Nutritional and antioxidant potential of Chilean native fruits: lleuque (*Prumnopitys andina*) and copihue (*Lapageria rosea*)

CLAUDIA GIOVAGNOLI-VICUÑA – PATRICIA VELÁSQUEZ – GLORIA MONTENEGRO – JAIME ESPEJO – MIGUEL GÓMEZ – GUSTAVO CABRERA-BARJAS – ADY GIORDANO

Summary
Nutritional and antioxidant properties of pulp and seeds of lleuque (*Prumnopitys andina*) and copihue (*Lapageria rosea*) were investigated. Proximal analysis revealed that the pulps are rich in carbohydrates, whereas the seeds are a good source of fibre. Both pulps exhibited higher phenolics content (*PC*) and flavonoids content (*FC*), as well as antioxidant capacities, compared to seeds. The data showed that *PC* correlated with antioxidant capacity the strongest, followed by *FC* and anthocyanins content. It was observed that global antioxidant score and relative antioxidant capacity index represented the antioxidant capacity behaviour of the extracts. According to the polyphenol antioxidant coefficients calculation, *PC* was an important contributor to antioxidant capacity of all extracts. The results of this study confirmed that the native Chilean fruits lleuque and copihue are an excellent source of antioxidants.

Keywords
underexplored fruits; composition; valorization; bioactive

Territory of Chile is a long, narrow strip of land and, due to its length and geographical location, it has a wide variety of climates from tropical to polar, presenting large diversity in its ecosystems and rich biodiversity [1]. This provides an insight into the dimensions of sustainability in the bio-economy of the country using natural resources such as Chilean native berries. The Chilean native berries were food and medicinal source for pre-Columbian indigenous people from Coquimbo Valley to Patagonia [2]. Therefore, current studies on native Chilean fruits are focusing on antioxidants and their main bioactive compounds with possible medicinal effects.

Lleuque or "Andean grape" (*Prumnopitys andina*) is a native Chilean fruit that has been used since pre-Hispanic times as raw food and later to prepare jams and preserves. This fruit grows in the central provinces of Chile, from Linares to Cautín, its fruiting period spanning from January to March. Lleuque is a bright-green fruit, approximately 2 cm long and 1.5 cm wide, weighing approximately 3.6 g (Fig. 1A). Seventy percent of the fresh weight of fruit is represented by the sweet fleshy aril (edible part) and contains a single seed [3]. Likewise, the Copihue fruit *(Lapageria rosea*) is a native Chilean fruit known as "cucumber" in the south of Chile. The indigenous people (Mapuches) call it copín or copiu, which name they also give to the whole plant. This fruit is a smooth-oblong green berry that is approximately 2.5 cm long and 2–2.5 cm wide (Fig. 1B). Its fruiting period spans from November to February. The pulp is whitish and sweet, and it contains several seeds [4]. It is commonly consumed raw due to its refreshing taste [5].

Currently, there is a growing interest in valorization of natural residual sources, since the waste or by-products of food processing, such as seeds and the peel of some fruits, make up a significant portion of the fruit [6]. These wastes or by-products were shown to be a greater source

Claudia Giovagnoli-Vicuña, Patricia Velásquez, Ady Giordano, Inorganic Chemistry Department, Faculty of Chemistry and Pharmacy, Pontificia Universidad Católica de Chile, Avenida Vicuña Mackenna 4860, Macul, 7810000 Santiago, Chile.
Gloria Montenegro, Miguel Gómez, Department of Plant Science, Faculty of Agronomy and Forestal Engineering, Pontificia Universidad Católica de Chile, Avenida Vicuña Mackenna 4860, Macul, 7810000 Santiago, Chile.
Jaime Espejo, NeoArbor SpA, Sor Teresa de Los Andes No 331, Los Angeles, Chile.
Gustavo Cabrera-Barjas, Technological Development Unit (UDT), Universidad de Concepción, Av. Cordillera 2634, Parque Industrial Coronel, 4190000 Coronel, Chile.

Correspondence author:
Ady Giordano, e-mail: agiordano@uc.cl

Fig. 1. Photographs of Chilean native fruits and their fruit plants.

A – lleuque (*Prumnopitys andina*), B – copihue (*Lapageria rosea*), C – tree of lleuque, D – wall climber plant of copihue.

of antioxidants than the edible part of the fruit, for example, the jackfruit peel and papaya seed have a higher antioxidant activity than their pulp [7, 8]. In general, fruits contain many various antioxidants and it is not easy to determine each antioxidant compound individually. Thus, various methods were developed to assess total antioxidant capacity in fruits or other plants and animal tissues [9]. Analytically, the different methods used in radical deactivation can be divided into two groups based on their major mechanism of action, namely, those that use hydrogen atom transfer (HAT) or single electron transfer (SET). However, the results are the same in both mechanisms, but are different in terms of the kinetics and potential for side reactions. These mechanisms occur simultaneously and are determined by the antioxidant structure and pH [10].

The present research was focused in two non-domesticated, native Chilean fruits with only scarce scientific data and none related to the nutritional value [3, 4, 11]. This was an opportunity to valorize the native Chilean species as potential sources of bioactive compounds. Hence, the main aim of this research was to evaluate bioactive compounds, nutritional and antioxidant properties of pulp and seeds from lleuque and copihue.

MATERIALS AND METHODS

Samples

Lleuque (*P. andina*) and copihue (*L. rosea*) (Fig. 1) were picked in May and December in 2019, respectively, in the south-central regions of Chile. The criterion used to collect the fruits was the time of fruiting or fruit maturation period. For each fruit, 40 samples were chosen at optimal quality, without mechanical or microbiological damage. We collected the samples from a single tree for lleuque fruit and several wall-climber plants for copihue fruit. The pulp and seeds of fruits were manually separated and ground at room temperature (20 ± 2 °C). The ground parts of the fruits were stored at 4 °C until analysis for a maximum 24 h.

Physical characterization of fruits

Ten fruits, their pulp and seeds, were weighed separately using an analytical balance (BAS 31 plus; Boeco, Hamburg, Germany). The mean weight of the fruit, pulp, seed, percentage of pulp and ratio fruit/seed were calculated. In addition, length and diameter of the fruit and seeds were measured [12].

Proximate composition

Macronutrients of the samples were determined according to Association of Official Analytical Chemists (AOAC) methods, which involved moisture content (934.06), crude protein (960.52), lipid content (960.39), crude fibre (962.09) and crude ash (923.03) content [13].

