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Research Article

Preliminary Studies of Chemical and Physical Properties of Two Varieties of Avocado Seeds Grown in Chile

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In Chile, the most cultivated avocado varieties are Hass and Negra de la Cruz. The increase in the production of fast food preparations, salads, and avocado oil has generated large amounts of seeds and husks as waste, which may constitute a possible new source of bioactive compounds, of great interest to the food and pharmaceutical industry. Therefore, the objective of this study was to determine different nutritional, biochemical, antimicrobial, and physical properties of the SH and SNC seeds grown in Chile. In SH and SNC, their nutritional composition, lipophilic components, and total phenols (PTs) were determined. The antioxidant and antibacterial activity of extracts were measured in different solvent combinations. In addition, the color change of seeds (ΔE) during the browning, the mechanical resistance to the cut, and their thermal transitions by differential calorimetry (DSC) were determined. The results indicated that SH has a higher protein content and lipophilic components. In the ethanol extract, SNC showed greater antiradical activity and is an inhibitor of Gram (+) bacteria. The SNC browning given by ΔE was greater and adjusted well to kinetic and enzymatic models. The physical analyses of the seeds indicated that SNC presented higher cut resistance and lower transition temperature (T_g) with a lower thermal fusion of its lipids, which would be due to its higher unsaturated composition. These properties of SH and SNC can be useful for the agrifood, pharmaceutical, and chemical industries.

1. Introduction

In recent decades, there is a tendency to replace synthetic preservatives by derivatives from natural origin that are obtained from waste or discards of vegetables from the food industry [1]. In this way, most researchers have devoted themselves to the search for new bioactive compounds present in fruits and their waste that demonstrate an optimal antioxidant and antibacterial power [2]. One fruit that has relevance in the diet of consumers due to its health properties is avocado. The avocado (*Persea americana* Mill.) belongs to the family Lauraceae, which includes approximately 150 species, and its habitat is mainly tropical America. In Chile, the production of avocados is focused in the central zone of the country, and approximately 40,000 ha are destined to this product and is distributed between the IV and VI regions with production areas located close to both the coast and the hills. In this region, the most cultivated varieties are Hass and Negra de la Cruz. Negra de la Cruz takes that name because it is abundantly cultivated in the sector of La Cruz (Region of Valparaiso, Chile); also, it is known as *Prada* or *Vicencio*. It is believed that it was originated by natural hybridization, with the influence of the Mexican variety, *Leucaria*, so it could be considered, therefore, as a Mexican Guatemalan hybrid. The fruit distinguishes itself from other fruits due to its high lipid content and is consumed as a food and used as an ingredient

in the formulation of nutraceutical and cosmetic products. Furthermore, several beneficial medicinal properties of compounds present in the avocado pulp, seed, and peel have been reported [3]. In addition, seeds and peel of Hass avocado cultivated in USA, Mexico, Portugal, and Australia have been shown to possess antioxidant and antimicrobial properties [4–6]. Therefore, it would be interesting to determine how the phytochemicals, biochemistry, and physical properties differ in avocado varieties cultivated in areas of southernmost climatic conditions. Furthermore, due to the increase in avocado production, a considerable amount of the fruit is used for fast food preparations, salads, and oil, which generates large volumes of residues from seeds and peel of the fruit, which may constitute a possible source of antioxidants and antimicrobials of great interest in food and pharmaceutical industry. Determination of the nutritional value, antioxidant and antimicrobial activity, browning, and physical properties of avocado seeds cultivated in Chile has not been evaluated, and knowledge of these properties would contribute to the development of new products and food ingredients derived from the waste of avocado industry. The main objective of this study was to analyze and compare different properties (chemical composition, antioxidant and antibacterial activity, browning, and physical properties) of Hass (SH) and Negra de la Cruz (SNC) avocado seeds cultivated in Chile.

2. Materials and Methods

2.1. Plant Material. The avocado seeds were obtained from the processing of avocado "Hass" and "Negra de La Cruz" varieties to produce avocado oil, harvested during the month of October 2017; these were supplied by Agroindustrial Razeto SA, commune of Quillota, Valparaíso Region (Chile). Five kg of each seed type were washed, ground in a kitchen grinder (Somela FP500, Santiago, Chile), and sieved to a size of 0.8 ± 0.2 mm. The samples were then stored under refrigeration (5°C), in plastic containers, with a double cover, lined with aluminum foil.

2.2. Preparation of Raw Extracts. To differentiate the extraction efficiency of polar components such as polyphenols and at the same time their antioxidant and antimicrobial activity, extractions with decreasing polarity solvents such as methanol, ethanol, and ethyl acetate were used. Ten grams of avocado seeds were weighed into a flask lined with aluminum foil and then was stirred in a shaker (Wrist Action, Burrell, USA), with 100 mL of each of the following extractant solutions: E/W = ethanol/water (50/50% v/v), M/W = methanol/water (50/50% v/v), and EA = ethyl acetate (of 99.9% purity) for 1 h. This procedure was subsequently repeated to the solid phase produced in the first extraction with 50 mL of each extractant and shaken for 30 min. The extract was filtered through cotton and deposited in a 250 mL glass bottle lined with aluminum foil. The six raw extracts obtained were preserved under refrigeration and then used to quantify the TP and antioxidant capacity (DPPH* and FRAP).

2.3. Chemical, Physical, and Microbiological Analyses

2.3.1. Nutritional Analysis. Following the Association of Official Analytical Method [7], moisture content, ash, proteins ($N \times 6.25$), and fiber were determined. Carbohydrate contents were obtained by difference of the rest of nutrients [8].

2.3.2. Fatty Acid. The composition of fatty acids was determined from oil by gas chromatography FID detection using the official method AOCS [9], previous preparation of the fatty acid methyl esters derivatives. Gas chromatography was performed using a Hewlett–Packard model 5890 series II chromatograph (using H_2 as the carrier gas) connected to a Hewlett–Packard 3397 integrator. The capillary column used was of BPX-70 fused silica (length 100 cm and internal width 0.25 mm, with a film thickness of 0.2 μm). The oven temperature was programmed from 160°C to 220°C with a heating rate of 2°C/min. The temperature of the injector and detector was fixed at 250°C.

