 2005), and the results are expressed in g/100 g of dry weight (DW). The energy was determined according to Chahdoura et al. (2015).

Dietary fiber

Soluble dietary fiber (SDF) and insoluble dietary fiber (IDF) were determined according to the enzymatic gravimetric method (993.19 and 991.42) from AOAC (Association of Official Analytical Chemists International, 2005). Results are expressed as g/100 g DW.

Mineral content

For the determination of the minerals, 0.5 g sample was weighed and digested with a diacid mixture (HNO₃:HClO₄). The concentration of P was measured by colorimetry of the phosphovanadomolybdate complex, according to the method described by AOAC (Association of Official Analytical Chemists International, 2005), and reading at 470 nm (Spectrophotometer Milton Roy, model Spectronic 20, USA). The concentration of K, Ca, Mg, Fe, Mn, Zn, Cu, Na, Se and B were quantified by atomic emission spectroscopy (Varian, model 725-ES, Mulgrave, Australia). The results are expressed as mg/100 g DW.

Amino acid profile

The amino acid content in the optimized mixtures was determined according to Li et al. (2012), Nielsen et al. (1985) and Morales de León et al. (2005). An acid hydrolysis was performed, then we used a cation-exchange separation column of 4.6 x 150 mm (Sykam GmbH, LCA K06/Na, Germany) with post column ninhydrin derivation, using an amino acid analyzer (Sykam GmbH, Germany). The results are reported in g/100g of protein.

2.4 Antioxidant compounds

Samples preparation

To determine the content of bioactive compounds and antioxidant capacity, 0.1 g of optimized samples were mixed with 10 mL of acetone (carotenoids and chlorophylls) or with 10 mL of 80% methanol (v/v) (phenols, flavonoids, DPPH, ABTS and FRAP). Then, mixtures were sonicated for 20 min at 40 kHz and 25 °C (Ultrasonicator, LSS, 32V118A, China) and

centrifuged at 12500 xg for 10 min at 5 °C (Thermo-Scientific centrifuge, ST 16R, Germany).

Total carotenoids

Isochromatic fractions of red (RC= capsanthin and capsorubin) and yellow (YC= β -carotene, β - cryptoxanthin, zeaxanthin) carotenoids were evaluated according to Hornero-Méndez & Mínguez-Mosquera (2001). Sample absorbance was measured on a UV-Vis spectrophotometer (Jenway, 6715, USA), at 472 and 508 nm, respectively. The results are expressed as mg/g DW.

Chlorophyll

Alpha, beta and total chlorophyll content were determined according to Witham et al. (1971). The absorbance was measured at wavelengths of 645 and 663 nm, and the corresponding equations for each chlorophyll type were used. The results are reported as mg of chlorophyll per gram of dry weight (mg/g DW) (Equation 2).

$$\begin{aligned} \text{mg chlorophyll a / g powder} &= (12.7 \times A_{663} - 2.69 \times A_{645}) \times V / 100 \times W \\ \text{mg chlorophyll b / g powder} &= (22.9 \times A_{645} - 4.68 \times A_{663}) \times V / 100 \times W \end{aligned} \quad (2)$$

where A: absorbance; V: volume of chlorophyll extract and W: weight

Total phenols and flavonoids concentration

Phenols contents were determined using the Folin Ciocalteu method, described by Singleton & Rossi (1965), with some modifications. From the supernatant, 0.5 mL was mixed with 0.5 mL of Folin-Ciocalteu reagent diluted in water to a concentration of 50% (w/v). The mixture was left to rest for 7 min, then, 1.5 mL of sodium carbonate at 2% (w/v) was added and left to react in darkness for 60 min. The absorbance was measured at a wavelength of 725 nm. The results are expressed as mg of gallic acid equivalents per g of dry weight (mg GAE/g DW). Total flavonoids contents was determined according to Rosales et al. (2011); 0.5 mL from the supernatant was mixed with 0.15 mL of NaNO₂ (5% w/v) and 2 mL of distilled water and the mixture was left to rest for 5 min in total darkness. A quantity of 0.15 mL of AlCl₃•6H₂O (10% w/v) was added, along with 1 mL of NaOH (1M), and the mixture rested for another 15 min. The absorbance was measured at a wavelength of 415 nm. The results are expressed as mg of quercetin equivalents per g of dry weight (mg QE/g DW).

