Assessment of the Nutritional Value of Traditional Vegetables from Southern Chile as Potential Sources of Natural Ingredients

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Abstract

There is an increasing interest in consuming healthy foods motivated by the need of boosting the immune system naturally. In this sense, vegetables rich in bioactive compounds are a clear example of “superfoods” that promotes overall health and strengthen the immune response. Therefore, in this study eight traditional vegetables usually produced in southern Chile (pea, corn, carrot, leek, spinach, chard, coriander and parsley) were characterized in terms of their nutritional composition to evaluate their potential as lyophilized natural ingredients. Thus, chemical composition, amino acid profile, minerals, vitamins, carotenoids, polyphenols and pesticide residues were evaluated. Green leafy vegetables resulted to be an excellent source of proteins and dietary fibers as well as vitamins (ascorbic acid, choline, alpha-tocopherol and niacin), minerals (calcium, phosphorus and iron), carotenoids and polyphenols. Among the eight vegetables assessed spinach exhibited the more balanced nutritional profile. Moreover, 332 pesticide residues were analysed and only six were detected in a low concentration. Due to their nutritional properties, the present results suggest that vegetables produced in southern Chile could be considered as promising alternatives to develop natural food ingredients.

Keywords Nutritional value · Vegetables · Vitamins · Minerals · Antioxidants · Pesticide residues

Introduction

The current food trends alongside the scenario caused by the COVID-19 pandemic have renewed the interest in healthy lifestyles motivating the consumption of healthy foods. In this sense, vegetable intake has lately increased driven by the need of boosting the immune system naturally. In fact, existing evidence highlights that diet has a profound effect on people’s immune system and disease susceptibility. It has been demonstrated that specific nutrients or nutrient combinations may affect the immune system through the activation of cells, modification in the production of signalling molecules and gene expression [1]. According to World Health Organization (WHO), above 80% of the rural inhabitants around the world rely on traditional plants as a source of nutrients and primary health care [2].

Actually, nutritional deficiencies of energy, protein and specific micronutrients are associated with depressed immune function and increased susceptibility to infection [3]. While an adequate intake of iron, zinc and vitamins A, E, B6 and B12 is predominantly vital for the maintenance of immune function [4]. Therefore, the key to maintaining good health is necessary to carefully choose the foods that are consumed in order to avoid nutrient deficiencies.

Vegetables are important for human nutrition in terms of proteins and fibers, as well as vitamins, minerals and non-nutritive phytochemical compounds (phenolic compounds, flavonoids, bioactive peptides, etc.), which have proven health-promoting effects [5]. Several in vitro, pre-clinical and clinical investigations have revealed an inverse relationship between high consumption of vegetables and the incidence of chronic ailments such as cardiovascular and neurodegenerative diseases, ischemic stroke, arthritis, inflammatory bowel and some forms of cancers [6]. For instance, vegetables such as spinach, broccoli and onion...
are recognized as rich sources of health-promoting compounds [7].

In Chile, vegetables such as pea, corn, carrot and chard are consumed either as salads or cooked as vegetable omelettes, while parsley and coriander are used as raw ingredients in the preparation of traditional Chilean soups such as “cazuela” to incorporate flavour and aroma. However, large amounts of fresh vegetables must be consumed to attain maximum health benefits [8].

In this sense, lyophilization is an adequate technology to deliver high-quality and natural health-boosting green powder supplements. Therefore, green powders as dietary supplements might be advantageous since they can help people to reach suitable daily vegetable intake to boost the immune system, particularly under the current pandemic scenario, where COVID-19 is undermining nutrition across the world [3].

Consequently, this work proposed to characterize in detail the nutritional quality of traditional vegetables produced in southern Chile including pea, corn, carrot, leek, spinach, chard, coriander and parsley to expand the knowledge on the nutritional quality of these vegetables and further use them as sources of natural ingredients. Thus, the chemical composition, content of amino acids, vitamins, minerals, carotenoids, polyphenols and antioxidant capacity of eight lyophilized vegetables were evaluated. The results obtained from this study shed light on the feasibility of these lyophilized vegetable powders for the formulation of natural food supplements to meet the nutritional requirements and to strengthen human health, especially under the current global health emergency. Also, this work provides the initial information for the construction of a food database so far not available for small farmers and consumers, which could assist in the decision-making process towards improving plant-based supplement production and dietary habits.

Materials and Methods

Plant Materials

Eight vegetables were selected for the study namely pea (*Pisum sativum*), corn (*Zea mays*), carrot (*Daucus carota*), leek (*Allium ampeloprasum*), spinach (*Spinacia oleracea*), chard (*Beta vulgaricus* var. cicla), coriander (*Coriandrum sativum*) and parsley (*Petroselinum crispum*). Samples of these species were supplied by Asociación Gremial Hortícola de La Araucanía (Temuco, Chile), which unites small and medium vegetable producers in La Araucanía region with the aim of providing them better commercialization channels.