2.3.3. Tocopherol Analysis. Levels of total tocopherols were determined in lipid extracts by high-performance liquid chromatography (HPLC) by fluorescence detection, following the standard AOCS method [10]. The mobile phase was propan-2-ol in *n*-hexane (0.5 : 99.5 v/v) at a flow rate of 1 mL/min. Peaks were detected at 290 and 330 nm, excitation and emission wavelengths, respectively. Tocopherols were identified using external standards (Merck). Results are expressed in microgram tocopherol per gram lipid.

2.3.4. Analysis of Carotenoids. The content of lutein and β-carotene was determined by the open column chromatography technique, using MgO in Hyflo® Super-Cel® and neutral alumina, in addition to hexane, diethyl ether, and acetone among others, as elution solvents. The absorbance of the samples with β-carotene and lutein was read at 445 and 450 nm, respectively, according to the visible absorption spectrophotometric method [11].

2.3.5. Total Polyphenols. For the six crude extracts that could contain different types of soluble components (organic acids, free fatty acids, mineral salts, and among others), the content of total polyphenols (TPs) was determined. The TPs were evaluated by the Folin–Ciocalteu method [12]. The phenolic compounds were oxidized by the Folin–Ciocalteu reagent. The blue coloration was then measured in a spectrophotometer at a wavelength of 765 nm, and a calibration curve of gallic acid is used to transform the data to gallic acid equivalent (GAE mg/dry weight; dw).

2.3.6. DPPH Radical Scavenger Activity. DPPH was assayed according to the method proposed by Brand-Williams et al. [13]. Using this method, the antioxidant capacity was determined based on the capacity of the antioxidants in the sample to reduce the DPPH* radical. The purple coloration

was then measured in a spectrophotometer at a wavelength of 517 nm. The results were expressed as a function of the IC_{50}, which corresponded to the concentration of the extract capable of inhibiting 50% of the free radicals in the DPPH* solution. This was obtained from the equation of the line obtained by plotting percent inhibition versus concentration, in milligrams GAE per milliliter.

2.3.7. Determination of Antioxidant Activity with FRAP Assay. The ability of the extracts to reduce ferric iron (Fe^{+3}), which is present in a complex with 2,4,6-tri-(2-pyridyl)-s-triazine (TPTZ), to the ferrous form (Fe^{+2}) at low pH was determined using the FRAP assay. The blue color generated was quantified by colorimetry (593 nm) based on a ferrous sulphate pattern [14].

2.4. Browning Expressed as Color Kinetics. The change in color on the CIELAB scale was measured using a tristimulus colorimeter (CR-400, Konica Minolta Sensing, Osaka, Japan). The color parameters determined during the browning were L* (whiteness/darkness), a* (redness/greenness), and b* (yellowness/blueness). These values were also used to calculate the total color change (ΔE) of the SH and SNC seeds. For which, both seeds were cut in half and kept at room temperature (25°C) for 30 min. The change in color due to browning was measured as follows: the first 5 min at 30 s intervals and the remaining 25 min measured at 5 min intervals. The equipment was calibrated with a reference tile of white porcelain ($L_0 = 97.63$, $a_0 = 0.31$, and $b_0 = 4.63$), before the determinations. The color change (ΔE) was calculated taking as a reference the color change (ΔE) of the SH and SNC seeds of avocado at time zero, by means of the following equation:

$$\Delta E = \sqrt{\left((a^* - a_0^*)^2 + (b^* - b_0^*)^2 + (L^* - L_0^*)^2\right)}, \quad (1)$$

where L^* and L_0^* are the initial and final lightness, a^* and a_0^* are the initial and final parameters from green to red, and b^* and b_0^* are the initial and final parameters from blue to yellow.

2.4.1. Estimation of Browning Kinetics. The browning of both seeds based on the color change ΔE was estimated by two different methods:

(1) Michaelis–Menten-Type Kinetic Model. As the color change in time responds to the enzymatic browning reaction of SH and SNC, the ΔE values were plotted as a function of time and adjusted to a parabolic behavior (such as the classic kinetic model of Michaelis–Menten) [15] given by the following equation:

$$\Delta E = \frac{\Delta E_{\max} * [t]}{\beta + [t]}, \quad (2)$$

where ΔE = "distance" between two colors in the CIELAB color chart graph, $\Delta E_{\max}$ = "maximum distance" between two colors in the CIELAB color chart graph, $[t]$ = time, and β = time necessary to reach 1/2 of $\Delta E_{\max}$. Then, we sought to linearize this graph; for that, the linearization Lineweaver–Burk was used [16]:

$$\frac{1}{\Delta E} = \frac{\beta}{\Delta E_{\max}} * \frac{1}{[t]} + \frac{1}{\Delta E_{\max}}. \quad (3)$$

Graphing $1/\Delta E$ versus $1/t$ of equation (3) with this, we obtain the value of β and $\Delta E_{\max}$.

(2) First-Order Kinetics. On the contrary, to adjust the data to first-order kinetics, without considering the substrate concentration, a relative substrate concentration [Sr] was calculated, from the following relation:

$$[\mathrm{Sr}] = \frac{\Delta E_{\max} - \Delta E_t}{\Delta E_{\max} - \Delta E_0}, \quad (4)$$

where ΔE_0 is the variation of color at initial time (equal to zero) and ΔE_t is the color variation at a time "*t*."

Once [Sr] was calculated, the experimental data were adjusted to the linearized first-order kinetic model:

$$\ln[\mathrm{Sr}] = \ln[S_0] + \mathrm{kr} * t, \quad (1)$$

where kr = relative kinetic constant of the first order and parameter $[S_0]$ is the initial concentration of substrate in mg/ml.