Phenolic compounds analysis by HPLC

Phenolic acids and flavonoids were determined according to Aguiñiga-Sánchez et al. (2017). The content and type of metabolites present in each sample were identified by high performance liquid chromatography (Agilent Technologies 1100, USA) with a diode array detector (DAD). A Nucleosil 100-C18 of 125 x 4.0 mm column with an internal diameter of 5 μ m (Macherey-Nagel, Germany) was used for the phenols. For flavonoids, a Hypersil 100-C18 of 125 x 40 mm column with an internal diameter of 5 μ m (Agilent Technologies, USA) was used. In the HPLC analysis 8 phenolic acids were used

(HPLC Grade) as caffeic, gallic, chlorogenic, vanillic, *p*-coumaric, syringic, *p*-hydroxybenzoic and ferulic (Sigma Aldrich, EE.UU.). On the other hand, 7 HPLC grade flavonoid standards were used as rutin, phloridzin, myricetin, quercetin, naringenin, phloretin and galangin (Sigma Aldrich, USA). The results are expressed as mg/g DW.

Analysis of antioxidant activity

Antioxidant activity was determined by DPPH, ABTS and FRAP assays. The DPPH (2,2'-diphenyl-1-picrylhydrazyl) method was carried out according to Brand-Williams et al. (1995); the absorbance was measured at 517 nm (A517). For the ABTS [2,2'-Azino-bis (3-ethylbenzothiazoline-6-sulfonic acid)] method was according to Re et al. (1999); the absorbance was measured at 734 nm. The antioxidant capacity was determined by the ferric reducing ability of the plasma assay (FRAP) reported by Benzie & Strain (1996); the absorbance was measured at 593 nm. All of the results are expressed as μ M of Trolox equivalents per gram of dry weight (μ M TE/g DW).

Statistical analysis

All data were expressed as the mean \pm standard deviation ($n = 5$) and analyzed using analysis of variance (ANOVA) and a Tukey's multiple comparison of means test ($p \leq 0.05$). The SAS System for Windows version 9.4 was used for all the analysis.

3 Results and discussion

3.1 Nutritional content

A proximate composition of each optimized sample is shown in Table 1. OP1 has a higher carbohydrate content, followed by the OP2 sample, this is because purslane is richer in carbohydrates than quelite cenizo and quintonil. All samples show a low-fat proportion; nonetheless, the samples that contain purslane have greater fat content. Since purslane has a higher level of α -linolenic acid (18:3), and omega 3 fatty acid essential for human nutrition, than any other green leafy vegetable, and is also rich in docosahexaenoic acid (DHA) and docosapentaenoic acid (DPA) (Oliveira et al., 2009; Uddin et al., 2014). The OP1 sample could be a good source of essential fatty acids. Regarding protein content, OP3 has 44.5% more protein, compared to the OP2 sample, which is second in protein content. This is because quelites from *Chenopodium* spp. and *Amaranthus* spp. are rich in this macronutrient, being comparable or even greater than important cereals such as corn, rice and wheat (Vega-Gálvez et al., 2010). According to the proximal composition of the optimized samples we can say that, assimilable carbohydrates and proteins are the main source of energy that the mixtures can provide. The highest ash value was found in OP3, meanwhile, OP2 had the lowest ash value. These values are similar to those reported by Dixit et al. (2018), in seaweed used as supplements where ash concentrations of 24.92 to 43.18 g/100 g are found. According to the authors, the ash content determines the presence of a significant amount of minerals that are essential to human nutrition. Regarding to the dietary fiber content we found a good concentration in the OP3 sample.

Table 1. Determination of macronutrients, dietary fiber (g/100 g DW) and energy value (kJ/100 g DW) in three optimized mixtures based on quelites leaves.

