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Cistus ladanifer seeds: From ancient snack to novel and sustainable food ingredient

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ABSTRACT

Cistus ladanifer is a persistent, abundant and widespread underexplored resource in the Iberian Peninsula. The seeds have been used as food for centuries, although their nutritional value and potential as food ingredients have not been exploited until now. In this study seeds from natural shrubland were collected three times during summer for two consecutive years. Analytical evaluation of the macronutrient content, fatty acids, and mineral composition was performed. Regarding the macronutrients, seeds showed a carbohydrate content of 46.1 ± 1.6%, a fibre content of 20.9 ± 1.4%, a protein content of 16.2 ± 0.4%, a lipid content of 13.0 ± 1.1%, and an ash content of 3.87 ± 0.16%. The fatty acids were found to be mostly unsaturated (74.05 ± 0.59%). Potassium was the most abundant mineral (975 ± 53 mg/100 g) followed by phosphorous, magnesium and calcium. In conclusion, several nutrient-related label claims may be used for *C. ladanifer* seeds as food ingredient. Compared to common cereals, nuts and seeds, *C. ladanifer* seeds are close to flax and chia seeds in relation to nutritional composition, and to pine nuts in relation to mineral composition.

1. Introduction

Cistus ladanifer L., from the Cistaceae family, is a wild evergreen, usually erect shrub, present principally in the South Iberian Peninsula (Portugal and Spain) and North Morocco (Demoly and Montserrat, 1993). In Portugal, dense *C. ladanifer* shrublands occupy 7.5% (249,382 ha) of Portugal's mainland forest area which in turn occupies almost 40% of the mainland (Godinho-Ferreira et al. 2005). Even though this vast resource holds no value for most agricultural and forestry activities, in recent years some research contributed to its valorisation. Inclusion of *C. ladanifer* soft stems and leaves or extracts in ruminant diets has proven to be beneficial (Jerónimo et al. 2012; Dentinho et al. 2014). Herbaceous biomass is used for essential oil extraction and labdanum resin are used by the fragrance and perfume industry (Raimundo et al. 2018). Lignified biomass can be used for the bio-fuel industry (Carrión-Prieto et al. 2017; Fernandes et al. 2018; Alves-Ferreira et al. 2019). Combined, these proposed uses are starting to draw a biorefinery approach for *C. ladanifer* valorisation which, combined with its vast distribution, density, and resilience makes it a sustainable resource worth investigating.

Cistus ladanifer fruits are globular loculicidal capsules bearing globular-polyhedric 1 mm seeds (Demoly and Montserrat, 1993). Capsules are mature and exposed in early summer, starting seed dispersal mostly below the parent plant canopy for an 8–10-month period. Additional to deer and ants, pre-dispersal seed predation by *Lepidoptera larvae* (Noctiodae) and *Coleopteran larvae* (Bruchidae, Scarabeidae, Curculionidae) is known to be the major cause of fruit and seed loss (Serrano et al. 2001; Bastida and Talavera, 2002; Delgado et al. 2007;

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Table 1
Nutritional composition (% w/w) and energetic value of *C. ladanifer* seeds flour from early, middle, and late summer collection (ES, MS, LS, respectively) during two consecutive years (2019 and 2020).