2.5. Antibacterial Activity

2.5.1. Minimum Inhibitory Concentration. To determine the minimum inhibitory concentration (MIC), a specific volume of these extracts were dried (<35°C) and completely dissolved in sterile distilled water. Reference bacterial strains were used: *Escherichia coli type I*, *E. coli O157*, *Salmonella enteritidis*, *Staphylococcus aureus*, *Bacillus cereus*, and *Listeria monocytogenes*. Each studied strain was inoculated into the brain-heart infusion (BHI) broth and then incubated for 24 h at 37°C. The grown culture was diluted to turbidity equal to that of a tube of 0.5 from the MacFarland scale. Then, the culture was inoculated by spreading on the surface of Mueller–Hinton, nutritive, and Palcam agar (Mueller–Hinton agar was used for *E. coli O157*, *S. enteritidis*, and *B. cereus*; nutritive agar was used for *S. aureus*; and Palcam Agar was used for *L. monocytogenes*). Antibacterial activity was determined by the well diffusion method (gel perforation), which is a modification of the Kirby–Bauer method, in which the paper disc is replaced by wells in the solidified culture medium [17, 18]. In the solidified media, where the culture was spread to exhaustion, seven perforations of approximately 11 mm diameter were made with a sterile punch. Thereafter, 35 μL of each raw extract was added to each well and 35 μL of the corresponding extraction (E/W, M/W, EA, and water as control). Finally, samples were incubated for 24 h at 37°C.

2.6. Physical Properties

2.6.1. Mechanical Test. The cutting force test was determined in the LLOID model LRSK universal algorithm

that was coupled with a Warner and Bratzler blade. This test measures the force (Newton) needed to cut the SH and SNC seeds. The test cell consisted of a 3 mm thick steel blade that had a 40° section at its bottom edge and was installed through a 4 mm slot in a small table (like a guillotine with a cut in V on the blade). The edge of the blade was loosely adjusted in the groove of the table. The SH and SNC samples to be tested were placed on the table, under the V-shaped blade, and cut when the blade moved down with a constant speed through the table's slot (the parameters of the tests were speed prior to the test: 3.0 mm/s, test speed: 1.0 mm/s, and speed after the test: 3.0 mm/s). The down travel distance was 60 mm (since the probe can vary between 55 and 65 mm). The resistance of the samples to the cut was recorded every 0.01 times by a computer on a force deformation graph. The parameter was the breaking strength of the first layer of the seed bark and the maximum force of the cut, which is the highest peak of the curve, which is the maximum resistance of the raised leg [19].

2.6.2. Thermal Analysis. Small pieces (15–20 μg) of fresh tissues from the SH and SNC cotyledons were histologically extracted and placed in a sealed aluminum capsule for later analysis in a differential scanning calorimeter DSC 6000 brand PerkinElmer (USA). The temperature program used consisted of a cooling of 25°C to −70°C with a speed of −10°C/min, a maintenance period of 2 min, and then a warm-up to 200°C at a speed of 5°C/min. An air-sealed capsule was used as a reference, and the constant flow of liquid N_2 of 40 mL/min was maintained. Thermograms were analyzed using Pyres Player software to determine the enthalpies (calculated from the area of the endothermic peak under the baseline), presented in joules per gram fresh weight (J/g FW), of phase transitions (T_g) as well as the melt onset temperatures.

2.7. Statistical Analysis. Data on the chemical composition, fatty acid profile, tocol content, and carotenoids were expressed as the mean ± standard deviation of four determinations ($n = 4$). Means were compared using the least-squares difference (LSD) method. Analyses were performed using the Statgraphics Plus 5.1 program; differences among batches were considered significant for a confidence interval at the 95% level ($p < 0.05$) in all cases.

3. Results and Discussion

3.1. Nutritional Composition. Table 1 shows the chemical composition of the SH and SNC seeds. From these results, it was possible to determine that there were no significant differences ($p < 0.05$) in the carbohydrates and fiber between the seeds of the SH and SNC avocados. Conversely, the level of protein, lipid, and ash was higher ($p < 0.05$) in the SH than in the SNC. However, the nutritional composition of avocado SH and SNC cultivated in Chile (Table 1), coincides with the ranges reported for the avocado of different varieties and origins [1, 3, 20, 21]. Nevertheless, the nutritional composition of both seeds is different from that reported for the other fruit component fractions. For example, it has been shown that the lipid content of the seed is higher than that of the peel but lower than that of the pulp in the mature fruit [22, 23].

3.2. Lipid Components. Table 2 shows the fatty acid profile, tocol content, and carotenoid content in the lipid fraction of both seeds expressed as a percentage of methyl esters. From these results, we conclude that the avocado seed SNC variety contained a higher ($p < 0.05$) percentage content of palmitic acid (C16 : 0) compared with SH. The SH seed contains oil with a significantly higher ($p < 0.05$) percentage content of oleic acid (C18 : 1ω-9) compared with that of the SNC oil. Conversely, SNC oil was found to have a higher percentage content of linoleic acid (C18 : 2ω-6) than SH oil. These differences in percentage composition indicate that SH oil is predominantly monounsaturated, while the SNC oil is polyunsaturated. Furthermore, the content of stearic, oleic, and linoleic acids present in the SH oil was very similar to that described by Gutfinger and Letan for the SH avocado seed oil [24]. In addition, the lipid composition of monosaturated and polyunsaturated fats in SNC oil is consistent with that reported by Bora et al. [20]. These results suggest that both oils possess beneficial properties for health, mainly due to the amounts of ω-6 and ω-3 essential fatty acids, which are precursors of larger and more unsaturated fatty acids. The lipid coming from the SH and SNC varieties contain an ω-6/ω-3 ratio of 5.8 and 9.8, respectively, values that are within the nutritionally recommended (from 5 : 1 to 10 : 1 in weight) since they can contribute to the fetus through placental transport and the newborn through breast milk, at the requirement of long-chain polyunsaturated fatty acids, necessary for a normal development of the nervous and visual system. The percentage content of oleic acid in SH and SNC oils was lower than that reported for Hass avocado pulp oil (15 to 69%) and avocado pulp from other origins [20, 23, 25]. In addition, it should be noted that the oil content and composition of the different avocado fractions (pulp, seed, and peel) vary according to the origin of the fruit, seasonality, and postharvest conditions [25, 26] and the variety [27]. Both seeds were found to contain a high level of α-tocopherol and α-tocotrienol (Table 2). The contents of α-tocopherol, δ-tocopherol, and α-tocotrienol were significantly higher in SNC oil than in Hass avocado seed oil ($p < 0.05$). These results indicate that the SNC variety can produce oils with high antioxidant power [28], only comparable to wheat germ oil, widely used in the field of cosmetics [5]. The content of α-tocopherol exceeds the tocol content reported for avocado pulp oil (130 to 179 μg/g oil) although avocado pulp oil contains higher γ-tocopherol content (144 to 780 μg/g) [5]. γ and δ-tocopherol isomers are more stable against oxidation, and tocopherols have a higher antioxidant potential than tocotrienols [29, 30]. Finally, the content of lutein (131.51 μg/g oil or 2.62 μg/g fresh fruit) and β-carotene (111.88 μg/g oil or 2.22 μg/g fresh fruit) present in SH was higher ($p < 0.05$) than that in the SNC variety (with β-carotene; 54.13 μg/g oil or 1.08 μg/g fresh fruit and lutein; 59.88 μg/g oil or 0.77 μg/g fresh fruit). Therefore, it would be