Proximal chemical analysis	Mixtures based on quelites		
	OP1	OP2	OP3
Carbohydrates	22.35 ± 0.16 ^a	19.52 ± 0.05 ^b	16.97 ± 0.07 ^c
Fat	2.62 ± 0.01 ^a	2.35 ± 0.05 ^b	1.74 ± 0.07 ^c
Protein	26.31 ± 0.03 ^c	27.48 ± 0.05 ^b	39.22 ± 0.02 ^a
Ash	21.34 ± 0.02 ^b	20.26 ± 0.05 ^c	24.75 ± 0.02 ^a
Dietary fiber			
Insoluble dietary fiber	26.52 ± 0.05 ^b	26.68 ± 0.02 ^b	27.81 ± 0.10 ^a
Soluble dietary fiber	8.43 ± 0.15 ^a	7.35 ± 0.10 ^b	8.19 ± 0.08 ^a
Total dietary fiber	34.95 ± 0.09 ^b	34.03 ± 0.06 ^c	36.00 ± 0.05 ^a
Energy	1000.91 ± 1.13 ^b	956.71 ± 0.08 ^c	1013.82 ± 1.10 ^a

Mean ± standard deviation ($n = 3$) in optimized formulations. The values with the same letter within each row are the same according to the Tukey test ($p < 0.05$).

Fiber content is important because of its health properties, such as gastrointestinal tract regulation, feces consistency, hypolipidemic effects and its cardioprotective benefits (Rideout et al., 2008).

Table 2 shows the content of 11 minerals present in each of the previously mentioned quelites-based optimized samples; dietary reference intakes were included (World Health Organization, 2004, 2012a, b; Rainey et al., 1999; Pizzorno, 2015; Mancini et al., 2017). All three samples show a good amount of macroelements (P, Ca, Mg, K and Na) and microelements (Fe, Cu, Mn, Zn, B and Se). The content range of the macroelements varied between 8186.09 and 8973.68 mg/100 g DW, with OP2 being the sample with the lowest content, and OP1, the sample with the highest value. On the other hand, OP1 showed the lowest trace elements concentration (30.6 mg/100 g DW), and OP3 had the highest concentration of trace elements (95.22 mg/100 g DW).

Nonetheless, Ca values from the samples OP2 and OP3 are above the DRI, while Mg content in all samples is within the DRI; these minerals are important for bone growth, development and stability, as well as for the good health of the muscular system (World Health Organization, 2004; Frossard et al., 2000). Boron is a micronutrient that also plays a role in bone maintenance and proper development, along with calcium, magnesium and phosphorus metabolism (Rainey et al., 1999; Pizzorno, 2015). Boron presence was found mainly in OP3, followed by the OP1 sample. The Na/K ratio varied from 0.56 to 0.81, with the OP3 sample showing the best Na/K ratio, these results are interesting from a nutritional point of view, since a diet with a balanced Na/K ratio is crucial for hypertensive people (World Health Organization, 2004). Iron is a micronutrient required for oxygen-transporting protein synthesis, such as hemoglobin and myoglobin, and it is also required for enzymes that produce energy, for the immune system and thyroid enzymes (World Health Organization, 2004; Frossard et al., 2000). All optimized samples are within the DRI for iron, nonetheless, OP3 and OP2 are richer in this element, due to the presence of quintonil, which is a high source of Fe (Santiago-Saenz et al., 2018). The high concentration of Fe in OP2 and OP3 could also interact with flavonoids forming chelates, thus potentiating the antioxidant effect of the flavonoids (Malesev & Kuntic, 2007; Cherrak et al., 2016). Therefore, quintonil is the most important source to increase the content of Fe in the mixtures. On the other hand, besides iron, manganese is the second most abundant micronutrient in the samples, with

the OP3 sample having the highest Mn content. Manganese is important to several enzymes that depend on it to function, such as metalloenzymes that are involved in glycolysis and lipid and protein degradation (National Academy Press, 1989). Zinc is the third most abundant micronutrient in the OP samples, while copper is the least abundant element in our samples, nonetheless, both elements are in a greater proportion in OP2 samples. According to the literature (World Health Organization, 2004), Zn and Cu are essential cofactors for enzyme complexes (cytochrome C-oxidase) that stabilize the cellular membrane, for hormones and nucleic acids. On the other hand, the sum of the minerals K+Na+Mg+Ca expressed in mg/100g DW of the obtained mixtures is higher compared to vegetables that are commonly consumed as sweet corn (1342), potatoes (6015), tomatoes (3429) and carrots (3276) (Dixit et al., 2018). Therefore, the mineral concentrations found in the samples can contribute significantly to the dietary reference intakes (DRI).