Year	Season	Water Content (%)	Protein (% dw)	Lipid (% dw)	Ash (% dw)	Fibre (% dw)	Carbohydrate (% dw)	Energetic value (kcal)/100 g
2019	ES	5.89 ± 0.00^{c}	16.7 ± 0.1^{a}	14.2 ± 0.1^{a}	3.83 ± 0.03^{b}	21.9 ± 0.3^{a}	43.3 ± 0.3^{d}	387 ± 1^{b}
	MS	5.55 ± 0.02^{d}	16.0 ± 0.2^{b,c}	14.0 ± 0.0^{b}	3.74 ± 0.02^{b}	18.4 ± 0.7^{c}	47.8 ± 0.5^{a}	395 ± 1^{a}
	LS	5.22 ± 0.01^{e}	15.8 ± 0.3^{c}	13.5 ± 0.0^{c}	3.73 ± 0.01^{b}	20.3 ± 0.4^{b}	46.7 ± 0.1^{b}	390 ± 1^{b}
2020	ES	5.57 ± 0.07^{d}	16.7 ± 0.1^{a,b}	12.7 ± 0.0^{d}	4.01 ± 0.01^{a}	21.9 ± 0.0^{a}	44.7 ± 0.1^{c}	381 ± 1^{c}
	MS	6.36 ± 0.15^{b}	16.1 ± 0.2^{a,b,c}	11.7 ± 0.0^{e}	4.13 ± 0.05^{a}	21.0 ± 0.0^{a,b}	47.1 ± 0.0^{a,b}	374 ± 1^{d}
	LS	6.70 ± 0.07^{a}	15.7 ± 0.0^{c}	11.6 ± 0.0^{e}	3.78 ± 0.05^{b}	22.2 ± 0.0^{a}	46.8 ± 0.0^{b}	371 ± 0^{d}
Total		5.88 ± 0.53	16.2 ± 0.4	13.0 ± 1.1	3.87 ± 0.16	20.9 ± 1.4	46.1 ± 1.6	383 ± 8.9

n = 2 or 12 (total) (mean value ± standard deviation). Different upper-case letters represent significant (α < 0.5) statistical difference of means by the Tuckey's HSD post-hoc test, for each column parameter. % dw: in relation to the dry weight.

Table 2
Fatty acids composition (%) of *C. ladanifer* seeds flour from early, middle, and late summer collection (ES, MS, LS, respectively) during two consecutive years (2019 and 2020).

Year	Season	C14:0	C16:0	C16:1	C18:0	C18:1	C18:2 (w6)	C18:3 (w3)	C20:0
2019	ES	0.124 ± 0.003	19.4 ± 0.1^{a,b}	0.0773 ± 0.0022^{c}	4.56 ± 0.01	12.6 ± 0.0^{c}	47.6 ± 0.3^{a}	13.8 ± 0.1^{a}	0.434 ± 0.005^{c}
	MS	0.118 ± 0.002	20.1 ± 0.2^{a}	0.0753 ± 0.0026^{c}	4.54 ± 0.08	13.2 ± 0.2^{c}	46.9 ± 0.5^{a}	13.2 ± 0.3^{a}	0.455 ± 0.008^{c}
	LS	0.123 ± 0.001	19.4 ± 0.1^{a,b}	0.0603 ± 0.0021^{d}	4.50 ± 0.05	12.7 ± 0.1^{c}	47.4 ± 0.0^{a}	14.0 ± 0.1^{a}	0.454 ± 0.002^{c}
2020	ES	0.113 ± 0.002	18.2 ± 0.2^{b}	0.0777 ± 0.0010^{c}	4.46 ± 0.06	15.5 ± 0.2^{b}	46.0 ± 0.3^{a,b}	12.6 ± 0.2^{a,b}	0.526 ± 0.000^{b}
	MS	0.126 ± 0.001	18.3 ± 0.6^{b}	0.101 ± 0.001^{b}	4.76 ± 0.21	17.8 ± 0.5^{a}	44.5 ± 0.9^{b,c}	11.6 ± 0.6^{b}	0.570 ± 0.016^{a}
	LS	0.120 ± 0.003	18.6 ± 0.1^{a,b}	0.117 ± 0.003^{a}	4.84 ± 0.01	17.8 ± 0.0^{a}	44.0 ± 0.4^{c}	11.5 ± 0.1^{b}	0.587 ± 0.004^{a}
Total		0.121 ± 0.005	19.0 ± 0.8	0.0846 ± 0.0186	4.61 ± 0.16	14.9 ± 2.3	46.1 ± 1.5	12.8 ± 1.1	0.504 ± 0.060
Year	Season	C20:1	C22:0	C24:0	Saturated	Unsaturated	Monounsat.	Polyunsat.	w3/w6
2019	ES	0.119 ± 0.029	0.0575 ± 0.0047^{c}	0.0832 ± 0.0012^{d}	24.6 ± 0.2	74.3 ± 0.4	12.8 ± 0.1^{c}	61.4 ± 0.4^{a}	0.290 ± 0.001^{a}
	MS	0.130 ± 0.028	0.0908 ± 0.0025^{c}	0.0862 ± 0.0004^{c,d}	25.4 ± 0.5	73.5 ± 0.7	13.4 ± 0.2^{c}	60.1 ± 1.0^{a}	0.281 ± 0.04^{a,b}
	LS	0.125 ± 0.002	0.0480 ± 0.0059^{c}	0.0998 ± 0.0104^{b,c,d}	24.6 ± 0.2	74.2 ± 0.3	12.8 ± 0.1^{c}	61.4 ± 0.1^{a}	0.296 ± 0.002^{a}
2020	ES	0.157 ± 0.057	0.595 ± 0.007^{a}	0.121 ± 0.003^{a,b,c}	24.0 ± 0.3	74.4 ± 0.4	15.8 ± 0.1^{b}	58.7 ± 0.7^{a,b}	0.275 ± 0.003^{a,b}
	MS	0.244 ± 0.010	0.369 ± 0.035^{b}	0.131 ± 0.011^{a,b}	24.2 ± 1.0	74.3 ± 1.1	18.1 ± 0.5^{a}	56.1 ± 1.7^{b}	0.260 ± 0.009^{b}
	LS	0.271 ± 0.001	0.442 ± 0.012^{b}	0.147 ± 0.002^{a}	24.7 ± 0.2	73.6 ± 0.4	18.1 ± 0.0^{a}	55.4 ± 0.5^{b}	0.260 ± 0.000^{b}
Total		0.175 ± 0.067	0.267 ± 0.213	0.112 ± 0.024	24.6 ± 0.6	74.0 ± 0.6	15.2 ± 2.4	58.9 ± 2.6	0.277 ± 0.014