Reagents

HPLC grade acetonitrile (ACN), methanol, fluorescein sodium salt, Folin-Ciocalteu reagent, n-hexane, ethane, acetone and toluene were purchased from Merck (Darmstadt, Germany). *Na*₂*SO*₄, KOH and sodium phosphate were purchased from Winkler (Winkler®, Santiago, Chile). *Na*₂*CO*₃ was acquired from VWR (Solon, Ohio, USA), while AAPH was obtained from Cayman (Cayman Chemical, Ann Arbor, MI, USA). Trolox, gallic acid and standards minerals and vitamins were purchased from Sigma Aldrich Co. (St. Louis, MO, USA). All chemicals used in the analysis were of analytical grade.

Lyophilization of Vegetable Material

The selected vegetables were washed, cut, peeled and subsequently frozen at −18 ± 1 °C for 24 h. Later, the frozen vegetables were subjected to lyophilization using a Liobras lyophilizer (Liotop LP1280, São Carlos, Brazil) under a controlled temperature program in a controlled atmosphere. Subsequently, lyophilized vegetables were subjected to milling using a laboratory-scale mill (Fritsch Mill Pulverisette 14, Indar-Oberstein, Germany) at 7000 rpm and sieved through a 200 μm sieve. Finally, vegetable powders were packaged in a zipped aluminium foil bag and stored at room temperature until further analysis.

Proximate Composition

The protein content was determined by Dumas method (DUMATHERM® N PRO analyser, Königswinter, Germany) using a conversion factor of 6.25. The oil content was determined according to the AOAC official method 945.18. The crude fibre was measured using the gravimetric method according to the AOAC official method 978.10. The ash content was determined as per the AOAC official method 942.05 and carbohydrates (nitrogen-free extracts) were calculated by the difference.

Amino Acids Analysis

The amino acids (AA) analysis of vegetable samples was performed according to the AOAC Official Method 994.12. The samples were hydrolysed with 6 N HCl containing phenol for 24 h at 110 ± 2 °C in glass tubes followed by HPLC separation (Agilent 1200 HPLC, Agilent Technologies, Palo Alto, CA, USA). The chromatograms detected at 570 nm and 440 nm were integrated using dedicated software (Agilent Open Lab software, USA). For the sulfur-containing AA,
methionine was determined as methionine sulphone by oxidation with performic acid-phenol for 16 h at 0 °C prior to hydrolysis.

**Determination of Vitamins**

To quantify the vitamins present in the vegetables, lyophilized samples were analysed according to the following protocols: retinol (vitamin A) according to AOAC Vol. 79, N°6 (1996); thiamine (vitamin B1) was quantified according to BS EN 14122–2014; riboflavin (vitamin B2) according to EN 14152:2006; niacin (vitamin B3) according to EN 15652:2009; pantothenic acid (vitamin B5) according to AOAC 2012.16; pyridoxine (vitamin B6) according to EN 14164:2014; folate (vitamin B9) according to NMKL 111:1985; choline according to AOAC Official Method 999.14; ascobic acid (vitamin C) according to HPLC-DAD method; tocopherol (vitamin E) according to AOAC Official Method UPLC-UV/DAD 212.09; and vitamin K according to AOAC Official Method 2012.10.

**Determination of Minerals**

To quantify the minerals present in the vegetables, lyophilized samples were analysed by the following protocols: copper (Cu) according to AOAC Official Method 999.11; iron (Fe) according to AOAC Official Method 999.11; mercury (Hg) according to AOAC Official Method 977.15; selenium (Se) according to AOAC Official Method 986.15; zinc (Zn) according to AOAC Official Method 999.11; phosphorus (P) according to AOAC Official Method 965.17; calcium (Ca) according to AOAC Official Method 991.25; sodium (Na) according to AOAC Official Method 969.23; Arsenic (As) according to AOAC Official Method 986.15; Tin (Sn) according to NCh 2761:2005; and Lead (Pb) according to AOAC Official Method 999.11.

**Total Carotenoids Content**

Total carotenoids content of each lyophilized vegetable was determined by AOAC Official Method 970.64 with some modifications. 0.2 g of lyophilized sample was ground and homogenized with 7 mL of the extracting solvent (hexane:ethane:acetone:toluene, at 10:6:7:7); 0.6 mL KOH (30%, v/v) was added and a fast homogenization occurred in a warm bath at 50 °C for 20 min. After removal from the warm bath, the sample was immediately cooled in ice water for 1 h. Further, 7 mL hexane aliquots were added to the flasks and then calibrated at 7 mL with Na2SO4 (10% w/v), homogenized and kept in the dark for 1 h. An aliquot of the supernatant was read by HT Multi-Detection Microplate reader (Biotek Instruments Inc., Winooski, VT, USA) at wavelength 445 nm. Results were given in mg of β-carotene for 100 g of lyophilized vegetable.

**Total Polyphenols Content**

Total phenolic content was determined in crude extract through reaction with Folin-Ciocalteau reagent (FC), following the method described by Wandersleben et al. [9] with some modifications. Briefly, 0.5 g of each lyophilized vegetable was mixed with 20 mL of ethanol (1:40, w/v). Later, 50 μL were mixed with FC reagent (1:1) (which had previously been diluted 10-fold with distilled water) and allowed to stand at 22 °C for 5 min; 100 μL of Na2CO3 (0.2 g/L) (Sigma–Aldrich, Germany) and 800 μL of distilled water was added to the mixture. After heating the tubes for 15 min at 45 °C in a water bath, they were allowed to cool in the dark for 30 min, after which the absorbance was measured at 750 nm using an HT Multi-Detection Microplate reader (Biotek Instruments Inc., Winooski, VT, USA). The results were expressed as gallic acid equivalents per gram of dry weight vegetable (mg GAE/100 g).

