Extraction of antioxidant compounds from Espino maulino (*Vachellia caven*) fruit using supercritical CO_2 and accelerated solvent extraction in a serial process: characterization and application in a model lipid system

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Abstract

This study investigated the extraction of antioxidant compounds from hawthorn fruit (*Vachellia caven*), a species native to South America, using supercritical CO_2 (Sc-CO_2) and accelerated solvent extraction (ASE) method. The results revealed that according to the total phenols assay, pod had a richness of phenolic compounds (22568.49 GAE/100 g dry matter), and a strong antioxidant activity was demonstrated by 2,2-diphenyl-1-picrylhydrazyl free radical (362.27 μmol TE/g dry matter) and oxygen radical absorbance capacity (3049.39 umol TE/g dry matter) assays. The seed fraction was rich in tocopherols, particularly γ-tocotrienol, which is scarce and associated with good antioxidant activity (59.92 ug/g oil). The Sc-CO_2 extract contained a significant amount of unsaturated fatty acids (65–84%), including linoleic and linolenic acids, which are of nutritional interest. Hawthorn extracts, particularly the pod extract, demonstrated significant antioxidant properties in a refined sunflower oil model system, slowing the generation of primary and secondary lipid oxidation compounds in a thermo-oxidative process, indicating their potential as natural preservatives in food applications.

Keywords: *Vachellia caven;* supercritical CO_2; accelerated solvent extraction; antioxidant properties; oxidative stability

Introduction

Oxidative rancidity refers to the deterioration of oils and fats, leading to undesirable off-flavors and odors. This characteristic directly affects the storage life and, consequently, the stability of the product (resistance to oxidation) (Mehany *et al.*, 2024). Oxidative stability is a key quality criterion, because oxidation reduces the nutritional value and depreciates the final product (Flores *et al.*, 2024). Among the strategies to minimize lipid

deterioration are avoiding the presence of oxygen, avoiding high temperatures in fatty materials, and addition of antioxidant species of plant origin, especially when they are little-known species. Therefore, it is necessary to seek strategies to stop or minimize the deterioration of lipids that leads to a chain reaction.

Phenolic compounds exhibit antioxidant activity by neutralizing free radicals and reactive oxygen species (ROS). Their activity is influenced by factors, such as humidity, oxygen, temperature, and pH (Pasquet *et al.*, 2024). Therefore, it is crucial to evaluate the effect of the chemical nature of antioxidant compounds (lipophilic or hydrophilic) or their interaction with the environment in which these compounds act.

Consumers increasingly reject synthetic additives, evidencing a global trend toward the development of a green and sustainable economy as well as the introduction of natural additives that offer equal or superior benefits over synthetic ones (Amarachukwu, 2022), especially if these protective components are obtained from underutilized native plant species (*Vachellia caven*), which would contribute to food sustainability.

On the other hand, extraction processes often use solvents that are not suitable for the health of the population. Organic solvents have been reported to cause headaches, dizziness, fatigue, blurred vision, behavioral changes, loss of consciousness, and even death if ingested or inhaled in excess of the limit (Joshi and Adhikari, 2019). In this sense, technologies such as supercritical CO_2 (Sc-CO_2) extraction and accelerated solvent extraction (ASE) have emerged, where the first one stands out for its selectivity, low temperature, high diffusivity, moderate working pressures, preservation of nutritional properties, harmlessness, and fastness, among other qualities (Moreira *et al.*, 2023). On the other hand, ASE offers advantages such as low extraction cost, lower solvent consumption, compared to Soxhlet extraction or shaker extraction, short extraction time, and simplified extraction protocols, among other advantages (Zhang *et al.*, 2022). Both Sc-CO_2 and ASE are called eco-technologies that help to value agro-industrial waste, development of technological innovations, commercial applications, generation of patents, and applications in plant and marine substrates, among many other positive characteristics (Fraguela-Meissimilly *et al.*, 2023). Some reported applications involve the recovery of bioactive compounds such as antioxidants from the wastewater of apple and apricot processing (Argun and Argun, 2025; Argun *et al.*, 2025), among others.

Nowadays, efforts are made to obtain bioactive compounds from industrial crops, for example, olive leaves, natural vanilla extracts, olive-fruit-processing by-products (Briante *et al.*, 2002; Pyrka *et al.*, 2025; Shyamala *et al.*, 2007), and even extracts from food waste such as coffee grounds and black tea have been used to stabilize polyunsaturated oils such as fish oil (). Efforts are made to characterize and obtain antioxidant compounds from promising native species, such as obtaining antioxidant extracts of different polarity from maqui leaves, microencapsulated extracts of boldo leaves, among others (Flores *et al.*, 2019; Polanco *et al.*, 2024). Also, efforts are made to recover compounds of interest from waste parts of plant species, such as peel from red pitaya fruit or tomato skin, which can be used as functional food ingredients, pigments, or antioxidants, which would have an impact on environmental pollution and improve the added value of waste products (Méndez-Carmona *et al.*, 2022; Yu *et al.*, 2023). However, few efforts are devoted to obtaining bioactive compounds from the native species Espino maulino (*Vachellia caven*) from South America.

The Espino maulino inhabits areas with a Mediterranean climate and warm steppe, capable of withstanding prolonged periods of drought. The fruit is a dark brown sub-woody legume called quiringa that contains hard seeds arranged in rows (Gómez-Fernandez *et al.*, 2023). Despite the limited research on *Vachellia caven*, its seed are reported to contain a high concentration of proteins, which may be the basis for high-protein flours (Benedetti and Pavez, 2012).

Therefore, this research aimed to develop apolar extracts of *Vachellia caven* using supercritical fluid extraction, followed by polar extracts through ASE, applied sequentially on the same substrate. This dual extraction process was designed to maximize recovery of bioactive compounds, particularly for applications as additives, to protect nutritionally important lipids. The extraction conditions for both methods were optimized by applying an experimental design using a response surface method for obtaining phenolic compounds efficiently. The optimal extracts were considered for the evaluation of a protective effect in a lipid matrix with the presence of saturated, monounsaturated, and polyunsaturated fatty acids.