n = 2 or 12 (total) (mean value ± standard deviation). Different upper-case letters represent significant (α < 0.5) statistical difference of means by the Tuckey's HSD post-hoc test, for each column parameter.

Table 3
Mineral composition (mg/100 g) of *C. ladanifer* seeds flour from early, middle, and late summer collection (ES, MS, LS, respectively) during two consecutive years (2019 and 2020).

Year	Season	Sodium	Copper	Iron	Manganese	Zinc	Phosphorous	Magnesium	Calcium	Potassium
2019	ES	1.27 ± 0.04^{b}	1.48 ± 0.00^{a}	4.55 ± 0.09^{c,d}	9.88 ± 0.09^{a}	3.37 ± 0.00^{a}	452 ± 3^{a,b}	287 ± 57	221 ± 0	940 ± 4^{b,c}
	MS	2.15 ± 0.42^{a}	1.41 ± 0.01^{b}	6.26 ± 0.35^{a}	6.88 ± 0.04^{c}	3.35 ± 0.03^{a,b}	437 ± 7^{b}	237 ± 23	226 ± 2^{c}	931 ± 19^{b,c}
	LS	2.33 ± 0.22^{a}	1.48 ± 0.01^{a}	5.97 ± 0.00^{a,b}	9.25 ± 0.00^{b}	3.41 ± 0.04^{a}	445 ± 13^{a,b}	216 ± 5	223 ± 2^{c}	924 ± 9^{c}
2020	ES	2.33 ± 0.12^{a}	1.38 ± 0.02^{b}	5.28 ± 0.12^{b,c}	6.48 ± 0.09^{d}	3.15 ± 0.00^{a,b,c}	442 ± 6^{b}	221 ± 0	252 ± 3^{a,b}	972 ± 17^{b}
	MS	2.39 ± 0.13^{a}	1.38 ± 0.01^{b}	4.60 ± 0.18^{c,d}	5.54 ± 0.18^{e}	3.00 ± 0.15^{c}	458 ± 14^{a,b}	202 ± 16	259 ± 2^{a}	1059 ± 6^{a}
	LS	2.93 ± 0.01^{a}	1.49 ± 0.01^{a}	4.41 ± 0.18^{d}	8.90 ± 0.03^{b}	3.08 ± 0.06^{b,c}	479 ± 3^{a}	245 ± 25	248 ± 1^{b}	1021 ± 1^{a}
Total		2.23 ± 0.54	1.44 ± 0.05	5.18 ± 0.77	7.82 ± 1.67	3.22 ± 0.17	452 ± 16	232 ± 35	238 ± 16	974 ± 53