The amino acid content of the samples is shown in Table 3; dietary reference intakes were included (National Academy Press, 1989; World Health Organization, 2007; Institute of Medicine, 2005). The amino acid (a.a) profile indicates that all the samples have relevant concentrations of 17. Lysine and glutamic acid are the most abundant amino acids, while methionine is found in a lower proportion. It has been reported that essential amino acids like lysine should be ingested in the diet or by supplements (Tomé & Bos, 2007). According to Schneidenburger & Diederich (2015) the main role of lysine is in protein synthesis and as precursor for carnitine biosynthesis, which is important in beta-oxidation. On the other hand, glutamic acid is involved from amino acid catabolism to urea synthesis, and it is also a brain neurotransmitter (World Health Organization, 2007; Institute of Medicine, 2005; Tomé & Bos, 2007). It is worth noting that, despite methionine being the least abundant amino acid, the samples contain at least 77% of the daily recommended uptake of methionine. When comparing the optimized samples regarding the total essential amino acids, the OP1 sample turned out to be the best. However, when considering the contribution of essential amino acids per 100g of sample, the OP3 mixture (21.93 g) provides a higher content followed by the OP1 (15.11 g) and OP2 (15.08 g) samples. These values are comparable to other sources of essential amino acids of vegetable origin such as oat (13.7 g), wheat (18.0 g), soy (19.9 g) and brown rice (22.1 g) (Gorissen et al., 2018).

These results show that the optimized vegetable samples are a viable dietary option, not only for their general amino acid content, but also because they contribute to a very similar methionine DRI to that which its most common sources can provide.

3.2 Antioxidant compounds

Carotenoids and total chlorophyll in the optimized sample OP1 were 3.25 and 1.40 times higher respectively than the optimized sample OP3, which showed the lowest concentration of these

photosynthetic pigments (Table 4). The OP1 and OP2 samples are rich in these pigments due to the presence of purslane in their composition. This agrees with other investigations which mentioned that purslane has an elevated content of alpha and beta chlorophylls (Nurfaizah et al., 2015). Our results showed that foliage from OP1 samples is a good source of photosynthetic pigments; in addition, the proportion of carotenoids that OP1 contains are similar to other food supplements. On the market, there are capsules based on mixtures of fruits, vegetables and berries (Juice Plus⁺) that can provide to 3.06 mg of carotenoids

Table 2. Macroelements and microelements content (mg/100 g DW) in three optimized mixtures based on quelites leaves and dietary reference intakes (DRI).

Mineral composition	Mixtures based on quelites			*DRI
	OP1	OP2	OP3	
Macroelements				
P	376.83 ± 0.02 ^c	401.66 ± 0.01 ^a	398.94 ± 0.02 ^b	700mg/day
Ca	899.30 ± 0.10 ^c	1528.51 ± 0.04 ^a	1464.23 ± 0.01 ^b	1000-1300mg/day
Mg	1079.10 ± 0.03 ^c	1114.18 ± 0.01 ^b	1181.17 ± 0.01 ^a	190-260mg/day
K	6526.70 ± 0.02 ^a	5051.16 ± 0.01 ^c	5257.39 ± 0.06 ^b	3510mg/day
Na	91.75 ± 0.05 ^a	90.58 ± 0.02 ^b	64.53 ± 0.03 ^c	< 2g/day
Microelements				
Fe	13.93 ± 0.02 ^c	74.69 ± 0.01 ^b	78.18 ± 0.04 ^a	5.9-21.8mg/day
Cu	0.68 ± 0.07 ^a	0.67 ± 0.02 ^a	0.54 ± 0.01 ^b	2mg/day
Mn	7.71 ± 0.01 ^c	8.17 ± 0.05 ^b	8.25 ± 0.01 ^a	0.52-10.8mg/day
Zn	2.96 ± 0.05 ^b	3.53 ± 0.03 ^a	2.45 ± 0.01 ^c	7.5-20mg/day
B	2.47 ± 0.01 ^b	1.97 ± 0.06 ^c	2.67 ± 0.03 ^a	1-3mg/day (< 20mg/day)
Se	2.85 ± 0.01 ^b	1.46 ± 0.04 ^c	3.13 ± 0.01 ^a	50-200µg/day

Mean ± standard deviation ($n=3$) in optimized formulations. The values with the same letter within each row are the same according to the Tukey test ($p \leq 0.05$); *DRI: dietary reference intakes.

Table 3. Amino acid composition (g/100 g protein) in three optimized mixtures based on quelites leaves and dietary reference intakes (DRI).