Materials and Methods

Raw material

Pods (seed coats) and seeds of *Vachellia caven* were collected from wild plants in the summer of 2024 from native vegetation around Talca, Chile (35°, 25' S, 71°40' W). Figure 1 shows the fruit and seeds of *Vachellia caven*.

The plant material was separated into pod and seed, dried in a forced air oven at 40°C for 3 h for pod (9.6% humidity) and 40°C for 1 h for seed (6.9% humidity),

Figure 1. ***Vachellia caven*: (A) fruit and (B) seeds.**

and crushed using a 300-g electric chopper (model AD6011CL; Moulinex, France) and a shear mill. The samples were passed through a No. 20 sieve (particle size of 850 um) and placed in airtight low-density polyethylene (LDPE) bags and stored at room temperature.

Extraction Process

The extraction process was carried out using pods and seeds of *Vachellia caven* as raw material. First, experiments were designed based on the Response Surface Method (RSM) using the Statgraphics Centurion XV program.

This sequential design allowed optimizing and obtaining of lipophilic extracts, first by Sc-CO_2 and then by hydrophilic extracts by ASE in residual cake.

The uncoded values of independent variables for both Sc-CO_2 and ASE extractions are given in Tables 1 and 2, respectively.

Supercritical carbon dioxide extraction

The extraction was carried out as described by Basegmez *et al.* (2017) with some modifications. A laboratory supercritical extractor was used (Spe-ed SFE-2, model 7071; Applied Separations, Allentown, PA). The procedure for Sc-CO_2 extraction consisted of weighing the crunched pods and seeds according to the proportions of each experiment obtained by RSM. The 50-mL extractor cell was charged with 19 g each of pod, seed, and pod–seed mixture samples, each charge mixed with ¼ Celite 545 as a filtration aid (Merck, Darmstadt, Germany), that is, approximately 4.75 g. Liquid CO_2 (purity 99.99%; Indura SA, Chile) was used at a superficial velocity of 1 mm/s.

Table 1. Experimental design of Sc-CO_2 extraction.

Extraction No.	Temperature (ºC)	Pressure (Bar)	Seed–pod ratio (%)
1	35	300	20:80
2	35	350	10:90
3	35	350	30:70
4	35	400	20:80
5	45	400	10:90
6	45	300	10:90
7	45	300	30:70
8	55	300	20:80
9	55	350	10:90
10	45	350	20:80
11	45	350	20:80
12	45	350	20:80
13	45	400	30:70
14	55	400	20:80
15	55	350	30:70

Table 2. Experimental design of ASE extraction.

Extraction No.	Temperature (ºC)	Ethanol–water ratio (%)
1	25	40:60
2	25	20:80
3	50	40:60
4	50	20:80
5	75	40:60
6	75	20:80
7	75	0:100
8	50	0:100
9	25	0:100

A Box–Behnken Design (BBD) was employed for Sc-CO_2 extraction, evaluating 15 experiments with three independent variables: temperature (35, 45, and 55°C), pressure (300, 350, and 400 bar), and pod–seed ratio (10:90, 20:80, and 30:70%). Oil yield and tocopherol concentration were used as response variables. The extraction yield of the oily fraction was considered according to Ortiz-Viedma *et al.* (2023), in addition to the concentration of tocopherols because of its effect on its stability.

Accelerated Solvent Extraction

The extraction process was carried out in a Dionex ASE300® unit (Thermo Fisher Scientific, MA, USA) according to the method proposed by Basegmez *et al.* (2017) with slight modifications. Once the residue was obtained from Sc-CO_2 extraction, 2 g of an optimized pod–seed mixed with a quarter of celite. This was taken to ASE equipment and adjusted to a temperature, ethanol–water ratio according to the experimental design, and pressure of 1,500 psi. The number of extraction cycles was obtained considering the evaluation of total phenols and dry extract yield (optimal values of 75°C; and ethanol–water ratio: 40:60), adjusting to six cycles of 5 min. The experimental design was carried out with a three-level Factorial design 3^2 that considers nine extraction experiments with two independent variables: temperature (25, 50, and 75°C), ethanol–water ratio (0:100, 20:80, and 40:60%), and as response variable the weight of dry extract (yield) and the amount of total phenols. The extraction yield of the aqueous fraction was considered as an optimization parameter according to Ortiz-Viedma *et al.* (2023), in addition to the concentration of total phenols because of its protective effect.

Chemical analysis

Nutritional characterization

The sifted pods and seeds were subjected to a proximal analysis according to the official methods of Association of Official Analysis Chemists International (AOAC, 2005). The moisture and ash content were determined by gravimetric methods, lipids by the Soxhlet method, proteins by the Kjeldahl method, and total carbohydrates were obtained as the remaining fraction at 100%.

Fatty acid profile of the extract obtained by Sc-CO_2 extraction

The fatty acid profile was performed according to the official AOAC (1992a) Ce 1-62 method, using gas chromatography, which was expressed as the percentage of methyl esters. An HP-5890 gas chromatograph (Hewllet-Packard, CA, USA) with a 50-m long bpx-70 fused silica column, 0.25-µm film thickness, and 0.25-mm internal diameter, with a flame ionization detector (FID), and a split injection system, calibrated as 90:10, was used. The derivatization of fatty acids was performed by a pre-treatment of extract, methylation with boron trifluoride (BF_3) in 12.5% methanol. Lipid extract, 100 mg, was weighed and 5 mL of 0.5-N NaOH in methanol was added, heated for 5 min to boiling point, cooled, and 5 mL of BF_3 in 12.5% methanol was added. Then, it was heated to a boiling point for 3 min. It was cooled and methyl esters were dissolved in 1.5 mL of petroleum ether. Saturated NaCl was added up to 2/3 of the tube, shaken gently, and left to stand for 1 h. By observing phase separation, fatty acid methyl esters (FAME) dissolved in petroleum ether were extracted.