Energy value

The protein, fat and carbohydrate content were used to calculate the energy value (*E*) [14]. *E* was calculated according to Eq. 1:

$$E = 17P + 37L + 17C \quad (1)$$

where *E* is the energy value expressed in kilojoule per kilogram of food, *P* is protein content, *L* is lipid content and *C* is carbohydrate content (all expressed as grams per kilogram).

Contribution rate of pulp and seeds

The fruits consist of pulp and seeds, so contribution of these two components to the fruits was calculated in the contribution rates way. The contribution rates were calculated according to Eq. 2 [15]:

$$CR_A = C_A \times \frac{W_A}{(C_A W_A + C_B W_B)} \times 100 \quad (2)$$

where CR_A is the contribution rate of *A* in percentage, C_A is the content of nutritional component in *A*, W_A is the weight of in the sample (in percentage), C_B is the content of nutritional component in *B*; *W* is the weight of the component in the sample (in percentage).

Extraction protocol

Thirty grams of samples were sonicated using ultrasonic bath (model 2200; Branson, Danbury, Connecticut, USA) with 90 ml of methanol (Merck, Darmstadt, Germany) at 20 Hz during 1 h, then centrifuged at 5 000×*g* for 15 min (MSE Super minor, Fisons, Ipswich, United Kingdom). The supernatants of samples were transferred into a round bottom flask and evaporated using rotary evaporator (Büchi, Flawil, Switzerland) with a vacuum pump. The dry extracts were diluted and filtered through a 0.45 μm Clarinert syringe mixed cellulose membrane filters (Bonna-Agela Technologies, Wilmington, Delaware, USA) [16].

Polyphenols content

The polyphenols content (*PC*) of samples was evaluated by the Folin-Ciocalteu procedure [17]. One hundred microliters of samples were vortex-mixed for 30 s with 500 μl of the Folin-Ciocalteu reagent (1 : 10, v/v, Merck) and stored in darkness at room temperature (20 ± 2 °C) for 8 min. Then, 1.2 ml of 75 g·l^{-1} sodium carbonate was added and the mixture was stored in darkness for 1 h at room temperature (20 ± 2 °C). The absorbance was measured at 760 nm in a UV–vis spectrophotometer Agilent 8453 (Agilent Technologies, Santa Clara, California, USA). PC was calculated using calibration curve with gallic acid (Sigma-Aldrich, St. Louis, Missouri, USA) as the standard and expressed as milligrams of gallic acid equivalent (GAE) per kilogram of dry weight.

Flavonoids content

Flavonoid content (*FC*) of samples was determined by the aluminium chloride procedure [18]. Five hundred microliters of samples were vortex-mixed (30 s) with 500 μl of 2% $AlCl_3$ ethanolic solution and stored during 1 h at room temperature (20 ± 2 °C). The absorbance was measured at 420 nm in a UV–Vis spectrophotometer Agilent 8453. *FC* was calculated using calibration curve with quercetin (Sigma-Aldrich) as the standard and expressed as milligrams of quercetin equivalent (QE) per kilogram of dry weight.

Anthocyanins content

The anthocyanins content (*AC*) was determined using a pH-differential protocol [19].

UV-Vis spectrophotometer Agilent 8453 was used to measure the absorbance at 520 nm and 700 nm. The results were expressed as milligrams of cyanidin-3-glucoside (Cy-3Gluc, Sigma-Aldrich) per kilogram of dry weight using molar extinction coefficient of 26 900 $l \cdot cm^{-1} mol^{-1}$.

DPPH radical-scavenging test

The antioxidant capacity of samples was determined using the 2,2-diphenyl-1-picrylhydrazyl (DPPH) test [20]. Fifty microliters of sample were vortex-mixed with 950 μl of 100 $\mu mol \cdot l^{-1}$ DPPH solution (Sigma-Aldrich) and stored during 30 min at room temperature (20 ± 2 °C). The absorbance was measured at 517 nm in a UV–Vis spectrophotometer Agilent 8453. The antioxidant capacity (A_{DPPH}) was expressed using calibration curve with Trolox (Sigma-Aldrich) as the standard and expressed as micromoles Trolox equivalents per kilogram of dry weight.

Ferric reduction antioxidant power test

The antioxidant capacity of samples was determined using ferric reduction antioxidant power (FRAP) test [20]. One hundred microliters of sample were vortex-mixed with 900 μl of FRAP reagent, which contained 2.5 ml of a 10 $mmol \cdot l^{-1}$ 2,4,6-tripyridyl-*s*-triazine (TPTZ) solution in 40 $mmol \cdot l^{-1}$ HCl with 2.5 ml of 20 $mmol \cdot l^{-1}$ $FeCl_3$ and 25 ml of 0.3 $mol \cdot l^{-1}$ acetate buffer at a pH of 3.6 (the reagents were purchased from Merck and Sigma-Aldrich). This freshly made solution was warmed to 37 °C and stored during 30 min at room temperature (20 ± 2 °C). The absorbance was measured at 593 nm in a UV-Vis spectrophotometer Agilent 8453. The antioxidant capacity (A_{FRAP}) was expressed using calibration curve with Trolox as the standard and expressed as micromoles Trolox equivalents per kilogram of dry weight.

ABTS radical-scavenging test

The antioxidant capacity of samples was determined using the 2,2'-azino-*bis*(3-ethylbenzothiazoline-6-sulphonic acid) (ABTS) radical-scavenging test [21]. Fifty microliters of sample were vortex-mixed with 1450 μl of ABTS solution (Sigma-Aldrich) and stored during 30 min at room temperature (20 ± 2 °C). The absorbance was measured at 732 nm in a UV-Vis spectrophotometer Agilent 8453. The antioxidant capacity (A_{ABTS}) was expressed using calibration curve with Trolox as the standard and expressed as micromoles Trolox equivalents per kilogram of dry weight.

Relative antioxidant capacity index

Relative antioxidant capacity index (*RACI*) was calculated according to PETROVIC et al. [22]. *RACI* was obtained by averaging the standard scores (*SS*) transformed from the raw data. The *SS* was calculated by using Eq. 3:

$$SS = \frac{x - \mu}{\sigma} \tag{3}$$

where x is the raw data, μ is the mean and σ is the standard deviation.

Polyphenol antioxidant coefficient

Polyphenol antioxidant coefficient (*PAC*) was calculated according to PETROVIC et al. [22]. *PAC* was calculated as ratio between antioxidant capacity and polyphenols content.