Table 1: Nutritional composition of SH and SNC versus bibliographic values [1, 3, 20, 23].

Variety	Component (g/100 g sample fresh)					
	Moisture	Protein	Lipid	Ash	Carbohydrates	Fiber
SNC	58.70 ± 0.20	0.60 ± 0.20[b]	1.32 ± 0.61[b]	1.10 ± 0.01[b]	33.51 ± 1.51	4.92 ± 2.71
SH	57.61 ± 3.61	1.91 ± 0.0[a]	2.02 ± 0.31[a]	1.52 ± 0.02[a]	32.04 ± 1.72	5.01 ± 0.21
SH [23]	54.45 + 2.33	2.19 ± 0.16	14.74 ± 0.32	1.29 ± 0.03	27.33*	—
SH [1]	55.76 + 4.34	2.19 ± 0.38	1.39 ± 0.54	0.70 ± 0.14	39.96*	—
SF [1]	52.69 + 1.49	2.22 ± 0.46	1.52 ± 0.83	0.83 ± 0.21	42.85*	—
SH [3]**	4.08 + 0.8	4.75 ± 0.01	4.38 ± 0.8	2.2 ± 0.14	79.10 ± 0.8	6.39 ± 0.50
SF [20]	56.04 + 2.58	1.95 ± 0.16	1.87 ± 0.31	1.87 ± 0.24	33.17 ± 1.37	5.10 ± 0.11

The data are expressed as the mean ± standard deviation ($n = 4$). Different letters indicate significant differences ($p < 0.05$) between samples SH and SNC. Samples without significant differences are not indicated with letters. SH = avocado seed Hass; SNC = avocado seed "Negra de la Cruz" variety; SF = avocado seed Fuerte variety. *Calculated by difference; **dry sample.

Table 2: Profile of fatty acids expressed as methyl esters (%) and content of tocols and carotenoids present in the waste seed of avocado SH and SNC varieties.

Fatty acids (%)	SH (a)	SNC
Saturated		
C14 : 0	1.01 ± 0.00	—
C16 : 0	18.40 ± 2.80	22.43 ± 2.60
C17 : 0	—	2.20 ± 0.04
C18 : 0	1.70 ± 0.03[a]	3.28 ± 0.00[b]
Total	20.11[a]	27.91[b]
Monounsaturated		
C16 : 1 $\omega 7$	3.50 ± 0.04[a]	6.77 ± 0.50[b]
C18 : 1 $\omega 9$	41.38 ± 0.50[a]	28.27 ± 0.60[b]
Total	44.88	35.04
Polyunsaturated		
C18 : 2 $\omega 6$	28.94 ± 2.03[a]	33.58 ± 0.90[b]
C18 : 3 $\omega 3$	5.02 ± 0.05[a]	3.42 ± 0.01[b]
Total	33.96	37.00
$\Omega 6/\omega 3$	5.8	9.8
Tocols (mg·kg $^{-1}$ oil)		
α-Tocopherol	425.66 ± 21.39[a]	1228.31 ± 68.56[b]
β-Tocopherol	8.27 ± 1.24	8.97 ± 1.77
γ-Tocopherol	2.33 ± 0.59	—
δ-Tocopherol	24.89 ± 7.88[a]	73.12 ± 11.02[b]
α-Tocotrienol	90.02 ± 9.55[a]	1948.02 ± 55.31[b]
δ-Tocotrienol	—	49.62 ± 6.79
Total	551.24	3308.03
Carotenoids (mg·kg $^{-1}$ oil)		
β-Carotene	111.88 ± 24.66[a]	54,13 ± 7.89[b]
Lutein	131.51 ± 16.87[a]	59.88 ± 8.56[b]
Total	243.39	114.01

Data are expressed as the mean ± standard deviation (n 4). Different letters indicate significant differences ($p < 0.05$) between samples. Samples without significant differences are not indicated by letters. SH avocado seed 9 Hass variety (a, preliminary unpublished laboratory studies, 2016 season); SNC avocado seed Negra de la Cruz variety.

Table 3: DPPH (IC50), FRAP antioxidant assay, inhibition positive (+), and minimum inhibitory concentration (MIC) of bacterial development of SH and SNC extracts.