**Oxygen Radical Absorbance Capacity (ORAC) Assay**

Antioxidant activities of samples were determined by the ORAC essay according to Casettari et al. [10] with some modifications. The vegetable solutions were prepared by mixing 0.5 g of each lyophilized vegetable with 20 mL of ethanol (1:40, w/v) and stirred for 1 h at room temperature. The assay was carried out using a Multi-Detection Microplate Reader (Biotek Sinergy HT). The incubator temperature was set at 37 °C. The reaction mixture for a hydrophilic assay was the following: 150 μL of 4 nM fluorescein sodium salt in 75 mM Na-phosphate buffer (pH 7.4), 25 μL of sample or Trolox. The blank was 75 mM Na-phosphate buffer (pH 7.4). The calibration curve was made each time with standard Trolox (50, 100, 200, 300, 400 and 500 μM). The reaction was initiated with 25 μL of 20 mg/mL AAPH. Fluorescence was read at 485 nm ex. and 528 nm em. until complete extinction. ORAC values were expressed as μmol Trolox equivalents per 100 g of dry weight vegetable (μmol Troloxeqv/100 g).

**Determination of Pesticide Residues**

General multi-residue methods for organochlorine and organophosphorous pesticides were used according to AOAC Official Methods 970.52, 985.22 and 2007.01 using a GC/MS Shimadzu (GM/MS-QP2020 NX, Shimadzu Co., Kyoto, Japan) and LC/MS/MS. Dithiocarbamates were analysed according to AOAC Official Method [11].
Statistical Analysis

Results are expressed as mean ± standard deviation of three biological replicates (n = 3). For each determination, triplicate samples were analysed. All data were subjected to one-way analysis of variance (ANOVA) using the Statgraphics Centurion XVIII (Statistical Graphics Corp., Herndon, USA). Duncan’s multiple range tests were performed for mean comparisons on each of the significant (p < 0.05) variables measured.

Results and Discussion

Proximate Composition

The proximate composition of eight lyophilized vegetables from southern Chile are shown in Table 1. Significant differences (p < 0.05) were observed in contents among different vegetables. Green leafy vegetables stand out for their high protein and dietary fiber content from 25.13 to 33.25 g/100 g dry weight (DW) and from 22.41 to 37.48 g/100 g DW, respectively, while their fat content is rather low. Conversely, carrot and corn are poor in protein concentration but rich in carbohydrates. The protein content found in coriander is higher than the values found by other authors [12] who found values between 21 and 30 g/100 g DW. Meanwhile, the protein content in spinach, chard and parsley were comparable to other reported values [13, 14]. It is widely held view that green leafy vegetables are rich sources of protein and dietary fiber.

The dietary fiber in the selected vegetables ranged from 11.35 to 37.48 g/100 g DW, with green leafy vegetables having the highest values of this macronutrient. Dietary fiber is essential for effective digestion and bowel movement. Thus, selected green leafy vegetables, especially parsley and coriander, could be used as high-fiber sources that could prevent obesity, constipation, diabetes, lower the serum cholesterol, reduce the risk of coronary heart disease, hypertension, colon and breast cancer [15].

The content of fat in this study ranged from 1.11 to 3.37 g/100 g DW. The investigated vegetables exhibited a low-fat content, with parsley showing the highest value. Contrary to these results, lower fat contents have been previously reported in parsley, mainly explained as an effect of cultivar origin and soil characteristics [16]. The main fatty acids found in parsley leaves are α-linolenic, linoleic and palmitic acid, while polyunsaturated fatty acids (PUFA) are the most abundant class [16]. This makes parsley leaves a potential source of essential fatty acids renowned for their multiple health benefits.

The ash contents in the studied vegetables ranged from 3.14 to 20.32 g/100 g DW, with the green leafy vegetables exhibiting the highest content. This is indicative that green leafy vegetables contained a high concentration of minerals, which would provide a considerable amount of mineral in diet.

Evaluation of Amino Acid Profiles

The amino acid profiles of the eight edible vegetables are shown in Table 2. It is important to note that leek, spinach, chard, parsley and coriander contained all of the essential amino acids. The relevance of this result is that in contrast to plants, humans and animals are unable to synthesise these amino acids necessary for protein biosynthesis. Therefore, the synthesis of proteins containing essential amino acids is only possible through protein-rich food intake [17]. Thus, the quality of a protein source is primarily based on its amino acid profile and digestibility [18], so these vegetables could be envisioned as potential sources of high-quality proteins for the development of novel food formulations.

Among essential and conditionally essential amino acids, the most abundant is arginine in pea. Arginine is a conditionally essential amino acid profile and digestibility [18], so these vegetables could be envisioned as potential sources of high-quality proteins for the development of novel food formulations.