Global antioxidant score

For each sample, the average of *T*-scores (*TS*) was used to calculate the global antioxidant score (*GAS*) value. *TS* was calculated according to Eq. 4 [23]:

$$TS = \frac{(X - X_{min})}{(X_{max} - X_{min})} \tag{4}$$

where X is antioxidant capacity of variable among the studied samples of the same extract group, X_{min} and X_{max} represent the smallest and largest antioxidant capacity values of variable X, respectively.

Statistical analysis

Analysis of variance was used to analyse the results using Tukey's HSD method with 95% confidence (Statgraphics Centurion XV 15.02.05 software; Statpoint Technologies, The Plains, Virginia, USA). All analyses were carried out in triplicate and the results were expressed as mean and standard deviation. In addition, to express the correlations and their significance, the Pearson correlation coefficient (R) and P-value were used. The criterion of statistically significant difference $p < 0.05$ was adopted.

RESULTS AND DISCUSSION

Physical characterization of fruits

Tab. 1 shows the mean physical characteristics of the fruit and seed(s) of lleuque and copihue. The weight, length, and diameter of the lleuque and copihue fruits were 4.5 g and 16.7 g, 1.3 cm and 5.1 cm; and 1.1 cm and 1.3 cm, respectively. The weight of the lleuque and copihue pulp constituted approximately 25 % and 80 % of the to-

Tab. 1. Physical characteristics of fruits and seeds of lleuque and copihue.

Physical characteristics	Lleuque		Copihue	
	Fruit	Seed	Fruit	Seed*
Weight [g]	4.5 ± 0.3	1.3 ± 0.4	16.7 ± 1,4	0.02 ± 0.00
Length [cm]	1.3 ± 0.2	1.2 ± 0.4	5.1 ± 0.8	0.20 ± 0.05
Diameter [cm]	1.1 ± 0.1	0.8 ± 0.2	1.3 ± 0.2	0.05 ± 0.01
Length/diameter ratio	1.2	1.5	3.9	4.0

Values represent mean ± standard deviation (n = 10).
* – weight of a seed, the fruit contains approximately 100 seeds.

tal weight of each fruit, respectively. The weight, diameter and length of seed(s) were 3.3 g and 0.02 g, 1.2 cm and 0.2 cm, and 0.8 cm and 0.05 cm for the lleuque and copihue fruits, respectively.

Proximate composition

The proximate composition of pulp and seeds of lleuque and copihue fruits is shown in Fig. 2A. In general, the pulp of both fruits is rich source in carbohydrates, whereas the seeds are a good source of fibre. The pulp lleuque and copihue fruits contained considerable amounts of carbohydrates (65.3 % and 65.8 %) and low amounts of lipids (0.7 % and 7.1 %). The lleuque seeds were found to contain high amounts of fibre (54.5 %) and low amounts of carbohydrates (7.7 %). On the other hand, copihue seeds contained high amounts of lipids (22.9 %), carbohydrates (50.6 %) and had low ash content (3.1 %). It should be noted that some variations can be observed in the values of the nutritional composition due to fruit growing location, temperature and exposure time to sunlight, stage of maturity, agricultural and post-harvest handling practices [24].

The results are comparable with those reported by Boeri et al. [25] for Berberis fruits, where moisture content for the pulp and seeds of 93.3 % and 0.0 %, protein content of 1.3 % and 13.6 %, lipid content of 1.7 % and 18.9 %, together with ash content of 3.6 % and 2.2 %, respectively, were reported. The authors did not report the fibre content. Likewise, the results of the present study are similar to those reported by Uribe et al. [26] for Chilean papaya fruit, where for the pulp moisture content of 91.6 %, protein content of 0.9 %, lipid content of 0.3 %, fibre content of 1.1 % and ash content of 0.6 % were reported. For the Chilean papaya seed, the values observed by Briones-Labarca et al. [8] were moisture content 3.5 %, protein content 31.8 %, lipid content 30.5 %, fibre content 24.4 % and ash content 4.0 %. Zura-Bravo et al. [27] reported the chemical composition

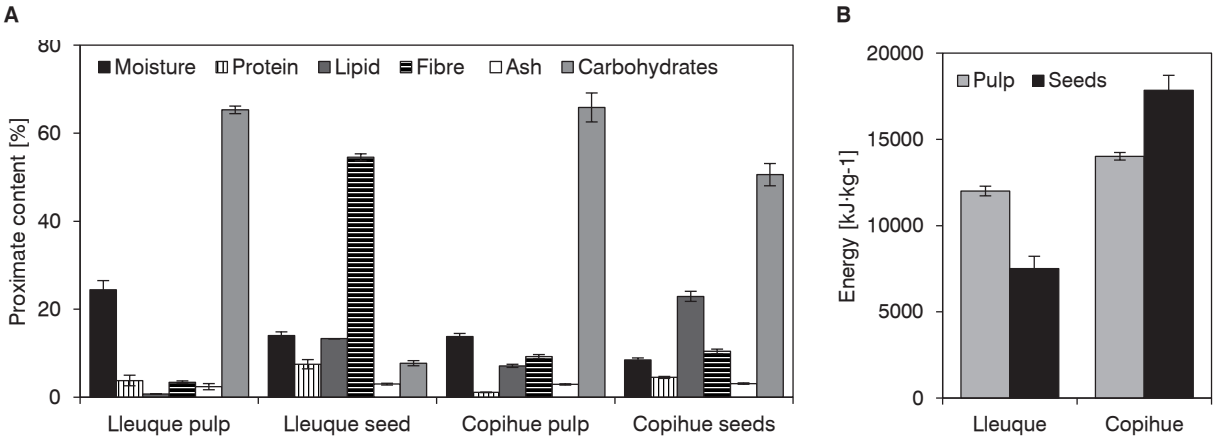

Fig. 2. Proximate composition and energy value of the pulp and seeds of lleuque and copihue.

A – proximate composition, B – energy values.
Values represent mean ± standard deviation (n = 3). Carbohydrate content was obtained by difference.