n = 2 or 12 (total) (mean value ± standard deviation). Different upper-case letters represent significant (α < 0.5) statistical difference of means by the Tuckey's HSD post-hoc test, for each column parameter.

Narbona et al. 2010).

The edible use of *C. ladanifer* seeds, eaten raw as walnut-like taste snack or ground into flour for bread and cake making, is documented through ethnobotanical studies and surveys across the Iberian Peninsula (Tardío et al. 2005; Tardío et al. 2006; Aceituno Mata, 2010), with no indications of toxicity. However, currently on the EU market there is no evidence of the use of such seeds as food. According to EU regulation 2015/2283 concerning novel foods, food produced or isolated from plants, or their parts is only considered novel food if there is no evidence of unsafe food use and if it is not produced by traditional propagation practices. Thus, *C. ladanifer* seeds do not need an authorization procedure as a novel food ingredient.

According to the European Commission *Health Promotion Knowledge Gateway* (https://ec.europa.eu/), most EU countries recommend the consumption of nuts and seeds as a part of a healthy diet. Although distinct in composition, nuts are important in diets because they have been linked to reducing the risk of type 2 diabetes and cardiovascular diseases (Kim et al. 2017).

Upcoming environmental changes such as low availability and high salinity of water will reduce fruit, nut and seed yields, which could have a negative impact on population health, thus justifying the need for an adaptation where sustainable and resilient food systems are supported and safeguarded (Alae-Carew et al. 2020). In this regard, given the potential biorefinery-like valorisation and the abundance of *C. ladanifer*, its seeds are worth evaluating as food and an additional valorisation path.

This work aims to characterize the nutritional composition of *C. ladanifer* seeds collected at three harvest seasons in two consecutive years in terms of macronutrient and mineral composition.