Tocopherols in Sc-CO_2 extraction

Tocopherols were determined by high-performance liquid chromatography (HPLC) according to the official AOAC (1992b) method Ce 8-89. HPLC was performed using a Merck-Hitachi L-6200 A pump (Merck) coupled with a Rheodyne 7725i injector fitted with a 20-µL sample loop, a Merck-Hitachi F-1050 fluorescence detector, and a Merck-Hitachi D-2500 chromatographic integrator. A standard solution containing α-, β-, γ-, and δ-tocopherols was prepared using reference compounds obtained from Calbiochem Merck (Darmstadt, Germany).

Total carotenoids in Sc-CO_2 extraction

Carotene was determined by a colorimetric method, using a Thermo Scientific Multiskan GO spectrophotometer, with a wavelength of 300–770 nm and the SkanIt GO software, according to the methodology described by Rodriguez-Amaya (2001). For this, the oil was diluted in hexane, and the carotenoid concentration was calculated using the following equations:

$$x1\left(ug\right)=\frac{A\times y\times 10^{6}}{A_{1cm}^{1\%}\times 100}, \tag{1}$$

$$x2\left(ug/g\right)=\frac{x}{weight\ of\ the\ sample}, \tag{2}$$

where $x1$ is the weight (ug) and $x2$ is the concentration (ug/g) of total carotenoid, y is the volume of the solution (mL) that gives an absorbance A (nm) at a specific wavelength, and $A_{1cm}^{1\%}$ is the absorption coefficient of carotenoid in the solvent used.

Total phenols in ASE extraction

Total phenols were determined by using the Folin–Ciocalteu method according to Bordeu and Scarpa (1998). Extract sample, 0.1 mL, was added to a 10-mL volumetric flask with 4.9 mL of distilled water and 0.5 mL of Folin-Ciocalteau reagent. It was left to stand for 3 min. Then, 1.7 mL of sodium carbonate solution (Na_2CO_3) was added. It was mixed and topped up to 10 mL with distilled

water at rest for 30 min. The absorbance was measured in a spectrophotometer (UV/Vis, UV3-200 model, Unicam, Brand, Cambridge, UK) at 765 nm. The concentration of total phenols was determined by a calibration curve with gallic acid solutions between 50 and 800 µg/mL, and the results were expressed in gallic acid equivalents per gram of extract (mg GAE/100 g).

2,2-Diphenyl-1-picrylhydrazyl free radical (DPPH) test in ASE extraction

The antiradical capacity was measured by the DPPH test, described by Brand-Williams *et al.* (1995). A stock solution of 1 mg/mL DPPH radical was prepared by diluting 1 mL in a 50-mL volumetric flask and interpreting against blank (methanol) at 517 nm in a spectrophotometer (ATI Unicam UV/Vis spectrometer UV3-200). Then, a curve was prepared with Trolox reagent using 25-mg stock solution in a 50-mL flask, and sufficient aliquots of this stock solution were taken to a volume of 10 mL with DPPH. Subsequently, 0.1 mL of each extract and 3.9 mL of 1 mg/mL DPPH solution were added to 10 mL tubes covered with aluminum foil. The tubes were left in the dark for 30 min at room temperature and measured spectrophotometrically. The results were expressed in µmoles Trolox equivalent/gram dry matter.

Oxygen Radical Absorbance Capacity (ORAC) method in ASE extraction

The antioxidant capacity was determined by the ORAC method, according to the methodology described by Huang *et al.* (2002) with slight modifications, using a FLx800-BID fluorometer (BioTek, Winooski, VT, USA). In all. 25 µL of sample and 150 µL of fluorescein solution were added to each well of the microplate and incubated at 37°C for 30 min. Then, 25 µL of 4.6% 2,2'-azobis(2-methylpropionamidine) dihydrochloride (AAPH solution) in phosphate buffer was added to start the reaction. The fluorescence intensity was recorded every minute using a 485-nm excitation filter with a 20-nm bandwidth and a 528-nm emission filter of the same bandwidth. The antioxidant capacity was calculated by interpolating the net area derived from the variations in fluorescein fluorescence intensity of the samples into a linear regression curve constructed from the areas under the curve (AUCs) of fluorescein kinetic decay in the presence of different concentrations of the Trolox standard (6-hydroxy-2,5,7,8-tetramethylchroman-2-carboxylic acid). The results were expressed as µmoles Trolox equivalent/g dry matter.

Total anthocyanins—differential pH method in ASE extraction

The differential pH method proposed by Lee *et al.* (2005) was used to measure anthocyanins. The authors prepared dilutions of the extracts with pH 1.0 buffers of 0.025-M potassium chloride (1.864 g/L) and pH 4.5 buffers of 0.4-M sodium acetate (32.812 g/L), adjusting the pH of both solutions with HCl. The absorbance of dilutions was then measured at 530 nm and 700 nm in a spectrophotometer (ATI Unicam UV/Vis Spectrometer UV3-200),

$$A = (A530\text{-}A700)\ pH\ 1.0 - (A530\text{-}A700)\ pH\ 4.5, \quad (3)$$

$$AT\ (\text{cyanidin-3-glucoside equivalents}) = A \times PM \times FD \times \frac{1{,}000}{\varepsilon x 1}, \quad (4)$$

where:

A is the absorbance obtained in Eq (3); PM is the molecular weight of cyanidin-3-glucoside (449.2 g/mol); FD is the dilution factor of extract vs. buffer (10 in general); 1,000 is the conversion factor from g to mg; ε is the molar extinction coefficient of cyanidin-3-glucoside 26900 L/mol × cm; and 1 is the path length of spectrophotometer cuvette in cm.

Dilution of 4-mL quartz cuvette: 0.4 mL of the extract was taken in a reaction tube with a lid, and 3.6 mL of the corresponding buffer was added and absorbance measured against the blank (the same buffer).

Total tannins in ASE extraction

The tannin content was determined according to the method proposed by Bossu *et al.* (2006) in a spectrophotometer (ATI Unicam UV/Vis Spectrometer UV3-200) with modifications. In all, 5 mL of 2.5% (w/v) KIO_3 solution was heated at 30°C for 10 min and then 1 mL of the sample was added. The mixture was kept at the same temperature for an additional 30 min before measuring its absorbance at 550 nm. The calibration curve was prepared with tannic acid.