Table 1 Proximate composition (g/100 g DW) of some traditional vegetables from southern Chile

| Vegetables   | Protein  | Fat         | Carbohydrate | Soluble fiber | Insoluble fiber | Dietary fiber | Ash       |
|--------------|----------|-------------|--------------|---------------|-----------------|---------------|-----------|
| Pea          | 26.28 ± 0.11<sup>c</sup> | 2.45 ± 0.15<sup>c</sup> | 41.84 ± 0.34<sup>b</sup> | 3.95 ± 0.09<sup>c</sup> | 21.87 ± 0.06<sup>b</sup> | 25.82 ± 0.15<sup>d</sup> | 3.46 ± 0.11<sup>f</sup> |
| Corn         | 15.05 ± 0.06<sup>f</sup> | 2.44 ± 0.10<sup>c</sup> | 68.79 ± 1.19<sup>a</sup> | 1.12 ± 0.50<sup>c</sup> | 10.23 ± 0.36<sup>d</sup> | 11.35 ± 0.14<sup>d</sup> | 3.14 ± 0.24<sup>f</sup> |
| Carrot       | 8.46 ± 0.12<sup>f</sup> | 1.11 ± 0.09<sup>d</sup> | 63.02 ± 4.06<sup>a</sup> | 8.52 ± 0.06<sup>e</sup> | 14.63 ± 0.26<sup>d</sup> | 23.15 ± 0.20<sup>f</sup> | 7.04 ± 0.03<sup>e</sup> |
| Leek         | 21.12 ± 0.03<sup>e</sup> | 1.17 ± 0.00<sup>d</sup> | 41.46 ± 0.61<sup>b</sup> | 6.22 ± 0.43<sup>b</sup> | 22.73 ± 0.35<sup>b</sup> | 28.95 ± 0.07<sup>c</sup> | 7.66 ± 0.13<sup>d</sup> |
| Spinach      | 29.36 ± 0.49<sup>b</sup> | 2.80 ± 0.11<sup>b</sup> | 29.72 ± 0.51<sup>d</sup> | 3.70 ± 0.06<sup>d</sup> | 22.00 ± 0.45<sup>b</sup> | 25.70 ± 0.50<sup>c</sup> | 12.58 ± 0.12<sup>c</sup> |
| Chard        | 28.38 ± 0.04<sup>b</sup> | 2.68 ± 0.12<sup>c</sup> | 33.49 ± 1.18<sup>c</sup> | 0.87 ± 0.01<sup>c</sup> | 21.54 ± 0.16<sup>c</sup> | 22.41 ± 0.18<sup>c</sup> | 13.87 ± 0.03<sup>b</sup> |
| Parsley      | 25.13 ± 0.06<sup>d</sup> | 3.37 ± 0.09<sup>a</sup> | 24.85 ± 0.60<sup>c</sup> | 3.98 ± 0.04<sup>c</sup> | 29.71 ± 0.79<sup>a</sup> | 33.69 ± 0.75<sup>b</sup> | 12.70 ± 0.09<sup>c</sup> |
| Coriander    | 33.25 ± 0.13<sup>a</sup> | 2.45 ± 0.07<sup>c</sup> | 7.30 ± 1.35<sup>f</sup> | 7.57 ± 0.42<sup>b</sup> | 29.91 ± 0.49<sup>a</sup> | 37.48 ± 0.06<sup>e</sup> | 20.32 ± 0.13<sup>a</sup> |

All values given are means ± standard deviation (n = 3)
Different superscript letters in the same column indicate a significant difference between groups (p < 0.05)
Table 2  Amino acid profile of some traditional vegetables from southern Chile