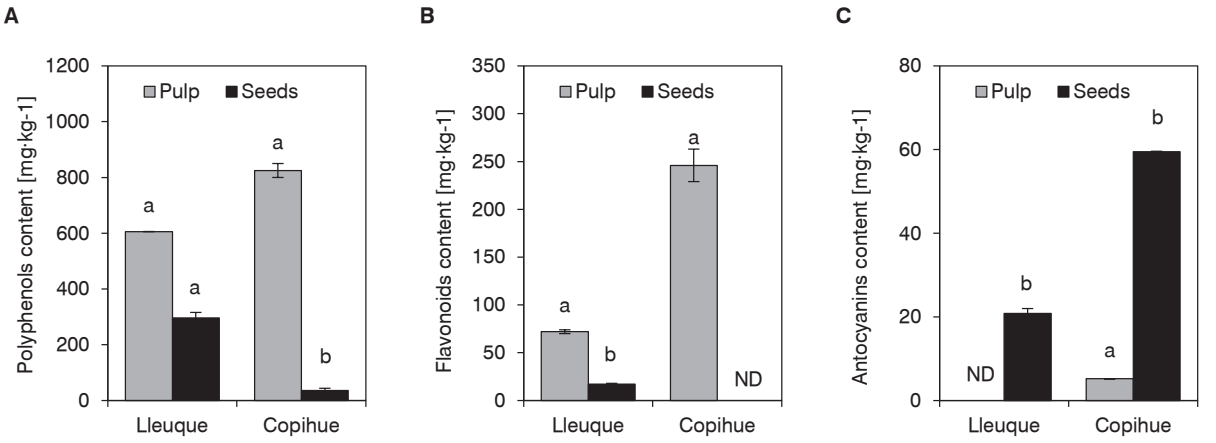

Fig. 3. Antioxidant compounds contents of the pulp and seeds of lleuque and copihue.

A – polyphenols, B – flavonoids, C – anthocyanins.
Values represent mean ± standard deviation (n = 3). Different lowercase letters above bars indicate significant differences between mean values ($p < 0.05$). Polyphenols content is expressed as milligrams of gallic acid equivalent per kilogram of dry weight. Flavonoids content is expressed as milligrams of quercetin equivalent per kilogram of dry weight. Anthocyanins content is expressed as milligrams of cyanidin-3-glucoside per kilogram of sample
ND – not detected at the conditions tested.

of myrtleberry, also known as Chilean guava, murtilla, or murta, with a moisture content of 80.3 %, crude protein of 1.1 %, total lipids of 0.3 % crude fibre of 3.4 %, crude ash of 0.7 % and carbohydrates of 17.7 %. The proximate analysis of murta seeds exhibited significant fibre content (64.0 %), lipid content (14.0 %), crude protein (12.0 %), and low levels of ashes (1.5 %) [28].

Energy values of the pulp of lleuque and copihue fruits were 12 004 kJ·kg^{-1} and 14 018 kJ·kg^{-1}, respectively (Fig. 2B). For the seed, the values were 7 510 kJ·kg^{-1} and 17 842 kJ·kg^{-1} for lleuque and copihue fruits, respectively. Therefore, these fruits are a good source of energy.

The data showed that the contribution rate of lleuque pulp to the carbohydrate content was high (95.4 %), while lleuque seeds contributed greatly to the lipid content (by 88.5 %) and a only little to the carbohydrate content (by 4.6 %). Fibre, moisture and ash contents were 86.6 % 18.9 %, and 33.5 %, respectively. In the case of copihue fruit, pulp presented a considerable fibre contribution rate with 86.7 %. The copihue seed had a higher impact on the nutritional components content, with contribution rate percentages of moisture content 7.7 %, protein content 35.8 %, lipid content 30.4 %, fibre content 13.3 %, ash content 12.7 % and carbohydrate content 9.5 %.

Antioxidant compounds

The main contributors to the antioxidant capacity of fruits are polyphenol compounds, such as flavonoids, anthocyanins and phenolic acids [29]. These compounds can improve or help human health, for example, in the metabolic regulation, chronic disease or cell proliferation [30]. The polyphenols, flavonoids and anthocyanins contents of the pulp and seeds of lleuque and copihue fruits are shown in Fig. 3. The results showed that copihue pulp presented the highest *PC* (825 mg·kg^{-1}, expressed as milligrams of GAE), followed by lleuque pulp, lleuque seed and copihue seeds (Fig. 3A). There were significant differences ($p < 0.05$) for polyphenols between the pulp and seeds for that fruit. *FC* showed the same behaviour as *PC* (Fig. 3B). Fig. 3C shows that the anthocyanins content of copihue seeds was much higher than that of the pulp of that fruit. For the lleuque seed, a considerable value of 21 mg·kg^{-1} (expressed as milligrams of Cy-3Gluc) was found for *AC*. However, it should be pointed out that *AC* of the lleuque pulp was not detected at the conditions tested. In general, there were much higher *AC* levels in seeds than in the pulp of the fruits. These data provide evidence of the difference in antioxidant compounds among the pulp and seed(s) of both of these Chilean native fruits.

A similar situation was observed by Onivogui et al. [31] for *Anisophyllea laurina* R. Br. ex Sabine fruits, where *PC* and *FC* levels in the pulp extract were higher than in the seed extract. On the other hand, an opposite situation was observed in extracts of Annona fruits, where *PC* and *FC* levels in the seed extract were higher than in the pulp

extract [32]. A previous work of Jiménez-Aspee et al. [3] indicated that *PC* of a lleuque extract (retained in the resin Amberlite XAD7) ranged from 20 g·kg^{-1} to 73 g·kg^{-1}, while *FC* ranged from 13 g·kg^{-1} to 53 g·kg^{-1} (expressed as grams of catechin equivalent). For the different parts of the fruits, namely pulp and seeds from lleuque and copihue, no reports have been published so far. However, Seguel Benítez et al. [4] reported *PC* of the whole copihue fruit to be 3 600 mg·kg^{-1}.

In the case of *AC*, our results have a trend similar to that of Ali et al. [33] and Orqueda et al. [34], where it was reported that the seed(s) extract showed a higher *AC* than the pulp of pomegranate and red chilto fruits, respectively. For the whole copihue fruit, in 2016 *AC* of 140 mg·kg^{-1} was reported (expressed as milligrams of malvidin-3-glucoside) [4]. That value is consistent with those found in this work.

Choi et al. [35] indicated that the weight of the seed affects the content of anthocyanins, isoflavones and phenolics, as well as the antioxidant activities in black soybeans. Pastrana-Bonilla et al. [36] investigated ten cultivars of muscadine grapes (five bronze-skin and five purple-skin) and reported that the total *AC* of the seeds of purple grapes was 1.3-fold higher than that of bronze grape seeds. These authors explained the higher *AC* of the seeds by possible migration of pigments from the skin to the pulp or some tinting from ruptured skin cells during the process of separation of the fruit into its parts. On the contrary, Snyder et al. [37] observed that the seeds of raspberry contained almost no anthocyanins.