Thermo-oxidation test

The thermo-oxidation test was based on the NCh 5-19 method described in American Oil Chemists' Society (AOCS, 1993) manual. Briefly, two tubes were used for thermo-oxidation: the first tube with 3 g of pure refined sunflower oil (PS), without antioxidants (donated by Camilo Ferrón Chile, S.A.) as a control (PS); the other tube consisting of 3 g of PS added to an extract volume with 0.13-mg GAE. Mixed extract lipid–aqueous fraction (Sc-CO_2 extract–ASE extract 1:10 ratio). The samples were shaken prior to heating at 60°C in the dark and covered in a Rancimat apparatus used for heating. The absorption parameters K232 and K270 were performed at every 30 and 60 min and up to 3 h of heating. PS was used as a control.

Statistical analysis

The results were analyzed using the Statgraphics Centurion software. They were subjected to ANOVA tests, which were used to compare mean values of each parameter, using the Tukey's Honestly Significant

Difference (HSD) test with a confidence level of 95%. Analyses were conducted in triplicate, except for the literature data taken directly from their references for comparative purposes. The results were expressed as mean and standard deviation (SD).

Results and Discussion

Nutritional composition

Curiosity in under-exploited plant species located in South America has increased in recent years, especially if the species are grown in particular agronomic conditions, such as low water availability, which enhances the development of components of interest to humans (Barba-Ostria *et al.*, 2024; Ortiz-Sempértegui, *et al.*, 2024). Nutritional composition is important for the health of the population, because it informs the consumer about the composition of plant material and the proportion of its composition. Particular attention is given to poorly studied species.

As shown in Table 3, *Vachellia caven* seeds exhibited a higher protein content (approximately 40%) compared to quinoa, amaranth, linseed, and cañihua seeds. However, a protein percentage of slightly higher than 40% in cotyledons was reported in the past (Benedetti and Pavez, 2012). Ash content in *Vachellia caven* pods and seeds was similar to those reported for quinoa, amaranth, and linseed, although lower than those for cañihua. However, the fat content in *Vachellia caven* seed was significantly lower than that of the four mentioned species. On the other hand, the total carbohydrate content of *Vachellia caven* pod stands out, reaching 94.35%, exceeding the values for the seeds of other species and for *Vachellia caven* seeds as recorded in the literature.

Optimization for total phenol and tocopherol content

Figure 2(A) shows the estimated response surface for the response variable gamma tocopherol, with a coefficient of determination, $R^2 = 0.92$, both found as a function of temperature (°C), pod–seed mixture (%), and a high pressure value for Sc-CO_2 optimization process. Table 4 shows experimental results of the extraction design for Sc-CO_2. Figure 2(C) shows the estimated response surface, considering total phenols as a response variable (measured in mg GAE per 100 g of sample) as a function of the ethanol–water ratio (%) and temperature (°C), with $R^2 = 0.93$ for ASE optimization process, each one with its respective pareto diagrams. Table 5 shows experimental results for the aqueous fraction obtained by ASE.

It is observed in Figure 2A that at low temperature and low proportion of pod–seed mixture (10:90%), there is a greater response of gamma tocopherol evidenced in the diagram in red with a high desirability. The highest gamma tocopherol concentration of 460.658 ppm was obtained at a temperature of 35.05°C, pressure of 400 bar, and a pod–seed ratio of 10:90%. Therefore, these conditions are considered optimal point for Sc-CO_2 extraction. The pareto diagram shows a significant negative effect of temperature, which reflects an inverse relationship between temperature and gamma tocopherol concentration. This relationship can be attributed to the fact that at higher temperatures the compounds undergo degradation.

Figure 2C shows that at higher temperatures (75°C) and a higher ethanol–water ratio (40:60), a greater quantity of total phenols, 8995.11 mg GAE/100 g, is obtained. Therefore, these conditions are considered theoretical optimal point for ASE extraction. The pareto diagram shows a significant positive effect ($P < 0.05$) of ethanol–water ratio; on the contrary, temperature and interactions do not significantly affect the response variable.

The theoretical optimum oil yield per Sc-CO_2 was 2.49%, slightly higher (about 2%) than the experimental optimum yield of 2.44%. On the other hand, the experimental optimum yield of 352.9 ppm of gamma tocopherol

Table 3. Results of proximal analysis of *Vachellia caven* pods and seeds, compared to average values of quinoa, amaranth, linseed, and cañihua seeds from literature, measured as percentage on dry basis.

Samples/nutritional composition	Proteins (%)	Fat (%)	Ash (%)	Total carbohydrates (%)	Total (%)
Vachellia caven pod	0.48 ± 0.02	0.52 ± 0.02	4.65 ± 0.03	94.35 ± 0.01	100
Vachellia caven seed	39.17 ± 0.05	2.63 ± 0.01	4.57 ± 0.03	53.63 ± 0.07	100
Whole wheat quinoa flour[a]	17.32	6.70	3.31	72.67	100
Amaranth flour[b]	15.84	7.41	3.00	73.75	100
linseed flour[c]	22.24	40.60	3.63	33.53	100
Cañihua flour[d]	18.89	8.60	8.37	64.14	100

Notes: [a]Cervilla *et al.*, 2012; [b]Dicao *et al.*, 2023; [c]Hussain *et al.*, 2008; [d]Moscoso-Mujica *et al.*, 2024.