| Amino acids (g/100 g DW) | Pea    | Corn   | Carrot  | Leek   | Spinach  | Chard    | Parsley   | Coriander   |
|-------------------------|--------|--------|---------|--------|----------|----------|-----------|-------------|
| Essential amino acids   |        |        |         |        |          |          |           |             |
| Histidine (His)         | 2.12 ± 0.13b | 2.72 ± 0.21a | <0.01   | 1.38 ± 0.11c | 1.83 ± 0.19bc | 1.52 ± 0.12c | 0.85 ± 0.09d | 0.81 ± 0.10d |
| Isoleucine (Ile)        | 5.83 ± 0.17b | 8.15 ± 0.34a | 2.10 ± 0.31cd | 2.07 ± 0.21d | 2.52 ± 0.18e | 1.84 ± 0.13d | 2.53 ± 0.22c | 1.74 ± 0.14d |
| Leucine (Leu)           | <0.01   | <0.01   | <0.01   | 3.09 ± 0.32ed | 3.62 ± 0.29c  | 5.15 ± 0.30b  | 4.32 ± 0.25b | 4.56 ± 0.29ab |
| Lysine (Lys)            | 5.27 ± 0.19a | 1.81 ± 0.07a | 2.01 ± 0.15de | 4.13 ± 0.14b | 4.22 ± 0.20d | 3.29 ± 0.14c | 3.26 ± 0.18c | 2.24 ± 0.21d |
| Methionine (Met)        | 0.78 ± 0.07de | 1.82 ± 0.13a | <0.01   | 0.95 ± 0.04bc | 1.07 ± 0.08e | 0.85 ± 0.09ed | 0.77 ± 0.06de | 0.67 ± 0.04f  |
| Phenylalanine (Phe)     | 2.90 ± 0.21ab | 2.41 ± 0.19f | 1.49 ± 0.07f | 1.76 ± 0.07e | 3.14 ± 0.22f | 2.29 ± 0.08de | 2.63 ± 0.11bc | 1.62 ± 0.11gf  |
| Threonine (Thr)         | 1.42 ± 0.06c | 0.39 ± 0.03f | <0.01   | 1.10 ± 0.03ed | 1.92 ± 0.11bc | 1.11 ± 0.10d | 3.06 ± 0.04a  | 0.56 ± 0.03c  |
| Valine (Val)            | 3.08 ± 0.14ab | 3.12 ± 0.28ab | 3.24 ± 0.27a | 2.67 ± 0.23bc | 3.18 ± 0.20c  | 2.61 ± 0.07c | 3.06 ± 0.19ab | 2.08 ± 0.17d  |
| Conditionally essential amino acids |        |        |         |        |          |          |           |             |
| Arginine (Arg)          | 7.45 ± 0.22a | 2.36 ± 0.13d | 2.45 ± 0.17d | 2.88 ± 0.21c | 3.75 ± 0.19b  | 2.47 ± 0.17d | 2.69 ± 0.19ed | 1.89 ± 0.11c  |
| Glycine (Gly)           | 2.42 ± 0.11b | 1.77 ± 0.21c | 1.28 ± 0.08d | 2.05 ± 0.11c | 3.25 ± 0.27a  | 2.52 ± 0.11b | 2.68 ± 0.16b  | 1.81 ± 0.12c  |
| Proline (Pro)           | 2.38 ± 0.10c | 5.63 ± 0.17a | 0.92 ± 0.06f | 1.77 ± 0.11d | 2.78 ± 0.22b  | 1.98 ± 0.17d | 2.55 ± 0.11bc | 1.54 ± 0.08f  |
| Tyrosine (Tyr)          | 1.66 ± 0.07b | 1.94 ± 0.10l | <0.01   | 1.25 ± 0.08cd | 1.97 ± 0.11a  | 1.81 ± 0.12bc | 1.09 ± 0.08d  | 0.88 ± 0.03c  |
| Non-essential amino acids |        |        |         |        |          |          |           |             |
| Alanine (Ala)           | 3.34 ± 0.27b | 4.23 ± 0.32a | 3.04 ± 0.27bc | 3.11 ± 0.21b | 3.54 ± 0.38b  | 2.69 ± 0.17c | 2.68 ± 0.12c  | 1.77 ± 0.19d  |
| Aspartic acid (Asp)     | 6.63 ± 0.43a | 4.37 ± 0.33a | 6.05 ± 0.32ab | 5.61 ± 0.33a | 6.07 ± 0.52ab | 4.75 ± 0.23c | 3.38 ± 0.22d  | 3.05 ± 0.27d  |
| Hydroxyproline (Hypr)   | <0.01   | <0.01   | <0.01   | 0.41 ± 0.03a | 0.38 ± 0.08ab | 0.38 ± 0.11ab | 0.31 ± 0.03b  | 0.22 ± 0.10f  |
| Glutamic acid (Glc)     | 9.75 ± 0.33ab | 10.2 ± 0.47a | 8.12 ± 0.44c | 8.76 ± 0.43c | 8.91 ± 0.73c  | 6.37 ± 0.46d | 8.47 ± 0.63c  | 4.83 ± 0.13c  |
| Serine (Ser)            | 3.28 ± 0.17a | 2.98 ± 0.11ab | 2.41 ± 0.11c | 2.87 ± 0.09b | 3.10 ± 0.20ab | 2.42 ± 0.18c | 2.27 ± 0.15c  | 1.73 ± 0.17d  |
| Taurine (Tau)           | <0.01   | <0.01   | <0.01   | <0.01   | <0.01   | <0.01   | <0.01   | <0.01   |

All values given are means ± standard deviation (n = 3)
Different superscript letters in the same row indicate a significant difference between groups (p < 0.05)

Amino acid under stress conditions and catabolic states when the capacity of endogenous amino acids synthesis is exceeded [17]. The great importance of the availability of this amino acid is that arginine is indispensable for children’s growth, and low arginine bioavailability plays a pivotal role in the pathogenesis of a growing number of varied diseases, including sickle cell disease, thalassemia, malaria, acute asthma, cystic fibrosis, pulmonary hypertension, cardiovascular disease, certain cancers and trauma, among others [19]. Meanwhile, lysine is the most abundant essential amino acid found in leek. Lysine is a biologically determinant amino acid that helps the body absorb calcium, so that plays a crucial role in skeletal metabolism [20]. Isoleucine is the most abundant essential amino acid in corn, while leucine is the most abundant in carrot and green leafy vegetables (spinach, chard, parsley and coriander). Isoleucine, leucine and valine are branched amino acids, which are stored directly in muscle tissue, serving as scaffolds to support new growing muscle and increase strength. Therefore, these branched amino acids are of great biological value in the elderly for the prevention of sarcopenia since these amino acids increase muscle protein synthesis [21].

Vitamin and Mineral Composition

Table 3 depicts the profile of vitamins and minerals of vegetables investigated. As expected, the results indicated that green leafy vegetables are rich sources of vitamins and minerals. The most abundant vitamins in the studied vegetables were choline, ascorbic acid (vitamin C), niacin (B3) and α-tocopherol (vitamin E). In fact, vegetables are recognized as good sources of vitamin C and vitamin E [22].