Antioxidant capacity

Various methods are available for evaluating the antioxidant capacity of foods. However, to establish authenticity of the antioxidant capacity of food, it is recommended to use two methods, because of the complex reactivity of bioactive compounds present in food [38]. Thus, the antioxidant capacity of pulp and seeds from lleuque and copihue fruits was measured using three methods, DPPH radical-scavenging test, FRAP test and ABTS radical-scavenging test. The results obtained for the Chilean native fruits in the different parts (pulp and seeds) showed that these fruits present high antioxidant capacity by all the three methods (Tab. 2).

Lleuque fruit pulp had the highest antioxidant capacity value A_{ABTS} (9 161 μmol·kg^{-1}; $p < 0.05$), followed by A_{DPPH} (1 992 μmol·kg^{-1}) and A_{FRAP} (1 413 μmol·kg^{-1}). The same behaviour was observed for the copihue pulp, however, no antioxidant capacity was determined by the DPPH method. This can be explained by the fact that the nitrogen radical, DPPH, has a long mean reaction time and has no similarity to the transient and strongly reactive peroxyl radicals that promote lipid oxidation. Therefore, several antioxidant compounds that react rapidly with peroxide radicals may react slowly or be inert to DPPH radical [39]. Tab. 2 shows that the lleuque and copihue fruit seeds are a good source of antioxidant components.

The seeds of the lleuque fruit presented the highest antioxidant capacity values A_{ABTS} (1 527 μmol·kg^{-1}), A_{FRAP} (720 μmol·kg^{-1}) and A_{DPPH} (257 μmol·kg^{-1}). In the case of the seeds of the copihue fruit, no antioxidant capacity was detected by the DPPH method. However, by the ABTS and FRAP methods, the values of the total antioxidant capacity were found above 500 μmol·kg^{-1}. In summary, the part of the fruits notably influences the total antioxidant capacity of the whole fruit. A comparison of the antioxidant capacity of the lleuque and copihue fruits corroborates the finding of Jiménez-Aspee et al. [3] and Fuentes et al. [40], who observed comparable values of the antioxidant capacity in lleuque extract (retained in the resin Amberlite XAD7) and other native fruits (Maqui, Murta, Calafate, Arrayán and Chilean strawberry).

Tab. 2. Antioxidant capacity of the pulp and seeds of lleuque and copihue determined using different methods.

Antioxidant capacity	Lleuque		Copihue	
	Pulp	Seeds	Pulp	Seeds
A_{ABTS} [μmol kg^{-1}]	9 161 ± 153 ^{a}	1 527 ± 94 ^{a}	12 330 ± 297 ^{a}	963 ± 149 ^{a}
A_{FRAP} [μmol kg^{-1}]	1 992 ± 0 ^{b}	720 ± 39 ^{b}	3 951 ± 153 ^{b}	546 ± 0 ^{b}
A_{DPPH} [μmol kg^{-1}]	1 413 ± 0 ^{c}	257 ± 82 ^{c}	IN	IN

Values represent mean ± standard deviation (n = 3). Antioxidant capacity is expressed as micromoles of Trolox equivalents per kilogram of dry weight. Different lowercase letters in rows indicate significant differences between mean values ($p < 0.05$). A_{ABTS} – antioxidant capacity determined by ABTS radical-scavenging test, A_{FRAP} – antioxidant capacity determined by ferric reduction antioxidant power test, A_{DPPH} – antioxidant capacity determined by DPPH radical scavenging test, IN – inactive.

Tab. 3. Correlation coefficients of antioxidant compounds contents and antioxidant capacity.

	A_{ABTS}	A_{FRAP}	A_{DPPH}	*PC*	*FC*	*AC*
A_{ABTS}	1					
A_{FRAP}	0.953*	1				
A_{DPPH}	0.998*	0.999*	1			
PC	0.962*	0.931*	0.999*	1		
FC	0.863*	0.986*	0.998*	0.920*	1	
AC	–0.748*	–0.748*	–0.529	–0.902*	–0.996*	1

* – correlation was established as statistically significant at $p < 0.05$.
A_{ABTS} – antioxidant capacity determined by ABTS radical-scavenging test, A_{FRAP} – antioxidant capacity determined by ferric reduction antioxidant power test, A_{DPPH} – antioxidant capacity determined by DPPH radical scavenging test, PC – phenolics content, FC – flavonoids content, AC – anthocyanins content.

The correlation coefficients of antioxidant capacities, *PC*, *FC* and *AC* are presented in Tab. 3. *PC*, *FC* and *AC* were correlated significantly ($p < 0.05$) with A_{ABTS}, A_{FRAP} and A_{DPPH}, except for *AC* and A_{DPPH} that did not present significant correlation. These values showed that *PC*, *FC* and *AC* have an important contribution to the antioxidant capacity. In general, the results showed that *PC* had the highest correlation with antioxidant capacity, followed by *FC* and *AC*. For *AC*, the correlation coefficients were less than zero (negative values), that is, the antioxidant capacity, *PC* and *FC* were inversely related with *AC*. Therefore, when the *AC* was high, the antioxidant capacity (A_{ABTS}, A_{FRAP} and A_{DPPH}), *PC* and *FC* were low. This implies that *AC* could be the main contributor to the antioxidant capacity of seeds of the two native Chilean fruits.

GAS and *RACI* are summarized in Fig. 4. *GAS* allowed us to find the sample with the highest antioxidant capacity. The results showed that lleuque pulp presented the highest antioxidant capacity, followed by lleuque seed, copihue pulp and copihue seeds (Fig. 4). On the other hand, *RACI* shows the comparison of the antioxidant capacity values obtained from various procedures such as A_{ABTS}, A_{FRAP} and A_{DPPH}. This dimensionless index calculates the range between the mean and the raw data by standard deviation units [41]. Based on the *RACI* values of the four extracts, Fig. 4 shows that the lleuque and copihue pulps had the highest values (0.99 and 0.98, respectively), followed by copihue seeds and the lleuque seeds (0.95 and 0.88, respectively). In addition, the behaviour of *RACI* matched with the values obtained by the methods measuring antioxidant capacity (A_{ABTS}, A_{FRAP} and A_{DPPH}). Sun and Tanumihardjo [42] observed that there was a correlation between *RACI* and each method of measurement of antioxidant capacity. Thus, this index makes it possible to represent the antioxidant capacity of a food obtained by different methods.