2. Materials and methods

2.1. Chemicals

Kjeltabs Cu-3.5 (*FOSS*, *Micro Atomo*); sulphuric acid (*Prolabo*, *VWR*),

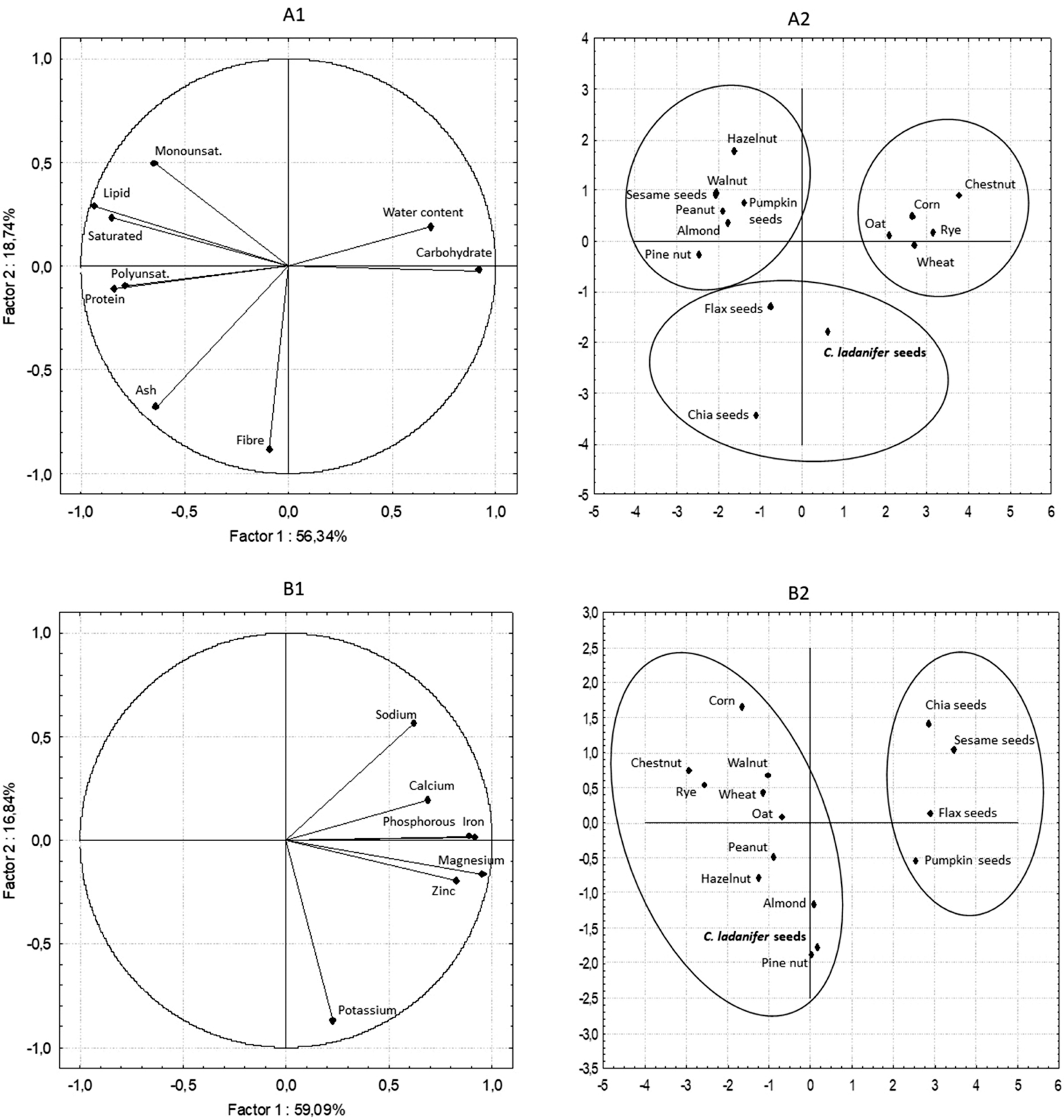

Fig. 1. Projection of the nutritional variables (1) and of the nut/cereal values (2) on the two-factor plot obtained by the Principal Component Analysis of the general nutritional composition (A, 75.08% of the variance) and of the mineral composition (B, 75.93% of the variance). Values for *C. ladanifer* seeds were obtained from Tables 1–3 as the mean total value, whereas values for the other nuts and seeds (whole grain, whole flour, or kernel) were obtained from the Portuguese Platform of Food Information (INSA, 2021).

0.1 M hydrochloric acid (*Prolabo*, *VWR*), 37% hydrochloric acid (*Prolabo*, *VWR*); petroleum ether (*Normapur*, *VWR*), *n*-hexane (*Normapur*, *VWR*), methanol (*Normapur*, *VWR*); potassium hydroxide (*Prolabo*, *VWR*), 67–69% nitric acid (*Prolabo*, *VWR*), 30% hydrogen peroxide (*Prolabo*, *VWR*), FAME Mix C8-C24 (CRM 18918, *Supelco*).

2.2. Capsule collection and processing

C. ladanifer subsp. *ladanifer* capsules were collected from natural shrubland located in Penha Garcia, Idanha-a-Nova, Castelo Branco, Portugal (GPS: N 40°1′43.4′' W 6°59′34.8′') for two years (2019 and 2020) at three different times: Early Summer (ES, end of June), Midsummer (MS, middle of August), and Late Summer (LS, beginning of October). After each collection, capsules were crushed to release seeds. Seeds were separated from the capsule parts by sieving with a metal mesh (ASTM no. 20, 850 μm). Seeds were stored in a dark and dry place until further analysis. Before evaluation, seeds were ground to a fine powder.