Choline was an abundant vitamin in all studied vegetables which presented values from 204 mg/100 g DW (pea) to 64 mg/100 g DW (corn and chard), niacin (B3) ranged from 2.7 mg/100 g DW (corn) to 9.25 mg/100 g DW (pea). Choline is an important vitamin found in many vegetables and used commonly as a food supplement. In addition, choline is an important component of breast milk on which the fetal development of a child is dependent during the early birth stages [23]. Studies have shown that a higher prenatal choline level in the mother’s body can safeguard the developing brain of the fetus from the adverse effects of SARS-CoV-2 infection [23].

The mean content of ascorbic acid varied significantly from 12.6 mg/100 g DW (corn) up to 618.2 mg/100 g DW.
Table 3  Vitamins and minerals composition of some traditional vegetables from southern Chile

| Vitamins (mg/100 g DW) | Pea     | Corn    | Carrot  | Leek    | Spinach | Chard    | Coriander | Parsley |
|------------------------|---------|---------|---------|---------|---------|----------|-----------|---------|
| A Retinol              | 0.27±0.11 | 0.28±0.07 | 28.1±2.98 | 1.23±0.11 | 4.87±0.67 | 3.87±0.33 | 3.37±0.56 | 5.21±0.78 |
| B1 Thiamine            | 1.030±0.164 | 0.211±0.034 | 0.175±0.033 | 0.405±0.065 | 0.370±0.059 | 0.278±0.045 | 0.325±0.055 | 0.151±0.024 |
| B2 Riboflavin          | 0.147±0.024 | 0.043±0.007 | 0.132±0.021 | 0.217±0.035 | 1.140±0.182 | 0.950±0.152 | 0.930±0.149 | 0.511±0.082 |
| B3 Niacin              | 9.25±1.29 | 2.70±0.38 | 3.89±0.55 | 4.70±0.66 | 4.11±0.57 | 3.15±0.44 | 4.22±0.59 | 5.55±0.78 |
| B3 D-Pantothenic acid  | 1.40±0.28 | 0.70±0.14 | 1.13±0.23 | 0.79±0.16 | 0.32±0.06 | 1.72±0.34 | 1.57±0.31 | 1.99±0.40 |
| Calcium pantothenate   | 1.52±0.30 | 0.76±0.15 | 1.23±0.25 | 0.86±0.17 | 0.35±0.07 | 1.87±0.37 | 1.70±0.34 | 2.16±0.43 |
| B6 Pyridoxine          | 0.626±0.088 | 0.148±0.021 | 0.360±0.050 | 1.170±0.172 | 1.311±0.183 | 0.806±0.113 | 0.860±0.120 | 0.646±0.090 |
| B7 Folate              | 0.489±0.147 | 0.058±0.017 | 0.126±0.038 | 0.643±0.193 | 0.922±0.276 | 1.130±0.340 | 0.661±0.198 | 0.682±0.205 |
| C Ascorbic acid        | 19.4±2.47 | 12.6±3.54 | 14±2.89 | 45.2±3.65 | 267±32.3 | 618±101 | 121±31.4 | 456±77.6 |
| E α-tocopherol         | ND       | <1      | 3.01±0.55 | 2.63±0.44 | 13.4±2.43 | 76.7±23.0 | 9.29±1.44 | 83.0±33.5 |
| δ-tocopherol           | ND       | ND      | ND      | ND      | ND      | ND       | ND        | ND      |
| γ-tocopherol           | 6.77±0.98 | 1.78±0.19 | ND      | <1      | 2.62±0.22 | <1       | 6.38±0.91 | ND      |
| K1 Phylloquinone       | 0.028±0.005 | 0.005±0.001 | 0.089±0.003 | 0.054±0.007 | 2.190±0.307 | 1.810±0.254 | 0.476±0.095 | 0.533±0.107 |
| Choline                | 204±19.4 | 64.3±4.37 | 72.8±5.88 | 126±11.6 | 108±8.73 | 645±4.59 | 104±9.33 | 124±7.42 |

| Minerals (mg/100 g DW) | Pea     | Corn    | Carrot  | Leek    | Spinach | Chard    | Coriander | Parsley |
|------------------------|---------|---------|---------|---------|---------|----------|-----------|---------|
| Ca Calcium             | 30.92±2.951 | 9.221±1.724 | 134.6±12.62 | 233.9±21.51 | 1211±102.5 | 737.8±98.62 | 647.5±97.91 | 854.6±73.72 |
| Cu Copper              | 1.01±0.05 | 0.38±0.07 | 0.42±0.07 | 0.39±0.03 | 0.61±0.04 | 0.65±0.03 | 1.01±0.07 | 0.74±0.05 |
| Fe Iron                | 8.06±1.11 | 1.68±0.12 | 1.17±0.22 | 5.15±0.34 | 38.6±2.72 | 9.61±0.99 | 38.4±3.77 | 9.74±1.01 |
| P Phosphorus           | 321±33.2 | 343±28.2 | 220±23.3 | 443±29.7 | 347±21.9 | 218±17.7 | 522±33.5 | 449±11.3 |
| Se Selenium            | <0.005   | <0.005   | <0.005   | <0.005   | <0.005   | <0.005   | <0.005   | <0.005   |
| Na Sodium              | 1.28±0.04 | 1.11±0.05 | 411±32.2 | 58.1±11.9 | 101±17.3 | 1441±103.4 | 62.9±11.2 | 447±13.6 |
| Zn Zinc                | 5.41±0.68 | 2.53±0.32 | 1.14±0.10 | 3.5±0.82 | 14.4±1.28 | 5.56±0.87 | 6.23±1.02 | 6.33±0.98 |
| As Arsenic             | <0.005   | <0.005   | <0.005   | <0.005   | <0.005   | <0.005   | <0.005   | <0.005   |
| Pb Lead                | <0.02    | <0.02    | <0.02    | <0.02    | <0.02    | <0.02    | <0.02    | <0.02    |
| Sn Tin                 | <0.5     | <0.5     | <0.5     | <0.5     | <0.5     | <0.5     | <0.5     | <0.5     |
| Hg Mercury             | <0.005   | <0.005   | <0.005   | <0.005   | <0.005   | <0.005   | <0.005   | <0.005   |