PAC calculations made it possible to estimate the contribution of the polyphenols present in the studied extracts for each method of antioxidant capacity measurement. As shown in Fig. 5, notable variations were observed between *PAC* values of the different antioxidant capacity methods for the same extract. The highest *PAC* values were obtained for ABTS assay, while *PAC* values for DPPH assay were much lower. These findings taken together suggest that polyphenols were an important contributor to antioxidant capacity in all extracts. It should be noted that high *PAC* is not associated with high antioxidant capacity of the extracts, but it rather contributes to the antioxidant capacity per sample and assay evaluated.

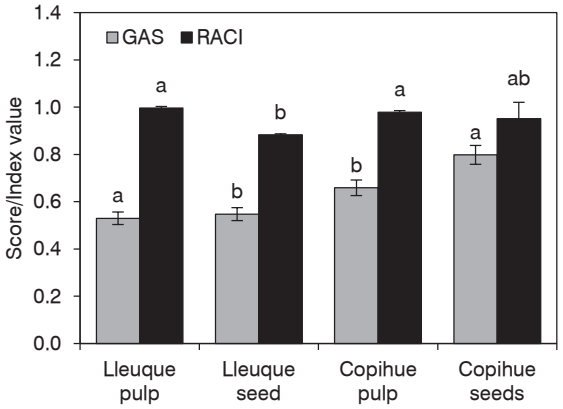

Fig. 4. Global antioxidant score and relative antioxidant capacity Index of the pulp and seeds of lleuque and copihue.

Different lowercase letters above bars in the same group indicate significant differences between mean values ($p<0.05$). *GAS* – global antioxidant score, *RACI* – relative antioxidant capacity index.

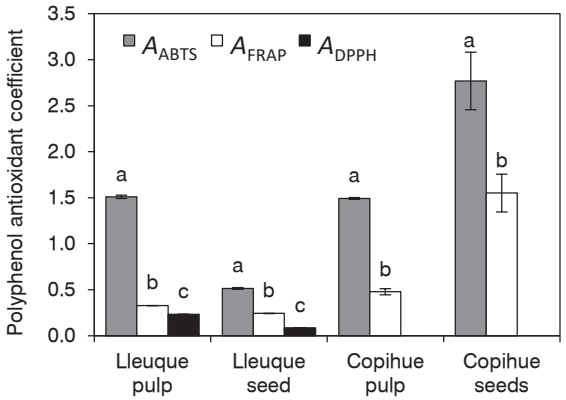

Fig. 5. Polyphenol antioxidant coefficients of the pulp and seeds of lleuque and copihue.

Different lowercase letters above bars in the same group indicate significant differences between mean values ($p < 0.05$). A_{ABTS} – antioxidant capacity determined by ABTS radical-scavenging test, A_{FRAP} – antioxidant capacity determined by ferric reduction antioxidant power test, A_{DPPH} – antioxidant capacity determined by DPPH radical scavenging test

CONCLUSIONS

This study showed that Chilean native fruits lleuque and copihue are rich in bioactive compounds, allowing to open opportunities for valorization of these non-domesticated species. In addition, we observed that each part of the fruit notably influences the content of bioactive compounds and the total antioxidant capacity. According to the statistics applied, *RACI* and *GAS* showed a strong match with antioxidant capacity, providing representative values for the seed and pulp extracts of lleuque and copihue fruits. On the other hand, *PAC* showed that polyphenols were an important contributor to antioxidant capacity in all extracts. The results suggest that consumption of these fruits may play a significant medicinal role, because they are a good source of antioxidants.

Acknowledgements

The authors acknowledge the funding provided by Research and Postgraduate Direction of the Faculty of Chemistry and Pharmacy, Pontificia Universidad Católica de Chile (Santiago, Chile).

REFERENCES

1. Mølgaard, P. – Holler, J. G. – Asar, B. – Liberna, I. – Rosenbæk, L. B. – Jebjerg, C. P. – Jørgensen, L. – Lauritzen, J. – Guzman, A. – Adsersen, A. – Simonsen, H. T.: Antimicrobial evaluation of Huilliche plant medicine used to treat wounds. Journal of Ethnopharmacology, *138*, 2011, pp. 219–227. DOI: 10.1016/j.jep.2011.09.006.
2. Schmeda-Hirschmann, G. – Jiménez-Aspee, F. – Theoduloz, C. – Ladio, A.: Patagonian berries as native food and medicine. Journal of Ethnopharmacology, *241*, 2019, article 111979. DOI: 10.1016/j.jep.2019.111979.
3. Jiménez-Aspee, F. – Theoduloz, C. – Pormetter, L. – Mettke, J. – Ávila, F. – Schmeda-Hirschmann, G.: Andean *Prumnopitys Andina* (Podocarpacae) fruit extracts: characterization of secondary metabolites and potential cytoprotective effect. Molecules, *24*, 2019, article 4028. DOI: 10.3390/molecules24224028.
4. Seguel Benítez, I. – Chahín Ananía, M. G. – Chait Mujica, E. (Eds.): Copihue: manejo, caracterización y usos. (Copihue: management, characterization and uses). Boletín INIA 341. Santiago : Instituto de Investigaciones Agropecuarias (INIA), 2016. ISSN: 0717-4829. In Spanish.
5. Gay, C.: Historia, física y política de Chile – Botánica – Tomo sexto (History, physics and politics of Chile – Botany – Volume six). Chile : Museo de Historia Natural de Santiago, 1853. In Spanish.
6. Shirahigue, L. D. – Ceccato-Antonini, S. R.: Agro-industrial wastes as sources of bioactive compounds for food and fermentation industries. Ciencia Rural (Santa Maria), *50*, 2020, article e20190857. DOI: 10.1590/0103-8478cr20190857.
7. Zhang, L. – Tu, Z. – Xie, X. – Wang, H. – Wang, H. – Wang, Z. – Sha, X. – Lu, Y.: Jackfruit (*Artocarpus heterophyllus* Lam.) peel: A better source of antioxidants and α-glucosidase inhibitors than pulp, flake and seed, and phytochemical profile by HPLC-QTOF-MS/MS. Food Chemistry, *234*, 2017, pp. 303–313. DOI: 10.1016/j.foodchem.2017.05.003.
8. Briones-Labarca, V. – Plaza-Morales, M. – Giovagnoli-Vicuña, C. – Jamett, F.: High hydrostatic pressure and ultrasound extractions of antioxidant compounds, sulforaphane and fatty acids from Chilean papaya (*Vasconcellea pubescens*) seeds: Effects of extraction conditions and methods. LWT – Food Science and Technology, *60*, 2015, pp. 525–534. DOI: 10.1016/j.lwt.2014.07.057.
9. Yilmaz, Y. – Göksel, Z. – Erdogan, S .S. – Öztürk, A. – Atak, A. – Özer, C.: Antioxidant activity and phenolic content of seed, skin and pulp parts of 22 grape (*Vitis vinifera* L.) cultivars (4 common and 18 registered or candidate for registration). Journal of Food Processing and Preservation, *39*, 2015, pp. 1682–1691. DOI: 10.1111/jfpp.12399.
10. Mareček, V. – Mikyška, A. – Hampel, D. – Čejka, P. – Neuwirthová, J. – Malachová, A. – Cerkal, R.: ABTS and DPPH methods as a tool for studying antioxidant capacity of spring barley and malt. Journal of Cereal Science, *73*, 2017, pp. 40–45. DOI: 10.1016/j.jcs.2016.11.004.
11. Chamorro, M. F. – Ladio, A.: Native and exotic plants with edible fleshy fruits utilized in Patagonia and their role as sources of local functional foods. BMC Complementary Medicine and Therapies, *20*, 2020, article 155. DOI: 10.1186/s12906-020-02952-1.
12. Salomón-Torres, R. – Ortiz-Uribe, N. – Valdez-