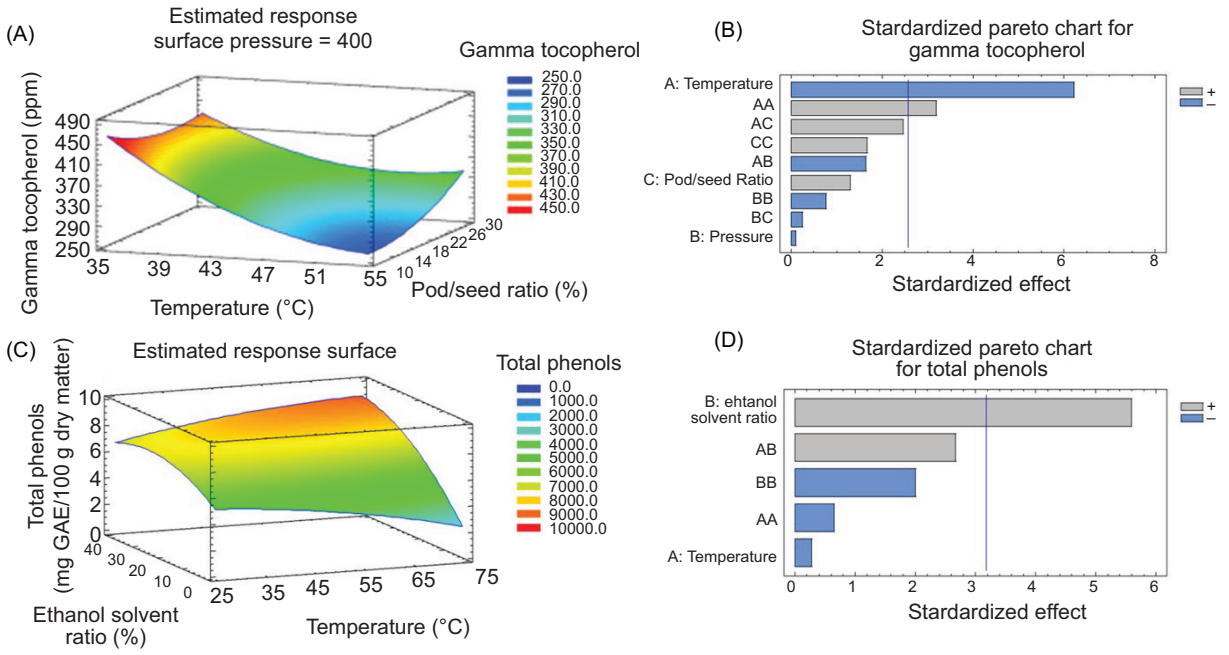

Figure 2. Estimated response surface in (A) Sc-CO_2 and (C) ASE extraction process, considering gamma tocopherol and total phenols as response variables, respectively; (B and D) their respective standardized pareto diagrams.

Table 4. Experimental results of Sc-CO_2 optimization process.

Extraction No.	Yield (%)	Gamma tocopherol concentration (ppm)
1	1.42 ± 0.02	400.9 ± 7.33
2	1.39 ± 0.04	319.6 ± 6.68
3	1.53 ±0.01	418.4 ± 14.00
4	2.69 ± 0.06	259.8 ± 3.44
5	1.74 ± 0.10	446.5 ± 2.26
6	1.67 ± 0.11	301.0 ± 2.39
7	1.51 ±0.05	400.0 ± 0.92
8	2.36 ± 0.12	370.8 ± 13.54
9	1.53 ± 0.08	303.2 ± 3.19
10	1.72 ± 0.06	327.1 ± 8.48
11	1.43 ± 0.05	340.8 ± 3.26
12	2.44 ±0.15	352.9 ± 1.30
13	1.47 ± 0.18	345.1 ± 2.60
14	1.49 ± 0.07	294.6 ± 0.66
15	1.41 ± 0.09	319.9 ± 2.96

was lowerthan the theoretical optimum yield for gamma tocopherol of 460.6 ppm (>30%).

The experimental total phenol concentration was 8698.88 mg GAE/100 g, slightly lower (<4%) than the theoretical optimum value of 8995.11 mg GAE/100 g, as well as the experimental yield of 0.18 g/g dry sample, much lower than the predicted theoretical yield (0.43 g/g dry sample), with >50% difference.

Table 5. Experimental results of ASE optimization process.

Extraction No.	Total phenols (mg GAE/100 g sample)	Dry extract (g/g of sample)
1	6613.70 ± 352.4	0.26 ± 0.02
2	8019.07 ± 665.2	0.30 ± 0.05
3	8139.07 ± 465.5	0.35 ± 0.03
4	6835.09 ± 444.5	0.32 ± 0.02
5	9317.58 ± 476.8	0.44 ± 0.04
6	6847.43 ± 505.1	0.32 ± 0.02
7	2239.64 ± 234.2	0.06 ± 0.01
8	4988.07 ± 412.1	0.31 ± 0.02
9	4388.62 ± 387.8	0.28 ± 0.03

Concentration of bioactive compounds

It is observed in Table 6 that the highest amounts of total tocos in the decreasing order are in seed extract by Soxhlet method; seed extract by Sc-CO_2 extraction; pod–seed mixture by Soxhlet method; pod extract by Soxhlet method; pod–seed mixture by Sc-CO_2 extraction; and pod extract by Sc-CO_2 extraction. In the seed extracts obtained by Sc-CO_2 extraction and Soxhlet method, a higher concentration of γ-tocopherol is observed, followed by α-tocopherol, δ-tocopherol and β-tocopherol. Statistically significant differences ($P < 0.05$) are found between the concentrations of all types of tocopherols in both methods for seed extracts, except in the case of δ-tocopherol where the same values are obtained.

Table 6. Concentration of bioactive compounds in the extracts of *Vachellia caven*: total tannins and total anthocyanins by ASE extraction; total carotenoids by Sc-CO_2 extraction; and tocopherols by Sc-CO_2 extraction and Soxhlet method.

Methods/ samples	Total tannins (mg EAT/g dry matter)	Total anthocyanins (mg C3G eq/g dry matter)	Total carotenoids (µg/g oil)	α-T (µg/g oil) by Sc-CO_2 extraction	β-T (µg/g oil) by Sc-CO_2 extraction	γ-T (µg/g oil) by Sc-CO_2 extraction	δ-T (µg/g oil) by Sc-CO_2 extraction	α-T (µg/g oil) by Soxhlet method	β-T (µg/g oil) by Soxhlet method	γ-T (µg/g oil) by Soxhlet method	δ-T (µg/g oil) by Soxhlet method	γ-T3 (µg/g oil) by Soxhlet method
Pod (P)	946.97 ± 23.8^a	0.01 ± 0.0^a	2.06 ± 0.29^a	24.62 ± 1.63^a	6.2 ± 0.52^a	2.57 ± 0.24^a	NF	221.81 ± 9.94^a	36.51 ± 1.54^a	164.93 ± 0.8^a	58.09 ± 0.33^a	59.92 ± 5.58
Seed (S)	305.64 ± 26.34^b	0.03 ± 0.0^a	0.66 ± 0.03^b	382.48± 1.28^b	10.28 ±0.43^c	609.79± 3.24^b	114.67± 4.64^b	510,9 ± 3.55^b	16.61 ± 0.68^b	939.32±10.69^b	123.6 ± 1.37^b	NF
Mix (P/S) (70/30)	317.52 ± 7.09^b	0.02 ± 0.01^a	0.65 ± 0.12^b	169.32±15.13^c	13.54 ± 0.82^c	121.43±10.09^c	90.3 ± 7.17^c	182.38 ± 0.59^c	11.43 ± 0.51^b	539.98± 0.4^c	93.65 ± 0.25^c	NF