2.3. Moisture content

Moisture content was determined using a gravimetric method. Samples (5.0 g) were dried, under vacuum, at 70 ± 3 °C for 4 h, until constant weight. Moisture corresponded to the weight loss during drying, reported in relation to the initial weight of the samples.

2.4. Ash content

Ash content was determined using a gravimetric method. Samples (3.0 g) were incinerated at 550 ± 25 °C overnight. Ash content corresponded to the weight left after the incineration, reported in relation to the initial weight of the samples.

2.5. Protein content

Protein content was determined by the Kjeldahl method. Samples were weighed (1.0 g) in a tared nitrogen-free weighing paper. Two kjeltabs Cu-3.5 catalyst tablets and 12 mL of 95% H_2SO_4 were added. The tubes with the mixture were placed in the digestion block (Tecator Digestor, FOSS) at 420 °C for 1 h. Then, tubes were left to cool for 15 min. Samples were automatically analysed in Kjeltec (8400, FOSS). Nitrogen was quantified by titration with HCl (0.1 M) and protein content was obtained using the conversion factor of 6.25.

2.6. Lipid content and fatty acid composition

Samples (2.0 g) were hydrolysed with HCl (6 N), on a Soxcap System extraction (2047, FOSS). Samples were dried at 65 ± 3 °C overnight. Pre-treated samples were subjected to extraction with petroleum ether (90 mL) in Soxtec (2055, FOSS). Lipid content corresponded to the weight of the extract, dried at 103 ± 2 °C for 1 h.

Fatty acid composition was assessed by methylation of the lipid extract by dissolving the dried extract (100 mg) in *n*-hexane and methanolic potassium hydroxide (2 N). Fatty acids were analysed on GC SSL-FID (7890 A, *Agilent*), HP5 column (30 m × 0.32 mm i.d. × 0.25 μm), helium flow rate 0.8 mL/min, detector temperature 300 °C and injector temperature 260 °C with a split ratio of 1:100. Temperature program: initial temperature 50 °C for 3 min, increased by 10 °C/min to 170 °C, increased by 2 °C/min to 200 °C for 10 min, increased by 10 °C/min to 250 °C for 15 min. Their relative composition was determined by the integration of the peaks. FAME Mix C8-C24 was used for identification of fatty acids.

2.7. Crude Fibre content

Because the total lipid content of seeds was higher than 5% (w/w) it was necessary to remove excess sample fat. Crude fat was removed by extraction from the dried sample using petroleum ether b.p. 40–60 °C in a Soxtec System HT1043 Extraction unit apparatus (Tecator, Hoganas, Sweden). After lipid extraction, the powder was dried in a ventilated chamber set at 103 °C.

The crude fibre was determined by the Weende method (Fibertec System1020, Tecator). During the acid digestion, the feed sample is boiled in 1.25% (v/v) H_2SO_4 (extraction of free sugar and starch) followed by alkaline digestion with 1.25% (w/v) NaOH (to remove proteins, some hemicelluloses and lignin). After each digestion, crucibles were washed with hot distilled water to remove acid and alkaline residues and neutralized. Finally, the samples were defatted again with acetone and the crucibles were placed in a muffle furnace for ash determination (EN ISO 6865 (AOAC 978.10)).

2.8. Carbohydrate content and energetic value

Carbohydrate content and the energetic value of the samples were calculated according to EU Regulation No 1169/2011.