Salas, B. – Rosas-González, N. – García-González, C. – Chávez, D. – Córdova-Guerrero, I. – Díaz-Rubio, L. – Haro-Vázquez, M. – Mijangos-Montiel, J. L. – Morales-Maza, A. – Mahadevan, P. – Krueger, R.: Nutritional assessment, phytochemical composition and antioxidant analysis of the pulp and seed of medjool date grown in Mexico. PeerJ, *7*, 2019, article e6821. DOI: 10.7717/peerj.6821.

13. Official methods of analysis of AOAC International. 15th edition. Arlington : Association of Official Analytical Chemists, 1990. ISBN: 0935584420.
14. Admassu, H. – Abera, T. – Abraha, B. – Yang, R. – Zhao, W.: Proximate, mineral and amino acid composition of dried laver (*Porphyra* spp.) seaweed. Journal of Academia and Industrial Research, *6*, 2018, pp. 149–154. ISSN: 2278-5213.
15. Xin, G. – Zhu, F. – Du, B. – Xu, B.: Antioxidants distribution in pulp and seeds of black and red goji berries as affected by boiling processing. Journal of Food Quality, *2017*, 2017, article 3145946. DOI: 10.1155/2017/3145946.
16. Velásquez, P. – Orellana, J. – Muñoz-Carvajal, E. – Faúndez, M. – Gómez, M. – Montenegro, G. – Giordano, A.: Biological activity of native *Myrtaceae* fruits from Chile as a potential functional food. Natural Product Research, published online 16 September 2021. DOI: 10.1080/14786419.2021.1940176.
17. Singleton, V. – Orthofer, R. – Lamuela-Raventós, R.: Analysis of total phenols and other oxidation substrates and antioxidants by means of Folin-Ciocalteu reagent. Methods in Enzymology, *299*, 1999, pp. 152–178. DOI: 10.1016/S0076-6879(99)99017-1.
18. Woisky, R. G. – Salatino, A.: Analysis of propolis: Some parameters and procedures for chemical quality control. Journal of Apicultural Research, *37*, 1998, pp. 99–105. DOI: 10.1080/00218839.1998.11100961.
19. Martínez-Cruz, N. S. – Arévalo-Niño, K. – Verde-Star, M. J. – Rivas-Morales, C. – Oranday-Cárdenas, A. – Núñez-González, M. A. – Morales-Rubio, M. E.: Antocianinas y actividad anti radicales libres de *Rubus adenotrichus* Schltdl (zarzamora). (Anthocyanins and anti-free radical activity of *Rubus adenotrichus* Schltdl (blackberry)). Revista Mexicana de Ciencias Farmaceúticas, *42*, 2011, pp. 66–71. ISSN: 1870-0195. In Spanish.
20. Thaipong, K. – Boonprakob, U. – Crosby, K. – Cisneros-Zevallos, L. – Hawkins Byrne, D.: Comparison of ABTS, DPPH, FRAP, and ORAC assays for estimating antioxidant activity from guava fruit extracts. Journal of Food Composition and Analysis, *19*, 2006, pp. 669–675. DOI: 10.1016/j.jfca.2006.01.003.
21. van den Berg, R. – Haenen, G. R. M. M. – van den Berg, H. – Bast, A.: Applicability of an improved Trolox equivalent antioxidant capacity (TEAC) assay for evaluation of antioxidant capacity measurements of mixtures. Food Chemistry, *66*, 1999, pp. 511–517. DOI: 10.1016/S0308-8146(99)00089-8.
22. Petrovic, M. – Suznjevic, D. – Pastor, F. – Veljovic, M. – Pezo, L. – Antic, M. – Gorjanovic, S.: Antioxidant capacity determination of complex samples and individual phenolics – Multilateral approach. Combinatorial Chemistry and High Throughput Screening, *19*, 2016, pp. 58–65. DOI: 10.2174/1386207318666151102094227.
23. Leporini, M. – Loizzo, M. R. – Tundis, R. – Torre, C. – La-Fazio, A. – Plastina, P.: Non-pungent *n*-3 polyunsaturated fatty acid (PUFA)-derived capsaicin analogues as potential functional ingredients with antioxidant and carbohydrate-hydrolysing enzyme inhibitory activities. Antioxidants, *8*, 2019, article 162. DOI: 10.3390/antiox8060162.
24. Vega-Gálvez, A. – Poblete, J. – Quispe-Fuentes, I. – Uribe, E. – Bilbao-Sainz, C. – Pastén, A.: Chemical and bioactive characterization of papaya (*Vasconcellea pubescens*) under different drying technologies: evaluation of antioxidant and antidiabetic potential. Journal of Food Measurement and Characterization, *13*, 2019, pp. 1980–1990. DOI: 10.1007/s11694-019-00117-4.
25. Boeri, P. – Piñuel, L. – Dalzotto, D. – Monasterio, R. – Fontana, A. – Sharry, S. – Barrio D. A. – Carrillo, W.: Argentine Patagonia barberry chemical composition and evaluation of its antioxidant capacity. Journal of Food Biochemistry, *44*, 2020, article e13254. DOI: 10.1111/jfbc.13254.
26. Uribe, E. – Delgadillo, A. – Giovagnoli-Vicuña, C. – Quispe-Fuentes, I. – Zura-Bravo, L.: Extraction techniques for bioactive compounds and antioxidant capacity determination of chilean papaya (*Vasconcellea pubescens*) fruit. Journal of Chemistry, *2015*, 2015, article 347532. DOI: 10.1155/2015/347532.
27. Zura-Bravo, L. – Rodriguez, A. – Stucken, K. – Vega-Gálvez, A.: Drying kinetics of probiotic-impregnated murta (*Ugni molinae* T.) berries. Journal of Food Science and Technology, *56*, 2019, pp. 103–113. DOI: 10.1007/s13197-018-3463-9.
28. Cabrera-Barjas, G. – Quezada, A. – Bernardo, Y. – Moncada, M. – Zúñiga, E. – Wilkens, M. – Giordano, A. – Nesic, A. – Delgado, N.: Chemical composition and antibacterial activity of red murta (*Ugni molinae* Turcz.) seeds: an undervalued Chilean resource. Journal of Food Measurement and Characterization, *14*, 2020, pp. 1810–1821. DOI: 10.1007/s11694-020-00428-x.
29. Niu, S. – Hao, F. – Mo, H. – Jiang, J. – Wang, H. – Liu, C. – Fan, X. – Zhang, Y.: Phenol profiles and antioxidant properties of white skinned grapes and their coloured genotypes during growth. Biotechnology and Biotechnological Equipment, *31*, 2017, pp. 58–67. DOI: 10.1080/13102818.2016.1258329.
30. Cory, H. – Passarelli, S. – Szeto, J. – Tamez, M. – Mattei, J.: The role of polyphenols in human health and food systems: A mini-review. Frontiers in Nutrition, *5*, 2018, article 87. DOI: 10.3389/fnut.2018.00087.
31. Onivogui, G. – Letsididi, R. – Diaby, M. – Wang, L. – Song, Y.: Influence of extraction solvents on antioxidant and antimicrobial activities of the pulp and seed of *Anisophyllea laurina* R. Br. ex Sabine fruits. Asian Pacific Journal of Tropical Biomedicine, *6*, 2016, pp. 20–25. DOI: 10.1016/j.apjtb.2015.09.023.
32. Benites, R. – Formagio, A. – Argandoña, E. –