Note: Data are expressed as mean ± standard deviation. Different superscript letters in the same column indicate statistically significant differences ($P < 0.05$). NF: not found.

The concentration of tocopherols in pod–seed mixture by both methods (Sc-CO_2 extraction and Soxhlet method) does not show significant differences ($P < 0.05$), except for γ-tocopherol. The pod extract obtained by Sc-CO_2 method exhibited the lowest tocopherol concentration, compared to other samples. According to Ishak *et al.* (2021), in the extraction of chia seed oil, a lower concentration of total tocopherols by Sc-CO_2 extraction was observed, compared to Soxhlet method, and a higher content for γ-tocopherol was also reported for both Sc-CO_2 extraction and Soxhlet method. According to Bozan and Temelli (2002), in linseed oil, the authors obtained a higher concentration of total tocopherols by Soxhlet method, compared to Sc-CO_2 extraction. In addition, the authors reported the highest concentrations of both β- and γ-tocopherols.

Table 6 shows that the lipid fraction obtained by Sc-CO_2 extraction contains γ-tocotrienol. This phenomenon is remarkable because vegetable oils generally contain few tocotrienols, compared to tocopherols (Ortiz-Viedma *et al.*, 2023). In addition, it is reported that in the studies of various oils and fats, γ-tocotrienol was more effective in inhibiting lipid peroxidation than α-tocopherol and other tocopherol isomers (Nakamura *et al.*, 2014).

In the case of chia oil and linseed oil as reported in the literature, a higher concentration of total tocopherols was observed using Soxhlet extraction method, compared to Sc-CO2 extraction (Bozan and Temelli, 2002; Ishak *et al.* 2021). This difference in results is attributed to endogenous factors of seeds, such as origin of seeds, exogenous factors of extraction processes, such as processing conditions, use of different solvents of different polarity, and/or presence of co-solvents in extraction processes.

It is observed in Table 7 that *Vachellia caven* oil obtained from pods, seeds, and pod–seed mixture has different proportions of saturated, monounsaturated, and polyunsaturated fatty acids according to Sc-CO_2 extraction and Soxhlet method. It is also noted that Sc-CO_2 extraction does not differ greatly in its total PUFA content, except for total PUFA from pods. Total PUFA content from different parts of *Vachellia caven* was from 20% to 50%.

According to the literature, linoleic acid and linolenic acid are not synthesized by the human body and must be consumed through diet (Petraru *et al.*, 2021). Therefore, the oily extract of *Vachellia caven* mixture extracted by Sc-CO_2 extraction could be a nutritious option in diet, similar to amaranth, which could motivate its inclusion in food formulations or in the functional food industry. This species is considered a good source of bioactive compounds because of its wide distribution in South American countries, in addition to availability of its fruits despite subjected to long periods of drought.

Table 7. Fatty acids expressed as percentage (%) of methyl ester in pods, seeds, and pod–seed mixture of *Vachellia caven* and samples of linseed and amaranth seeds obtained by Sc-CO_2 extraction and Soxhlet method as reported in literature.