2.9. Mineral analysis

Minerals were analysed by first digesting the samples (0.5 g) with 6 mL nitric acid (67–69%) and 2 mL hydrogen peroxide (30%) in a microwave digestion system (Ethos One, Milestone). Minerals were quantified by atomic absorption spectroscopy (ICE3000, Thermo Scientific) or Inductively Coupled Plasma–Optical Emission Spectrometry (Activa M, Horiba Jobin Yvon). Flame atomic spectrometric measurements using the optimal instrumental parameters for each element with the following wavelengths: 589.0 nm (Na), 766.5 nm (K) and 422.7 nm (Ca). The operating conditions of the inductively coupled plasma atomic emission spectrometry equipment were as follows: 1000 W plasma power, 15 L/min plasma gas flow, 0.02 L/min nebulizer air flow and 1.0 bar air pressure. The analytical wavelengths (nm) were: 327.395 (Cu), 259.940 (Fe), 257.610 (Mn), 213.857 (Zn), 213.618 (P) and 279.553 (Mg).

2.10. Statistical analysis

One- and Two-Way-ANOVA and the Tuckey HSD post-hoc test were performed using the IBM SPSS Statistics 27 Software. All the analyses were performed in duplicate (n = 2).

Multivariate data analysis, namely Principal Component Analysis (PCA) was performed on datasets considering nutritional and mineral composition of *C. ladanifer* and other seeds and nuts (almond, peanut, hazelnut, chestnut, walnut, pine nut, wheat, rye, oat, corn, pumpkin seeds, chia seeds, flax seeds, sesame seeds) using STATISTICA 7 Software. Data was compiled from the Portuguese Platform of Food Information (INSA, 2021, www.portfir.insa.pt) as absolute values and compared to mean values of *C. ladanifer* seeds obtained in the present study.

3. Results and discussion

The overall nutritional value of *C. ladanifer* seeds is shown in Table 1. Carbohydrates and fibre stand as the major macronutrients representing, respectively, 46.1 ± 1.6% and 20.9 ± 1.4% of the seed weight. Although not very clear, carbohydrate content were the highest at the MS collection and fibre content the lowest, in both years. Ash content (3.87 ± 0.16%) and the energetic value of seeds (384 ± 9 kcal/100 g) did not show a consistent season variability. In contrast, lipid (13.0 ± 1.1%) and protein (16.2 ± 0.4%) content decreased from the beginning of summer to its end, even at the dry weight basis, removing the effect of the water content variation (5.88 ± 0.53%) during summer. The lipid content of *C. ladanifer* seeds was reported to be 13.2% by Krollmann and Gülz (1983) and Borges (1986).

According to EFSA (2010a), total carbohydrates intake should range between 45% and 60% of the total energy intake for children and adults, regardless of gender and lifestyle. Total carbohydrates of *C. ladanifer* seeds, excluding fibres, comprises 45% of the total energy, making it an equilibrated source of carbohydrates. Fibre adequate intake are 25 g/day for adults and less for children, meaning that a portion of 132 g of *C. ladanifer* seeds is enough. Indeed, according to Regulation (CE) No 1924/2006, regarding food nutritional and health claims, these seeds are considered high in fibre (≥ 6 g/100 g). Fibre is a general term for polysaccharides that are indigestible for humans that, generally, influence gut microbiota and nutrient absorption by different mechanisms, with generally accepted health benefits (Dhingra et al. 2012; Fuller et al. 2016; Qi et al. 2018). Proteins contribute to 16% of the total energy of *C. ladanifer* seeds, which means that according to Regulation (CE) No 1924/2006, as food, those seeds can be considered as a source of protein. According to EFSA (2012), although several health outcomes are associated with protein intake, data is insufficient to estimate a dietary reference value, pointing out a population reference intake of 0.83 g/kg body weight. Total lipids comprise 29% of the total energy of the seeds, which is within the reference intake range for adults (20–35%) proposed by EFSA (2010b), thus *C. ladanifer* seeds are a balanced source of lipids.