Volobuff, C. – Trevizan, L. – Vieira, M. – Silva, M.: Contents of constituents and antioxidant activity of seed and pulp extracts of *Annona coriacea* and *Annona sylvatica.* Brazilian Journal of Biology, *75*, 2015, pp. 685–691. DOI: 10.1590/1519-6984.21313.

33. Ali, S. I. – El-Baz, F. K. – El-Emary, G. A. E. – Khan, E. A. – Mohamed, A. A.: HPLC-analysis of polyphenolic compounds and free radical scavenging activity of pomegranate fruit (*Punica granatum* L.). International Journal of Pharmaceutical and Clinical Research, *6*, 2014, pp. 348–355. ISSN: 0975-1556. <http://impactfactor.org/PDF/IJPCR/6/IJPCR,Vol6,Issue4,Article12.pdf>
34. Orqueda, M. E. – Torres, S. – Zampini, I. C. – Cattaneo, F. – Di Pardo, A. F. – Valle, E. M. – Jiménez-Aspee, F. – Schmeda-Hirschmann, G. – Isla, M. I.: Integral use of Argentinean *Solanum betaceum* red fruits as functional food ingredient to prevent metabolic syndrome: effect of in vitro simulated gastroduodenal digestion. Heliyon, *6*, 2020, article e3387. DOI: 10.1016/j.heliyon.2020.e03387.
35. Choi, Y. M. – Yoon, H. – Lee, S. – Ko, H. C. – Shin, M .J. – Lee, M. C. – Hur, O. S. – Ro, N. Y. – Desta, K. T.: Isoflavones, anthocyanins, phenolic content, and antioxidant activities of black soybeans (*Glycine max* (L.) Merrill) as affected by seed weight. Scientific Reports, *10*, 2020, article 19960. DOI: 10.1038/s41598-020-76985-4.
36. Pastrana-Bonilla, E. – Akoh C. C. – Sellappan, S. – Krewer G.: Phenolic content and antioxidant capacity of muscadine grapes. Journal of Agricultural and Food Chemistry, *51*, 2003, pp. 5497–5503. DOI: 10.1021/jf030113c.
37. Snyder, S. M. – Low, R. M. – Stocks, J. C. – Eggett, D. L. – Parker, T. L.: Juice, pulp and seeds fractionated from dry climate primocane raspberry cultivars (*Rubus idaeus*) have significantly different antioxidant capacity, anthocyanin content and color. Plant Foods for Human Nutrition, *67*, 2012, pp. 358–364. DOI: 10.1007/s11130-012-0319-8.
38. Malta, L. G. – Tessaro, E. P. – Eberlin, M. – Pastore, G. M. – Liu R. H.: Assessment of antioxidant and antiproliferative activities and the identification of phenolic compounds of exotic Brazilian fruits. Food Research International, *53*, 2013, pp. 417–425. DOI: 10.1016/j.foodres.2013.04.024.
39. Pokorná, J. – Venskutonis, P. R. – Kraujalyte, V. – Kraujalis, P. – Dvorák, P. – Tremlová, B. – Kopriva, V. – Oštádalová, M.: Comparison of different methods of antioxidant activity evaluation of green and roast *C. arabica* and *C. robusta* coffee beans. Acta Alimentaria, *44*, 2015, pp. 454–460. DOI: 10.1556/066.2015.44.0017.
40. Fuentes, L. – Figueroa, C. R. – Valdenegro, M. – Vinet, R.: Patagonian berries: Healthy potential and the path to becoming functional foods. Foods, *8*, 2019, article 289. DOI: 10.3390/foods8080289.
41. Faraone, I. – Rai, D. K. – Chiummiento, L. – Fernandez, E. – Choudhary, A. – Prinzo, F. – Milella, L.: Antioxidant activity and phytochemical characterization of *Senecio clivicolus* Wedd. Molecules, *23*, 2018, article 2497. DOI: 10.3390/molecules23102497.
42. Sun, T. – Tanumihardjo, S. A.: An integrated approach to evaluate food antioxidant capacity. Journal of Food Science, *72*, 2007, pp. R159–R165. DOI: 10.1111/j.1750-3841.2007.00552.x.

Received 3 August 2021; 1st revised 12 October 2021; accepted 16 November 2021; published online 2 December 2021.