All values given are means ± standard deviation (n = 3)
Different superscript letters in the same row indicate a significant difference between groups (p < 0.05)

(cheddars). Green leafy vegetables (spinach, chard, coriander and parsley) stood out with mean values higher than 121 mg/100 g DW. Hence leafy vegetables are a rich source of ascorbic acid [24]. Ascorbic acid (vitamin C) is a potent free radical scavenger and plays a vital role in maintaining a healthy lifestyle by acting as anti-carcinogenic and anti-atherogenic agent [25]. The antiviral effects of ascorbic acid are already recognized by the medical community. Several studies describe multiple mechanisms by which ascorbic acid enhances the function of leukocytes including chemokinesis and chemotaxis, phagocytosis, lysosomal enzyme production, generation of reactive oxygen species (ROS) and microbial killing, up-regulation of antibody response and increasing interferon [26]. In addition, the Shanghai Expert Consensus on COVID-19 Treatment has included ascorbic acid as a treatment for COVID-19-associated pneumonia [27]. For its part, niacin (vitamin B3) is the third vitamin present in quantity in all studied vegetables. Green leafy vegetables presented a similar content of niacin with values ranging from 3.15 to 5.55 mg/100 g DW. Although green leafy vegetables are considered a good source of niacin, the highest concentration was found in pea (Table 3).

Concerning minerals, vegetables are a rich source of minerals such as calcium, iron, phosphorus, sodium and zinc, which present many health benefits. In this respect, leafy vegetables stand out as a rich source of minerals [24]. Calcium is the most plentiful mineral found in vegetables. In the present study, green leafy vegetables showed a high concentration of calcium from 647.5 mg/100 g DW (coriander) up to 1,211 mg/100 g DW (spinach). Phosphorus is the second most abundant mineral highlighting the concentrations found in coriander, parsley, leek, spinach, corn and pea which presented concentrations over 300 mg/100 g DW. Also, the concentrations of iron and zinc observed in spinach, coriander and parsley are significantly high, which qualifies them as potential sources for the development of food supplements. The latter, it is of relevance due to deficiencies of the microelements cause significant health issues in vulnerable populations. Iron deficiency affects >60% of the global population and
hence is considered the most common micronutrient deficiency worldwide. While zinc deficiency is one of the most widespread micronutrient deficiencies in the world causing serious health issues [28].

One important dietary uptake pathway of toxic heavy metals could be through vegetable crops irrigated with metal-contaminated wastewater [29]. Therefore, the assessment of the concentration of these minerals is relevant for the utilization of these vegetables as a food supplement, especially when they are provided as lyophilized ingredients, drying process that might cause the unintended concentration of these minerals. However, the accumulation of heavy metals (arsenic, lead, tin and mercury) in the lyophilized vegetables was below the threshold of detection set for the used method. Therefore, they can be considered nutritionally safe food supplements for human health.

**Total Carotenoids, Polyphenols and Antioxidant Capacity (ORAC)**

Carotenoids and polyphenols represent the most abundant lipid and water soluble phytochemicals, respectively [30]. These compounds are plant-based molecules that have recognized benefit in human health as potent antioxidant and anti-inflammatory agents [31]. Table S1 (Supplementary Material) shows the total carotenoids and polyphenols content of the eight studied vegetables from southern Chile. Green leafy vegetables exhibited high concentrations of these bioactive compounds. Neder-Suárez et al. [13] have indicated that herbaceous plants such as spinach, chard, coriander and parsley have a high content of polyphenols. In addition, the ORAC assay employed to determine the antioxidant capacity of the vegetables showed that the highest antioxidant capacity was measured in parsley (76,270 μmol Trolox eqv/100 g), followed by spinach (24,079 μmol Trolox eqv/100 g) and coriander (16,961 μmol Trolox eqv/100 g). These values are much higher than previously reported 16,270 μmol Trolox eqv/100 g (parsley), 15,200 μmol Trolox eqv/100 g (spinach) and 2850 μmol Trolox eqv/100 g [32]. On the other hand, two vegetables (carrot and pea) showed the lowest antioxidant capacity (below 3700 μmol Trolox eqv/100 g).