Indispensable amino acids	Mixtures based on quelites			*DRI (mg/kg/w/d)	*DRI (g/100 g p)
	OP1	OP2	OP2		
Threonine (Thr)	6.33 ± 0.02 ^a	5.95 ± 0.03 ^b	6.32 ± 0.02 ^a	15	3.0-7.1
Methionine (Met)	1.49 ± 0.02 ^a	1.43 ± 0.02 ^b	1.42 ± 0.01 ^b	10	1.8-4.1
Valine (Val)	6.78 ± 0.03 ^a	6.74 ± 0.02 ^a	6.42 ± 0.02 ^b	26	4.0-9.1
Phenylalanine (Phe)	7.13 ± 0.03 ^a	6.66 ± 0.02 ^b	7.11 ± 0.04 ^a	25	3.4-7.7
Leucine (Leu)	12.63 ± 0.01 ^a	12.42 ± 0.01 ^c	12.47 ± 0.02 ^b	39	6.1-14.1
Isoleucine (Ile)	5.04 ± 0.01 ^a	5.10 ± 0.02 ^a	4.64 ± 0.05 ^b	20	3.6-8.2
Lysine (Lys)	15.85 ± 0.03 ^a	15.20 ± 0.04 ^b	15.13 ± 0.03 ^b	30	5.3-12.6
Tryptophan (Trp)	2.19 ± 0.01 ^b	1.41 ± 0.03 ^c	2.42 ± 0.02 ^a	4	0.9-2.1
Total	57.44	54.91	55.93		
Dispensable amino acids					
Histidine (His)	4.60 ± 0.02 ^a	4.31 ± 0.12 ^b	4.36 ± 0.03 ^b	10	2.2-5.2
Alanine (Ala)	7.62 ± 0.10 ^a	7.18 ± 0.05 ^b	7.28 ± 0.02 ^b	**	3.6-8.5
Tyrosine (Tyr)	4.27 ± 0.01 ^a	3.71 ± 0.03 ^c	4.16 ± 0.01 ^b	25	2.8-6.4
Aspartic acid (Asp)	13.78 ± 0.05 ^a	13.87 ± 0.01 ^a	13.89 ± 0.14 ^a	**	6.5-15.4
Cysteine (Cys)	2.03 ± 0.03 ^b	1.85 ± 0.05 ^c	2.22 ± 0.10 ^a	4.1	1-2.2
Glutamic acid (Glu)	21.81 ± 0.02 ^a	21.42 ± 0.04 ^b	20.93 ± 0.03 ^c	**	15-33.7
Glycine (Gly)	7.51 ± 0.05 ^b	7.31 ± 0.02 ^c	7.60 ± 0.03 ^a	**	3.2-7.8
Proline (Pro)	5.51 ± 0.05 ^a	5.16 ± 0.01 ^c	5.37 ± 0.03 ^b	**	5.2-12
Serine (Ser)	7.33 ± 0.02 ^a	7.28 ± 0.04 ^{ab}	7.21 ± 0.02 ^b	**	3.5-7.9
Arginine (Arg)	7.09 ± 0.01 ^c	7.25 ± 0.03 ^b	8.04 ± 0.05 ^a	**	4.2-10.1
Total	81.55	79.34	81.06		

Mean ± standard deviation ($n = 3$) in optimized formulations. The values with the same letter within each row are the same according to the Tukey test ($p \leq 0.05$); *DRI (dietary reference intakes): mg/kg/weight/day; DRI (dietary reference intakes): g/100 g protein; **Nd (no data).

Table 4. Carotenoids, chlorophyll, total phenols and total flavonoids content and antioxidant capacity by DPPH, ABTS and FRAP assays in three optimized mixtures based on Mexican quelites leaves.