Fatty acid (%)*	*Vachellia caven* (this study)						Literature			
	Sc-CO_2			Soxhlet method			Sc-CO_2		Soxhlet method	
	Pod	Seed	Pod–seed mix	Pod	Seed	Pod–seed mix	Linseed[a]	Yellow amaranth[b]	Linseed (a)	Amaranth spp. (Peckus Farm)[c]
Saturated fatty acids										
Myristic, C14:0	1.58 ± 0.22	-	-	-	-	-	-	0.17	0.10	0.28
Palmitic, C16:0	10.52 ± 0.39	9.83 ± 0.16	8.94 ± 0.17	7.89 ± 0.03	10.11 ± 2.9	10.09 ± 0.09	6.20	19.46	7.6	25.91
Margaric, C17:0	0.24 ± 0.07	-	-	-	-	0.05 ± 0.03	-	0.10	-	0.11
Stearic, C18:0	4.01 ± 0.11	6.06 ± 0.05	6.17 ± 0.31	3.77 ± 0.08	6.62 ± 1.9	6.53 ± 0.06	3.60	4.45	4.10	3.34
Arachidic, C20:0	5.54 ± 0.32	0.70 ± 0.08	0.59 ± 0.2	6.60 ± 0.23	0.96 ± 2.2	0.94 ± 0.04	-	0.86	-	0.53
Behenic, C22:0	4.48 ± 0.12	0.30 ± 0.07	-	4.81 ± 0.19	0.43 ± 0.15	0.35 ± 0.04	-	0.29	0.10	0.11
Tridecanoic, C23:0	6.71 ± 0.1	0.19 ± 0.26	-	1.49 ± 0.39	-	0.06 ± 0.11	-	-	-	-
Lignoceric, C24:0	1.38 ± 0.2	-	-	0.90 ± 0.13	-	-	-	0.19	-	0.23
Total saturated fatty acids	34.46	17.07	15.70	25.46	18.12	18.01	9.80	25.52	11.90	30.51
Monounsaturated fatty acids										
Palmitoleic, C16:1	0.79 ± 0.14	0.16 ± 0.03	-	-	0.14 ± 0.01	0.10 ± 0.02	-	0.46	0.10	0.42
Palmitoleic *trans*, C16:1*t*	0.42 ± 0.04	-	-	-	-	-	-	-	-	-
Margaroleic, C17:1	2.70 ± 0.04	0.12 ± 0.78	-	2.84 ± 0.18	0.26 ± 0.04	0.17 ± 0.02	-	0.76	-	0.68
Oleic C18:1, n-9	16.09 ± 0.11	31.12 ± 0.23	32.12 ± 0.1	16.65 ± 0.19	31.41 ± 0.09	31.76 ± 0.01	17.50	32.56	16.10	26.88
Oleic *trans*, C18:1*t*	1.51 ± 0.09	-	-	1.52 ± 0.17	0.07 ± 0.02	-	-	-	-	-
Vaccenic *cis*, C18:1	0.49 ± 0.65	0.68 ± 0.21	1.11 ± 0.24	0.47 ± 0.43	-	0.76 ± 0.08	-	-	-	-
Nonadecanoic, C19:1	7.42 ± 0.01	0.21 ± 0.03	0.19 ± 0.03	7.79 ± 0.06	0.34 ± 0.19	0.27 ± 0.1	-	-	-	-
Eicosenoic, C20:1, n-9	15.59 ± 0.11	0.80 ± 0.02	0.75 ± 0.04	9.13 ± 0.11	0.90 ± 1.37	1.84 ± 0.02	-	0.68	-	0.15
Docosenoic, C22:1 n-9	1.13 ± 0.04	0.29 ± 0.15	0.04 ± 0.03	1.41 ± 0.16	0.10 ± 0.01	0.10 ± 0.15	-	-	-	-
Nervonic, C24:1	0.33 ± 0.46	-	-	1.86 ± 0.74	-	-	-	-	-	-
Total monounsaturated fatty acids	46.46	33.39	34.21	41.67	33.21	35.00	17.50	34.46	16.20	28.13
Polyunsaturated fatty acids										
Linoleic, C18:2, n-6	17.77 ± 0.31	48.72 ± 0.7	49.24 ± 0.77	18,91 ± 0.17	47.54 ± 0.88	46.99 ± 0.19	16.20	38.32	14.40	40.17
Linoleic *trans,* C18:2, 9*t*,12*t*	0.47 ± 0.09	-	-	0.41 ± 0.39	-	-	-	0.40	-	-
Linolelaico, C18:2*t*								0.40		0.29
γ-Linolenic, C18:3	0.83 ± 0.08	-	-	0.66 ± 0.02	-	-	-	-	-	-
α-Linolenic, C18:3, n3		0.82 ± 0.3	0.85 ± 0.45	12.90 ± 0.17	1.13 ± 0.4	-	55.00	1.02	50.00	0.60
Arachidonic, C20:4			-	-	-	-	-	0.09		-
Total polyunsaturated fatty acids	19.08	49.54	50.09	32.88	48.67	46.99	71.20	39.83	64.40	41.06

Notes: *Fatty acid as methyl ester.
[a]Pradhan *et al.* (2010); [b]Kraujalis and Venskutonis (2013); [c]Kraujalis *et al.* (2013).

It is observed in Table 7 that *Vachellia caven* provides at least 18% of essential fatty acids, such as linoleic (omega-6) and linolenic (omega-3), depending on the part of the fruit. Some important functions are structural component of membranes, contribution to the eicosanoid chain (alpha-linolenic acid serves as a precursor of long chain fatty acids), and anti-inflammatory effect, thus establishing this native species of significant interest and its possible contribution to the health of the population (Valenzuela *et al.*, 2011). However, during heating processes, PUFAs have a greater tendency to degrade than MUFAs, followed by saturated fatty acids (SFA) (Flores *et al.*, 2019; Santos et al., 2002), which makes it essential to delve deeper into the strategies for the protection of unsaturated fatty acids.

Antioxidant properties

According to Table 8, *Vachellia caven* pod has the highest total phenol content (22568.49 mg GAE/100 g dry matter), followed by seed and pod–seed mixture, both showing intermediate level of phenol content (8722.83 mg GAE/100 g dry matter). However, seed alone has a significantly lower phenol content (2682.9 mg GAE/100 g dry matter).

Regarding antioxidant activity (DPPH assay), pod showed the highest DPPH radical scavenging activity (362.27 μmol TE/g) whereas seed and pod–seed mixture had a lower DPPH activity.

In the case of ORAC assay, pod again showed the highest ORAC value (3049.39 μmol TE/g), indicating a strong antioxidant activity, whereas seed had a moderate ORAC activity (393.31 μmol TE/g), with pod–seed mixture having intermediate ORAC values (1225.77 μmol TE/g). The above trials were directly correlated regarding high total phenol content versus high antioxidant activity, a phenomenon previously reported in a study on antioxidant properties of plant materials (Repajić, *et al.*, 2024).

The total phenolic content of *Vachellia caven* pod was remarkably high (>22,000 mg GAE/100 g dry matter; >3 times higher) than the phenolic content of pomace of Calafate berry from Patagonia, well known for its excellent antioxidant properties, as well as for other berries, such as blueberry, strawberry, and raspberry (Ortiz-Viedma *et al.*, 2023; Palka and Wilczyńska, 2023). Also, the total phenolic content of *Vachellia caven* pod was many times higher than he methanolic extract of maqui leaves (4100.9 ppm) (Flores *et al.*, 2021).

A similar trend was observed with antioxidant capacity; pods showed a higher antioxidant capacity by both DPPH and ORAC methods. The antioxidant capacity of pod was 30 times more than that of Maqui berry pomace by ORAC assay; 2–3 times higher than Ginkgo biloba seed extracts according to DPPH assay; and even several times higher than phenolic extracts of olive leaves according to ORAC assay (Huamán-Castilla *et al.*, 2024; Ortiz-Viedma *et al.*, 2023; Xu *et al.*, 2022). These high levels of antioxidant properties of *Vachellia caven* species are very promising, and it would be of great interest to evaluate its antimicrobial, antifungal, and antibacterial effects, among others. Therefore, it would be important to evaluate its performance as a natural antioxidant in complex matrices such as foods.