Seeds	Extract	Antioxidant activity			Antibacterial activity MIC (mg dw extract/mL)		
		TP (g·EAG/kg·dw)	IC50 (g·EAG/kg·dw)	FRAP (mmol·Fe^{+2}/kg·dw)	*S. aureus*	*B. cereus*	*L. monocytogenes*
SH	E/W	36.41 ± 4.81[a]	7.12 + 1.51[a]	0.40 ± 0.01[a]	(+) 1172 + 20[b]	(+) 1172 + 14[b]	(+) 1172 + 18[c]
	M/W	27.46 ± 2.14[b]	7.75 + 1.32[a]	0.30 + 0.01[b]	(+) 3642 + 21[c]	(+) 1821 + 31[c]	(+) 910 + 12[b]
	EA	10.12 ± 1.02[c]	12.01 + 1.71[b]	0.09 + 0.00[c]	(+) 88 + 13[a]	(+) 88 + 10[a]	(+) 22 + 3[a]
SNC	E/W	21.71 + 3.11[a]	4.94 + 0.98[a]	0.28 + 0.01[a]	(+) 4182 + 51[b]	(+) 522 + 17[b]	(+) 4182 + 45[c]
	M/W	18.66 + 2.53[b]	5.97 + 1.05[b]	0.22 + 0.00[b]	(+) 4137 + 34[b]	(+) 1034 + 21[c]	(+) 1034 + 14[b]
	E/A	5.93 + 1.01[c]	9.54 + 1.67[c]	0.05 + 0.01[c]	(+) 55 + 6[a]	(+) 28 + 3[a]	(+) 28 + 3[a]

SH = avocado seed Hass; SNC = avocado seed Negra de la Cruz; E/W = ethanol/water (50/50% v/v); M/W = methanol/water (50/50% v/v); EA = ethyl acetate; TP = total polyphenols; IC50 = 50% of capacity antiradicalary; FRAP = ferric ion-reducing antioxidant parameter; MIC = minimum inhibitory concentration. Data are expressed as the mean ± standard deviation ($n = 4$). Different letters (a, b, and c) within columns indicate significant differences ($p < 0.05$) between samples. Values without significant differences are not indicted with letters.

expected that the oil from SH would have important cosmetic and health properties because it is well known that carotenoids can block free radical-mediated reactions and play an important role in the prevention of various human degenerative diseases [1].

3.3. Total Polyphenol Content (TP). Table 3 shows the TP content of varieties of Chilean avocado seed SH and SNC. Of the three solvent mixtures used, the results indicate that E/W was more efficient at extracting polyphenol compounds in both avocado seed varieties. Conversely, the TP content of the E/W, M/W, and AE extracts obtained from SH was significantly higher ($p < 0.05$) than that obtained from SNC. A high amount of TP was detected with the E/W extract at 36.41 g EAG/kg dw (or 16.36 g EAG/kg fresh weight). These results are consistent with the content of TP reported by Kosińska et al. for different varieties of avocado seeds, within the range 19.2 to 51.6 g EAG/kg fresh weight [31]. Nevertheless, the TP content in Hass avocado identified in the present study was higher than that determined by Vinha et al. in Algarvian avocado "Hass" variety, which was 7.06 g EAG/kg for fresh fruits [23]. In addition, the higher extraction levels obtained with hydroalcoholic E/W and M/W solvents are consistent with previous findings of polyphenol levels in seeds of avocados from different origins [3, 28]. Differences in TP concentrations can be attributed to the different varieties of avocados and to the growing conditions of each variety, as well as the different polarities of the compounds involved, and therefore to the selectivity of the different solvents by the different analytes of interest [32]. Conversely, Pahua-Ramos et al. recently determined and quantified seven phenolic compounds, and protocatechuic acid was the main phenolic compound identified, followed by kaempferide and vanillic acid present in small amounts [3]. In addition, Rodríguez-Carpena et al. showed that the most representative compounds in avocado seeds are catechins, hydroxybenzoic acids, hydroxycinnamic acids, flavonols, and procyanidins [1]. In addition, Wang et al. [21] showed that, in Florida avocados, the largest amount of antioxidant capacities, phenolic content, and procyanidins was found in the seeds and that procyanidins are the most active.

3.4. Evaluation of Antioxidant Capacity. The antioxidant capacity as determined by the DPPH* method, expressed as IC50, and the FRAP method is shown in Table 3. From the values obtained for IC50, the SNC extracts were shown to have a higher capacity for capturing free radicals compared with SH. Likewise, in the FRAP test, SNC presented a greater effect of Fe^{+3}. Consistent with the DPPH method, the FRAP method shows that increasing the polarity of the extract solution increases the antioxidant capacity of extracts from both seeds in a directly proportional manner. However, a higher antioxidant capacity for the Hass variety was evident from this method, indicating that the compounds present in SH have a greater capacity to reduce the Fe^{+3}-TPTZ to a Fe^{+2} complex. This highlights the E/W extract of SH, with a value of 0.40 μmol·$FeSO_4$/g dry seed. The different results obtained by both methods may be due to the presence of tocols in conjunction with polyphenols, which would interact synergistically with an antioxidant process, thus increasing its antioxidant capacity [33]. The results for SH and SNC coincide with most reports [21, 23] on the antioxidant capacity of avocados and its components, with the seeds containing the highest TP content and antioxidant capacities and the pulp containing the lowest. The TP content in the seeds ranged from 19.2 to 51.6 GAE·mg/g [34].

3.5. Browning Expressed as Color Kinetics. In Figure 1(a), the results of the color parameters of the SH and SNC seeds are presented during the browning period at room temperature. In both SH and SNC, the luminosity parameter (L^*) decreased significantly ($p < 0.05$) with the browning time. On the contrary, in SH and SNC, the parameter L^* reached similar final values. Additionally, both samples showed an increase of the parameters a^* and b^*, with a high tendency, to acquire a red and yellow tone, and these changes have significant tonality ($p < 0.05$) higher in SNC. These color changes are similar to those observed during the browning of other types of seeds and food [15, 35–37]. In Figure 1, the evolution of the color change given by ΔE (Figure 1(a)) and the linearized curves (Figure 1(b)) and the adjustment to first-order kinetics for enzymatic browning (Figure 1(c)) of SH and SNC are shown. It is observed that the curves are similar to the typical ones produced in vegetable products by the polyphenol oxidase enzyme [38]. The increase of the ΔE product of the colors red and yellow would be due to the enzymatic oxidation (polyphenol oxidase) of the phenolic substrates present in SH and SNC. Phenols generate brown compounds known as melanins which are pigments that affect the color of vegetables. The enzyme peroxidase has two isoforms with different thermal stabilities, so it is believed that it is deactivated following two stages governed by the Lumry–Eyring mechanism.