Antioxidant compounds	Mixtures based on quelites		
	OP1	OP2	OP3
Carotenoids (mg/g DW)			
Red carotenoids	1.76 ± 0.20 ^a	0.90 ± 0.04 ^b	0.30 ± 0.04 ^c
Yellow carotenoids	0.84 ± 0.08 ^a	0.75 ± 0.05 ^a	0.50 ± 0.01 ^b
Total carotenoids	2.60 ± 0.05 ^a	1.65 ± 0.01 ^b	0.80 ± 0.01 ^c
Chlorophyll (mg/g DW)			
Chlorophyll <i>a</i>	17.06 ± 0.20 ^a	16.44 ± 0.17 ^b	11.92 ± 0.10 ^c
Chlorophyll <i>b</i>	44.01 ± 0.15 ^a	43.79 ± 0.05 ^a	31.45 ± 0.09 ^b
Total Chlorophyll	61.07 ± 0.12 ^a	60.23 ± 0.23 ^b	43.37 ± 0.15 ^c
Total phenols (mg GAE/g DW)	11.07 ± 0.03 ^b	11.30 ± 0.02 ^a	6.64 ± 0.06 ^c
Total flavonoids (mg QE/g DW)	13.45 ± 0.03 ^a	13.22 ± 0.02 ^b	12.86 ± 0.05 ^c
Antioxidant activity (μmol TE/g DW)			
DPPH	25.47 ± 0.07 ^a	25.51 ± 0.05 ^a	24.43 ± 0.10 ^b
ABTS	65.98 ± 0.02 ^a	62.18 ± 0.08 ^b	41.59 ± 0.01 ^c
FRAP	60.91 ± 0.13 ^a	60.44 ± 0.18 ^b	37.81 ± 0.09 ^c

Mean ± standard deviation ($n=3$) in optimized formulations. The values with the same letter within each row are the same according to the Tukey test ($p \leq 0.05$).

in form of β -carotene per gram of sample (Goldfarb et al., 2011). Likewise, the total carotenoid content of the optimized mixtures is higher than that reported in edible fresh vegetables such as lettuce (0.808 mg/g DW) (Bhaskarachary et al., 1995) and cabbage (0.82 mg/g DW) (Hulshof et al., 1997). Therefore, the mixtures based on quelites are an economic proposal for supplementation processes, incorporating some pigments especially carotenoids into the diet, which are characterized by their high antioxidant capacity that protects the cells from highly oxidizing radicals and singlet oxygen (Oroian & Escriche, 2015).

Phenolic compounds are plant secondary metabolites with an important role in human diet, due to their antioxidant capacity and free radical scavenging; they also have an indirect effect by protecting endogenous human enzymes, such as superoxide dismutase, catalase or glutathione peroxidase (Oroian & Escriche, 2015). Regarding to the phenolic content, we found that OP2 has a significant concentration of total phenols, while OP1 has a greater total flavonoids concentration (Table 4). It has been reported that quelites are a good source for these bioactive compounds, especially the quelite cenizo, when compared to the others (Santiago-Saenz et al., 2018; Santiago-Saenz et al., 2019). The phenolic content in the optimized samples was lower than that reported in a dietary supplement based on vegetables such as carrots, parsley, beet, kale, broccoli, cabbage, tomatoes, garlic, oat and spinach, which contained 50 mg of total phenols per g of powder (Bresciani et al., 2015). On the other hand, Wasek et al. (2015), analyzed the phenolic content of 28 commercial dietary supplements based on fruits and vegetables and their results were found in a range of 0.132 to 378.52 mg GAE. The phenolic content present in quelites based mixtures is higher than 25 of the dietary supplements reported by Wasek et al. (2015).

Chromatographic studies were carried out to identify the secondary metabolites in the optimized mixtures of Mexican quelites. Table 5 shows the phenolic acids present in the samples, these were gallic acid, chlorogenic acid, caffeic acid and *p*-coumaric acid. Chlorogenic acid was the most abundant phenolic acid in

the samples, especially in the OP2 mixture. According to the results of Table 5, the identified flavonoids were the following: rutin, phloridzin, myricetin, quercetin, naringenin, phloretin and galangin, except for OP2, where myricetin, naringenin and phloretin were not identified. Meanwhile OP3, did not show the presence of myricetin and galangin. On the other hand, in the OP1 and OP3 samples the flavonoid with the highest concentration was phloridzin. These two samples have in common the presence of quelite cenizo with respect to OP2 which does not included it. It could therefore be said, that the quelite cenizo is responsible for the presence of phloridzin in samples OP1 and OP3. These results are consistent with the literature where it is mentioned that the quelite cenizo is high in phloridzin in comparison with quintonil and purslane (Santiago-Saenz et al., 2018). Other important flavonoids regarding their concentration in the samples were phloretin, myricetin and naringenin. It is important to indicate that the concentration of phenols is higher than that quantified by HPLC, this may be due to the overestimation of these compounds when they are identified by colorimetry, in the specific case of total phenols the Folin reagent does not react exclusively with phenols, also with other components as ascorbic acid, aromatic amines and carbohydrates (Khoddami et al., 2013). On the other hand, it may also be due to the presence of other phenolic compounds present in the samples; but that were not identified, because the standards were not available. The presence of these secondary metabolites impact on the antioxidant activity of the mixtures of quelites. Particularly rutin, quercetin, galangin and catechin that are flavonoids that have been shown to be excellent antioxidants *in vitro* and *in vivo* mainly when chelating metal ions such as Cu, Fe and Zn (Malesev & Kuntic, 2007; Cherrak et al., 2016). Likewise, amino acids such as Glu, Asp and Lys possess the ability to chelate metal ions and therefore act as antioxidant peptides (Wang et al., 2009). On the other hand, the analysis of phenolic compounds by HPLC and the amino acid profile shows that mixtures based on quelites have important concentrations of these antioxidant compounds, which can be included in the