Thermostability

Figure 3 shows the thermo-oxidative evaluation of pure sunflower oil (PS) and oil samples enriched with phenolic compounds (0.13 mg gallic acid equivalents, GAE, added per 3 g of oil) using 1 mL of a 1:10 mixture of Vachellia caven pod extracts obtained by both supercritical CO_2 (Sc-CO_2) and pressurized solvent extraction (PSE) methods. The evaluation was performed based on the absorption parameters K232 and K270.

Figure 3 shows the evolution of (A) primary (K232) and (B) secondary (K270) oxidation compounds of model lipid. To avoid misinterpretation of these absorbances, it is advisable

Table 8. Antioxidant properties of pod, seed, and pod–seed mixture (30:70) in ASE extraction.

Methods/samples	Total phenols (mg GAE/100 g dry matter)	DPPH (μmol TE/g dry matter)	ORAC (μmol TE/g dry matter)
Vachellia caven pod	22568.49 ± 282.35[a]	362.27 ± 42.62[a]	3049.39 ± 239.70[a]
Vachellia caven seed	2682.9 ± 151.06[b]	207.96 ± 16.33[b]	393.31 ± 20.65[b]
Pod–seed mix (30:70)	8722.83 ± 150.4[c]	282.7 ± 4.53[c]	1225.77 ± 37.03[c]

Notes: ORAC: oxygen radical absorbance capacity; DPPH: 2,2-Diphenyl-1-picrylhydrazyl free radical.
Data are expressed as mean ± standard deviation.
Different superscript letters in the same column indicate statistically significant differences ($P < 0.05$).

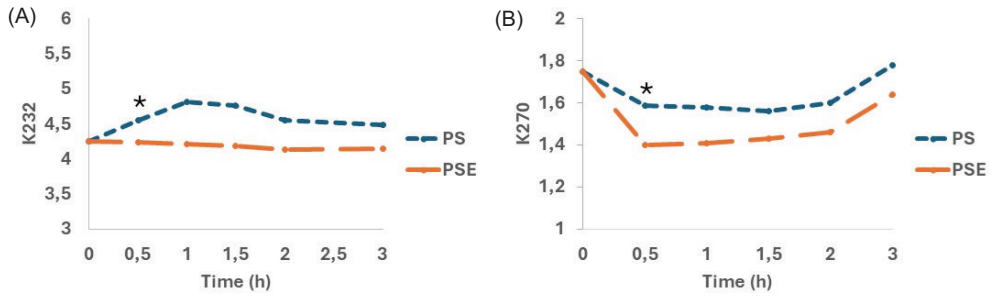

Figure 3. Evolution of (A) primary (K232) and (B) secondary (K270) compounds from the oxidation of pure sunflower oil (PS) and 3-g PS added with 0.13 EAG (PSE) from a 50% mixture of *Vachellia caven* pod extracts by Sc-CO_2 and ASE (PSE) extractions. *$P < 0.05$.

to have a lipid matrix without interferents (oleuropein or chlorogenic acid present in olive oil) (Pyrka *et al.*, 2025). For a better understanding of deterioration of lipids, it is necessary to monitor all the compounds formed, because primary compounds, such as hydropexides, are easily degraded, leading to misinterpretation of scientific research, contrary to the greater stability of secondary compounds (ketones and aldehydes) (Flores *et al.*, 2024). It was observed in both A and B cases that from 0.5 h of heating and throughout the heating process, there were significant differences ($P < 0.05$) in the control sample (PS) with respect to the fortified sunflower oil sample (PSE); in both cases, higher PS produced a greater amount of degradation products, compared to PSE, Therefore, the addition of the extract can be considered as a protective factor for the lipid material.

Conclusions

The results revealed that *Vachellia caven* pods and seeds contained various bioactive compounds, highlighting the presence of natural antioxidants, such as tocopherols and phenols, essential for oxidative stability in food items.

The optimization of the extraction processes allowed maximizing the yield of antioxidant compounds in different fractions, which represented a significant advancement for future industrial applications.

The optimum theoretical concentration of γ-tocopherol in Sc-CO_2 extraction was 460.658 ppm at a temperature of 35.05°C, pressure of 400 bar, and pod–seed ratio of 10:90, greater than the experimental optimum of 352.9 ppm of γ-tocopherol.

In the case of ASE extraction, it was evident that at 75°C and an ethanol–water ratio of 40:60, the concentration of experimental total phenols obtained was 8698.88 mg GAE/100 g, a slightly lower (<4%) than the theoretical optimum value of 8995.11 mg GAE/100 g.

The lipophilic extract presented a high proportion of unsaturated fatty acids. Also, the lipid fraction of *Vachellia caven* contained different types of tocotrienols, especially γ-tocotrienol. *Vachellia caven* pods showed a high concentration of carotenoids and total phenols, exceeding the values reported for Calafate berry, maqui leaves, and flaxseed and grape seeds; this positioned pods as a promising source of natural antioxidants. The antioxidant analysis confirmed that *Vachellia caven* pod extracts had a high antioxidant capacity, which was reflected in thermo-oxidative study, and therefore its application in stability and shelf-life studies of food products is suggested. It is projected that Sc-CO_2 and ASE technologies could be used efficiently to obtain bioactive compounds from medicinal plants or to recover compounds of interest for the health of the population from waste materials.

Author Contributions

Marcos Flores and Cielo Char: conceptualization; Katherine Cordero, Cielo Char, and Claudia Vergara: methodology; Claudia Vergara, Cielo Char, and Katherine Cordero: software; Marcos Flores, Claudia Vergara, and Jaime Ortiz-Viedma: validation; Katherine Cordero: formal analysis; Katherine Cordero: investigation; Marcos Flores: resources; Claudia Vergara: data curation; Marcos Flores: writing—original draft preparation; Cielo Char, Claudia Vergara, and Jaime Ortiz-Viedma: writing—review and editing; Cielo Char and Jaime Ortiz-Viedma: visualization; Jaime Ortiz-Viedma: supervision; Marcos Flores: project administration; Marcos Flores: funding acquisition. All authors had read and agreed to the published version of the manuscript.

Conflicts of Interest

The authors declared no conflict of interest.

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