The loss of activity of the stable isoform of the enzyme peroxidase consists of two phases: a reversible transformation in the first step that is very fast, followed by a slow and irreversible transformation of an intermediary [39]. The linearization of these curves (Figure 1(c)) was optimal with a coefficient of determination ($r2$) of 0.98 and 0.96 for SH and SNC, respectively, which allowed to obtain ΔE_{max} and the constant β. The lower value of β presented by SNC indicated that the browning reaction took a shorter time to reach 50% of the maximum color change (ΔE_{max}) in relation to SH, which is represented in the curves of Figure 1(a). However, at longer times, the ΔE_{max} values of both samples are equal. On the contrary, a good fit to first-order kinetics was obtained for SH and SNC reactions with $r2 = 0.83$ and 0.81 and relative kinetic constant values kr = −0.05 and −0.13 (min^{-1}), respectively. These results are similar to those related to enzymatic browning obtained in different vegetable products [15, 35].

3.6. Evaluation of Antibacterial Ability. Table 3 shows that the Gram (−) bacteria *E. coli type 1*, *E. coli O157*, and *S. enteritidis* were resistant to the different seed extracts of both

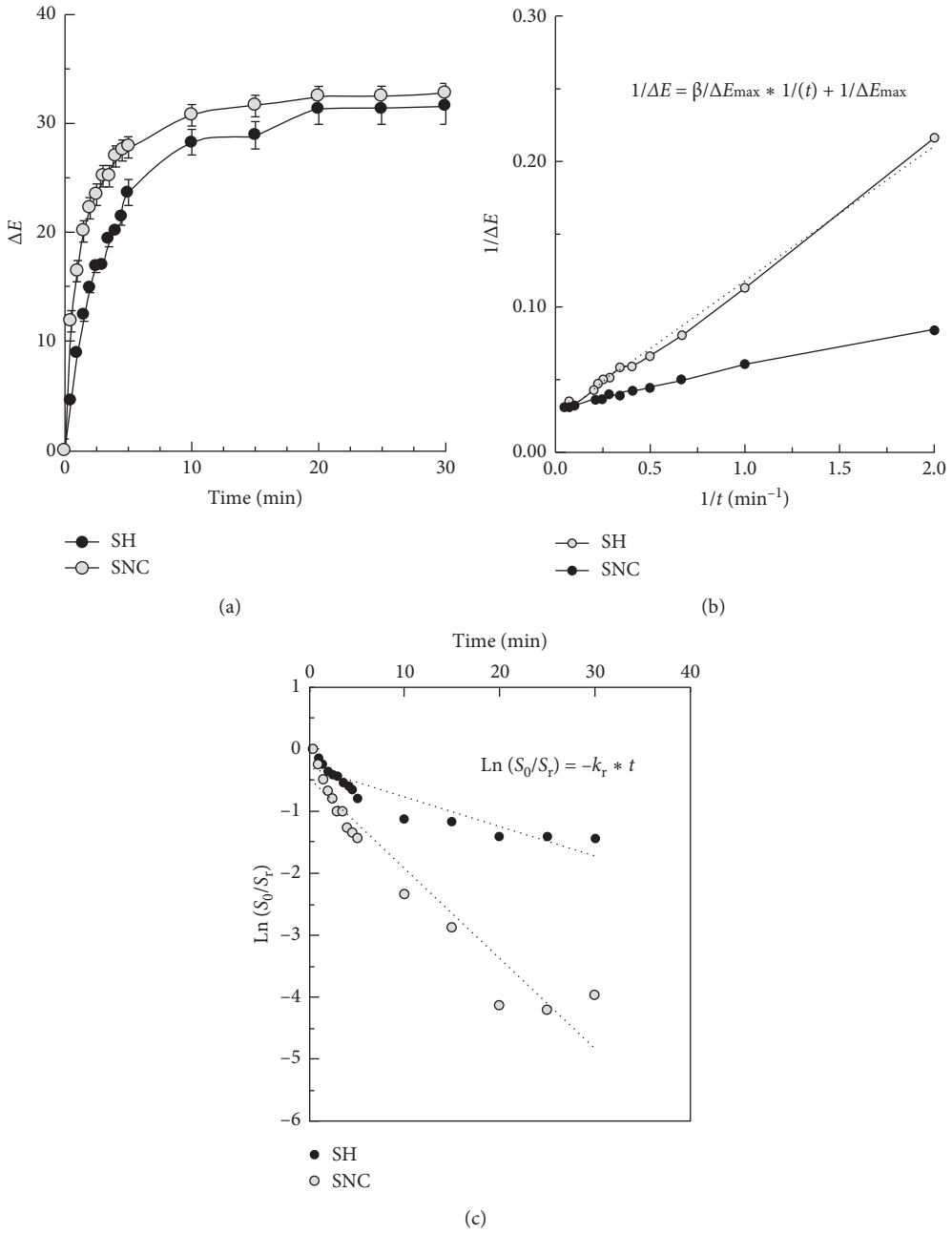

Figure 1: Evolution of the color change given by ΔE (a), the linearized Michaelis–Menten-type curves (b), and the adjustment to first-order kinetics for enzymatic browning (c) of SH and SNC.

avocado varieties. However, the Gram (+) bacteria *S. aureus, B. cereus,* and *L. monocytogenes* were susceptible to all SH and SNC extracts. This can be explained by the fact that the antimicrobial agents present in the seed extracts of both varieties of avocado interfere in cell-wall synthesis in Gram (+) bacteria. Thus, inhibiting the incorporation of new peptidoglycan units in the cell wall consequently weakens it, eventually leading to cell lysis and death [6]. This mechanism of action of the antimicrobial agents would be of the bactericida type. In contrast, Gram (−) bacteria are protected from this action because of the relative impermeability of the outer membrane, which would prevent these antimicrobial substances from reaching the cell wall [40]. According to Neeman et al., one of the isolated aliphatic compounds of avocado seeds, 1,2,4-trihydroxy-*n*-hepadeca-16-ene, can strongly inhibit Gram (+) bacteria, such as *S. aureus* and *B. cereus*, which was also observed with the avocado seed extracts used in the present study [6]. The inhibition halos on the bacteria reveal that the inhibition obtained with the ethyl acetate extract of the SNC variety was the most effective. In the decreasing order, a major effect was obtained against *L. monocytogenes*, followed by *B. cereus* and finally *S. aureus.* The MIC was expressed as micrograms dry raw extract per milliliter for both seed varieties on the bacteria