Table 5. Identification of phenolic compounds (mg/g DW) by HPLC in three optimized mixtures based on Mexican quelites leaves.

Phenolic compounds	Mixtures based on quelites		
	OP1	OP2	OP3
Phenolic acids			
Gallic acid	0.037 ^a	0.022 ^b	0.007 ^c
Chlorogenic acid	1.040 ^b	2.162 ^a	0.664 ^c
Caffeic acid	0.020 ^a	0.016 ^b	0.017 ^{ba}
Coumaric acid	0.022 ^a	0.019 ^b	0.008 ^c
Flavonoids			
Rutin	0.097 ^c	0.189 ^a	0.155 ^b
Phloridzin	11.793 ^a	0.088 ^b	11.801 ^a
Myricetin	0.660 ^a	*	*
Quercetin	0.048 ^a	0.032 ^b	0.026 ^c
Naringenin	0.521 ^a	*	0.438 ^b
Phloretin	0.447 ^b	*	0.879 ^a
Galangin	0.060 ^b	0.092 ^a	*

The values with the same letter within each row are the same according to the Tukey test ($p \leq 0.05$); *: not detected.

diet and have effects on the health of consumers. Bresciani et al. (2017), showed that the consumption of three capsules per day of 750 mg of powder based on a mixture of 36 fruits and vegetables high in polyphenols allowed the effective absorption of phenolic compounds in human plasma.

3.3 Antioxidant activity

Recently, antioxidants from natural sources and their benefits to human health have received much attention. Many medicine compositions are based on antioxidants in order to prevent and treat several complex diseases. Plants are a very good natural source of antioxidants, since they produce a wide variety of secondary metabolites with antioxidant capacities and elevated therapeutic activities (Oroian & Escriche, 2015). Table 4 shows the results from the DPPH, ABTS and FRAP assays, indicating the antioxidant characteristics of each quelites-based optimized sample. Our results show that the antioxidant capacities of the samples are as follows: OP1 > OP2 > OP3. The greater antioxidant capacity of OP1 respect to OP3 is due to the lower concentration of bioactive compounds in OP3 such as carotenoids, chlorophyll, phenolic acids and flavonoids. These compounds can interfere with oxidative cycles, inhibiting or retarding the oxidative damage in the cells (Oroian & Escriche, 2015). Therefore, these bioactive compounds are responsible for the antioxidant activity shown by the optimized samples. On the other hand, the values of the antioxidant activity of the mixtures are similar or even higher to different commercial dietary supplements based on vegetables and fruits (Wasek et al., 2015).

4 Conclusion

The combination of the Mexican quelites leaves resulted in an improvement in the nutritional and functional properties of the powder mixtures. The OP1 mixture prepared with *Portulaca oleracea* L. and *Chenopodium berlandieri* L. (1:1) has the highest content of antioxidant compound: total carotenoids, chlorophyll and phenolic compounds as phloridzin and chlorogenic acid, as well as macroelements and amino acids. Meanwhile, the highest content of microelements and macronutrients (protein and

dietary fiber) were found in the OP3 mixture. The best mixture was OP1, which had numerous nutrient properties. Therefore, the selection and analysis of the formulations is important as their collective properties can be enhanced, compared to their individual properties, when combined and can be potential sources of nutritive compounds and at the same time provide an alternative to improve the nutritional status of the populations if incorporated as food supplements.

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