It is known that heat treatments can induce significant changes in the chemical composition of perishable foods, particularly fresh vegetables, affecting the bioaccessibility, antioxidant capacity and contents of compounds such as vitamins, carotenoids and polyphenols [33]. Therefore, the results show that lyophilization is an adequate alternative to preserve the nutritional value of edible plants, thus making them suitable for the development of natural food supplements.

**Pesticides Residues**

The use of pesticides in the last year has increased because they have rapid action, decrease toxins produced by food infecting organisms and are less labour intensive than other pest control methods. However, improper and excessive use of pesticides during vegetable production has resulted in environmental pollution and augmented human health risks [34].

Therefore, it is important to consider the accumulation of these components especially when vegetables go through drying processes to concentrate healthy ingredients. It is likely that after processing the pesticide residues might remain and even increase their concentrations in the vegetables with potentially harmful effects to other non-targeted organisms than pests and diseases. In this regard, out of the 332 types of pesticide residues analysed, only six (1.8%) were detected (fenpropidin, cypermethrin, 2-phenylphenol, linuron, chlorpyrifos and mepanipyrim) (Table S2), and three (2-phenylphenol, chlorpyrifos and mepanipyrim) exhibited concentrations exceeding the maximum residue level (MRL) defined for fresh vegetables by the European Union (EU) regulation [35]. If the MRL is exceeded could represent health implications; however, the cumulative effect of pesticides residues represents a higher potential harmful risk for human health. Thus, numerous negative health effects, dermatological, gastrointestinal, neurological, carcinogenic, respiratory, reproductive and endocrine, have been associated with pesticide residues. It is worth stressing that for dehydrated food products there is a lack of official MRL regulation, which deserves attention since drying technologies are increasingly used considering their cost-effective benefits.

Solely leek did not show any type of pesticide residue, while corn and carrot did not surpass the MRL for insecticide and fungicide, respectively. It is noteworthy that the MRL defined by the EU and other international regulations apply to fresh fruits and vegetables, and there is a need to develop a specific regulation concerning pesticides limits for dehydrated food products, a market that is growing tremendously. The low percentage of pesticides present in these eight vegetables are in line with good agricultural practice coined by small farmers as a strategy to differentiate and promote an environmentally friendly and innocuous local production in southern Chile.

**Multivariate Analysis of Nutritional Information**

A hierarchical clustering and PCA-biplot analyses were conducted integrating all the nutritional information for the eight vegetables, where two groups of vegetables were identified (Fig. 1).
Group I included coriander, chard, spinach and parsley and exhibited high content of leucine, calcium, carotenoids, ascorbic acid and protein (Fig. 1a). Group II included carrot, leek, pea and corn and showed high quantities of 10 amino acids and carbohydrates (Fig. 1a, b). Corn was particularly high in eight amino acids including valine, glycine and methionine, while pea was specifically high in phenylalanine, cooper, arginine and choline. Among the eight vegetables assessed spinach exhibited the more balanced nutritional profile. Overall, the hierarchical clustering and PCA-biplot analyses permitted the identification of theoretical vegetable combinations to develop well-balanced natural health-promoting supplements.

Therefore, through an iterative method for solving a system of equations it is possible to obtain the most suitable combination of vegetables to respond to certain nutritional requirements. In this way, it is possible to generate different combinations of lyophilized vegetables for the development of food supplements that could remedy intake deficiencies or supplement specific nutritional requirements.

**Conclusions**

To the best of our knowledge, this is the first comprehensive evaluation of proximate, amino acids, vitamins and minerals composition, total carotenoids- and polyphenol-contents, pesticide residues and combination potential of eight traditional vegetables of southern Chile to design natural food supplements. The analyses revealed that lyophilized vegetables are promising sources of essential nutrients that could be exploited as ingredients for the preparation of food supplements to help obtain adequate amounts of nutrients with the overall benefits on health. The green leafy vegetables produced in southern Chile contain adequate quantities of proteins, essential amino acids, choline, ascorbic acid, alpha-tocopherol, niacin, calcium, phosphorus, iron and antioxidants. Thus, the intelligent combination of vegetables in specific proportions may contribute to providing health benefits due to their potential immune-boosting effect. Therefore, utilization of these vegetable powders is advised for the formulation of natural food supplements to meet the nutritional...
requirements and to strengthen human health, especially under the current global health emergency.

**Abbreviations**  
AA: Amino acid; AAPH: 2,2’-Azobis(2-methylpropanimidined) dihydrochloride; ACN: Acetonitrile; DW: Dry weight; FC: Folin-Ciocalteau; GAE/g: Gallic acid equivalents per gram of dry weight vegetable; GC/MS: Gas chromatography-mass spectrometry; HPLC: High performance liquid chromatography; HPLC-DAD: High performance liquid chromatography with diode-array detection; LC/MS/MS: Liquid chromatography with tandem mass spectrometry; MRL: Maximum residue level; ORAC: Oxygen radical absorbance capacity; PUFA: Polysaturated fatty acids; Trolox$_{eq}$/100 g: micro-moles of Trolox equivalents per 100 g of dry weight vegetable

**Supplementary Information**  
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**Data Availability**  
The data that support the findings of this study are available from the corresponding author, Mauricio Opazo-Navarrete upon reasonable request.

**Code Availability**  
Not applicable.

**Declarations**

**Conflict of Interest**  
The authors declare that they have no conflicts of interest.

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