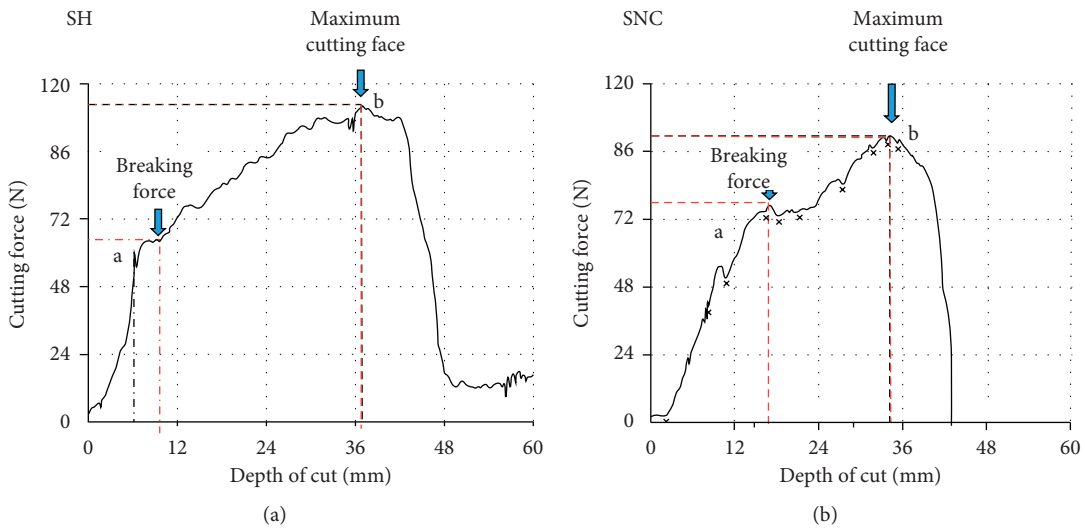

Figure 2: Cutting curves of SH and SNC obtained with the Wagner–Bratzler blade test.

that were susceptible to the extracts (Table 3). From these results, it can be concluded that a lower MIC was obtained with EA extracts from SH on *L. monocytogenes* (22 μg dry raw extract/mL), followed by the SNC extract obtained with ethyl acetate on *B. cereus* (28 μg dry extract/mL) and the SNC sample with the EA extract on *S. aureus* (55 μg dry raw extract/mL). These results indicate that the EA extracts of both avocado varieties have considerable antibacterial activity on the three bacteria studied compared with the other extracts. This may be due to the fact that this solvent is of lower polarity and extracts apolar compounds that have greater inhibitory effect on the studied bacteria; this behavior was also observed by Neeman et al. [6]. Conversely, the MIC obtained with the E/W extracts in the present study was greater than 500 μg/mL, which is similar to that obtained with ethanolic extracts reported by Raymond and Dykes [4], in *S. aureus* and *L. monocytogenes*. Finally, it is important to note that *L. monocytogenes* was the most susceptible strain to most extracts, followed by *B. cereus* and *S. aureus*. Nevertheless, although the MIC of the extract obtained with the hydroalcoholic E/W mixture was greater than the MIC of the EA extracts, it has the advantage of being more applicable. This is because it is considered as a food-grade solvent (GRAS), and is feasible for use in food preservation. The inhibitory power of bioactive avocado seed compounds has also been applied to different types of microorganisms, such as yeasts, fungi, and other pathogens. For example, Leite et al. successfully determined the toxicity, larvicidal and antifungal activity of hexane, and methanol extracts of avocado seeds, in *Artemia salina* and *Aedes aegypti*, and investigated their in vitro antifungal potential against strains of *Candida* spp, *Cryptococcus neoformans*, and *Malassezia pachydermatis* using a microdilution technique [41]. Furthermore, the results obtained were consistent with those of Ilozue et al. and Nwaoguikpe et al. who assayed avocado seed extracts with hot water and methanol and observed a higher antimicrobial effect than that obtained with cold water and ethanol extracts [42, 43]. This indicates the presence of an extract with a broad spectrum of antimicrobial activity. These extracts can be used as an ingredient in food preservatives or in the agronomic and chemical industry.

3.7. Mechanical Properties. Figure 2 shows the cut resistance curves of the SH and SNC seeds, which indicates that the Warner–Bratzler measurement moves during the test, and the sample is compressed to comply with the wall reference by the V-shape of sheet. The force increased in a nonlinear way that the sample was compressed until reaching a first breaking and finally a maximum cut. The first breaking of the samples would correspond to the area of the primary structure of seeds known as seminal cover (a) with SNC having the highest resistance compared to SH (80.6 ± 5.1 N vs 63.2 ± 2.3 N, respectively). On the contrary, the maximum strength of the cut (b) corresponding to the internal structure of both seeds should have the collapse of the internal structure of the seeds composed of cotyledons and embryonic axis, being the fractionation of greater resistance in SH than in SNC (102.6 N vs. 92.8 N, respectively); similar behaviors have been observed in other types of seeds [44]. Moistened seeds have no influence on the results, and physical evidence indicates that the industrial milling of the seeds is in a system of blades that must consume a greater energy in SNC.

3.8. Thermophysical Properties. Figure 3 shows the thermograms obtained for cotyledon tissues of SH and SNC, and Table 4 shows the data of each thermal event presented at low and high temperature. In previous studies, a high content of starch and fat in the cotyledons of avocado seeds has been determined [45], so we can consider that peak 2 corresponds to the melting of starch which occurs in most seeds starting at a temperature close to 50°C and end near 80°C. This range coincides with that determined in the cotyledons of SH and SNC that showed a transition with a maximum starch fusion at 72.22°C and 77.87°C for SH and SNC, respectively (Table 5). On the contrary, lipid melting would occur between 10°C and 30°C, which

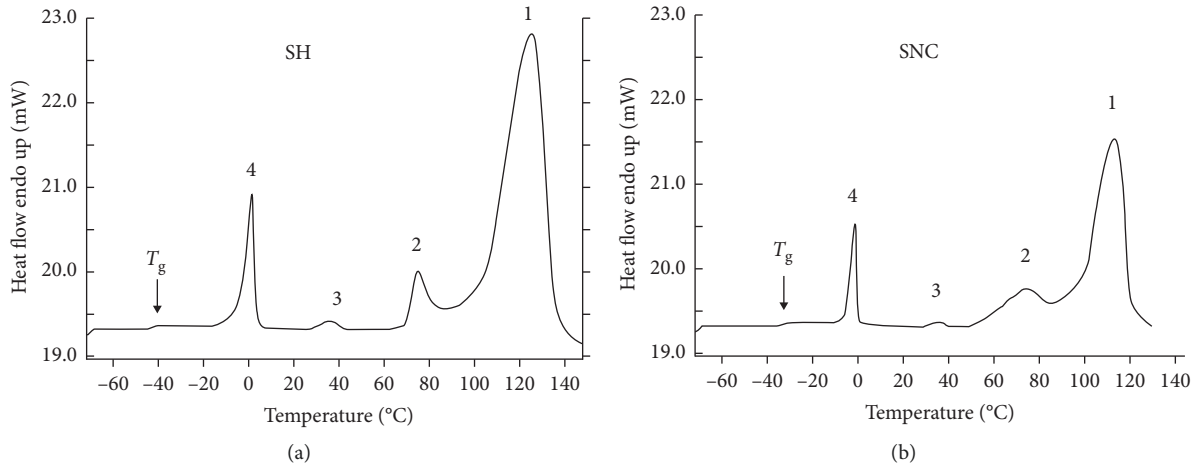

Figure 3: Thermal analysis of the plant tissue of the SH and SNC endosperm. Peak 1: evaporation of water; peak 2: fusion of starch; peak 3: lipid fusion; peak 4: fusion of frozen water. T_g: glass transition.

Table 4: Thermal transitions of the main components present in cotyledons of SH and SNC.

Sample	Transition	Temperature (°C)			Δh
		Onset	Maximum	End	(J/g)
SH	Starch melt	69.31 ± 7.65	72.22 ± 8.22	76.05 ± 6.67	2281 ± 343
	Lipid melt	28.28 ± 2.98	32.65 ± 4.31	35.45 ± 3.77	0.1941 ± 0.2301
	T_g	−35.69 ± 2.33			
SNC	Starch melt	75.69 ± 8.97	77.87 ± 7.99	79.80 ± 9.12	0.108 ± 0.121
	Lipid melt	14.22 ± 2.48	17.82 ± 3.27	21.27 ± 4.11	0.550 ± 0.022
	T_g	−38.44 ± 3.57			

Table 5: Parameters obtained from the adjustment of ΔE curves to a Michaelis–Menten type and first-order kinetic model curves.

	Michaelis–Menten-type model			First-order kinetics		
	β	ΔE_{max}	$r2$	kr (min^{-1})	S0 (mg EAG/mL)	$r2$
SH	3.70	39.84	0.98	−0.05	0.40	0.83
SNC	0.93	32.79	0.96	−0.13	0.68	0.81

coincides with the melting temperature of SH and SNC whose values were 32.65°C and 17.82°C, respectively. The differences in the onset temperatures of the SH and SNC lipids would be related to the degree of unsaturation of the fat, so, as the SNC fat is more polyunsaturated, it melts at a lower temperature. On the contrary, the higher enthalpy values of SH would indicate that it probably contains higher starch and nutrient content than SNC and would also depend on the water content, degree of germination of the seed, and the geographical origin of the varieties, which also has been observed in other types of seeds. The values of T_g for SH and SNC were low (−35.69 and −38.44°C) which can be attributed to the plasticizing effect of the high moisture content of both samples, which has also been observed in dehydrated samples of tomato and berries in which their water content gradually increased [46–48].

4. Conclusions

The findings of this study indicate the seeds of both varieties of avocados, Hass, and Negra de la Cruz grown in Chile have a good nutritional value and also essential components that can be harnessed technologically as ingredients for both human food and animal nutrition with the contribution of proteins and lipids superior in Hass seeds. The quality of the SNC oil presented a higher content of PUFAs and tocopherols than that of SH. However, SH oil presented a better carotenoid content. On the contrary, the hydrophilic extracts of E/W of SNC presented a higher TP content and antioxidant capacity than those of SH. However, the E/W extracts of SH presented a higher bacteriostatic power than the respective SNC. The enzymatic browning indirectly measured as a function of the color change indicated that SNC is faster than SH and that this change is similar with Michaelis–Menten's model and first-order kinetics. The best MICs of the SNC and SH extracts were those of EA followed by those of E/W. The E/W extracts are more applicable and have the advantage of being a GRAS-grade solvent and is feasible for using with the seeds of both varieties of avocado varieties to develop ingredients and agents as preservatives in order to enhance the shelf life of different types of foods and ensure their safety. According to the physical evidence,

the industrial grinding of the seeds that uses a system of blades in its process must consume a greater energy for the SNC rupture. On the contrary, the thermal analysis differs between the phase transitions of the tissues of the cotyledons of both types of seeds, especially the content of starch and water. The melting differences of the lipids would correspond to the differences in the degree of polyunsaturation of the fatty matter in the tissues of both samples.

Data Availability

The data used to support the findings of this study are included within the article.

Conflicts of Interest

The authors declare that there are no conflicts of interest regarding the publication of this paper.

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