The *C. ladanifer* seed fatty acid profile is presented in Table 2. Unsaturated fatty acids represent 74.1 ± 0.6% of the total fatty acids, distributed in 58.9 ± 2.3% for polyunsaturated and 15.2 ± 2.4% for monounsaturated. Specifically, oleic acid (C18:1, w9), linoleic acid (C18:2, w6) and linolenic acid (C18:3, w3) are the major fatty acids comprising 14.9 ± 2.3, 46.1 ± 1.5, and 12.8 ± 1.1%, respectively. Results obtained for the main fatty acids are in accordance with the values reported by Krollman and Gülz (1983) and very similar to the composition of some walnut cultivars for oleic and linolenic acids, and lower for linoleic acid that is usually higher than 50% for this nut (Martínez et al. 2010; Tapia et al. 2013). Therefore, according to Regulation (CE) No 1924/2006, claims such as "High omega-3 fatty acids", "high polyunsaturated fat" and "high unsaturated fat" may be used for *C. ladanifer* seeds as food. Additionally, the w3 to w6 ratio was observed to be 0.27 which matches the 1:4 recommendation (Kamal-Eldin and Pickova, 2008). Despite the total lipid seasonal variability observed (Table 1), for the year 2020, a similar decrease was observed for linoleic acid accompanied by an increase in oleic acid. Erucic acid (C22:1), reported as toxic (Chain et al. 2016), was not detected in *C. ladanifer* seeds. Krollmann and Gülz (1983) reported that approximately 10% of the seed lipids are free sterols, mainly β-sitosterol, which according to Sayeed et al. (2016), although in need of extensive research, may be a health-promoting molecule for consumers. (Table 3).

Regarding mineral composition, like ash content, no clear season and year content variability trends were observed for the individual minerals. According to Regulation (CE), (2006) No 1924/2006, *C. ladanifer* seeds may be claimed to constitute a source of calcium (238 mg/100 g) and zinc (3.22 mg/100 g) and a high source of phosphorous (452 mg/100 g), iron (5.18 mg/100 g) and magnesium (232 mg/100 g), besides "sodium-free" (2.23 mg/100 g). Despite these claims, potassium was the major mineral, found in the highest quantity (975 mg/100 g). Minerals are micronutrients and therefore are needed in low quantities in the human diet for the normal physiological function of the human body. However, many global food systems fail to provide them resulting in alarming deficiency disorders as demonstrated by Welch (2002). Palmer and Clegg (2016) discuss the benefits of high-potassium diets, namely blood pressure reduction, stroke risk decrease, and bone health improvement. In this regard, *C. ladanifer* seeds may be a fortifying food worth including in the human diet. However, risks associated with a high intake of micronutrients should be evaluated (Engle-Stone et al., 2019).

To find a position for *C. ladanifer* seeds among other nuts and cereals, Principal Component Analysis (PCA) was performed, comparing nutrient composition values extracted from the Portuguese Platform of Food Information (INSA, 2021, www.portfir.insa.pt) (Fig. 1). By PCA, it was possible to reduce the initial 9-dimensional hyperspace to a plane defined by the first two principal components (new axis), containing 75.1% of the variance of the original data matrix. The projection of the original variables of the nut/cereals on these planes is shown in Fig. 1. A clear separation between cereals and nuts was observed, the first ones higher in carbohydrates and water and the others higher in protein and lipid content. *Cistus ladanifer*, flax and chia seeds were however separated from those groups by the higher ash and fibre content. The *C. ladanifer* seeds additionally showed an intermediate carbohydrate, protein, and lipid content in relation to the two groups clearly formed by nuts and cereals, once more showing a balanced composition with regard to those macronutrients. The second PCA aimed to group the several nuts and cereals in relation to the mineral composition and explained 75.9% of the total variance using two components. From this analysis, two groups were clearly formed, this time clustering the seeds (chia, sesame, flax, and pumpkin seeds) in one group with higher general mineral content and clustering nuts, cereals, and *C. ladanifer* seeds in another group. Within the latter group, *C. ladanifer* and pine nuts are at extreme points mainly because of their high potassium content.

4. Conclusion

To our knowledge, this study is a first step in understanding the nutrient composition of *C. ladanifer* seeds and *Cistus* species. In general, these seeds have an intermediate compostion between the carbohydrate-rich cereals and lipid-rich nuts. They are also a source of protein, calcium, and zinc, and a high source of fiber, unsaturated fatty acids, phosphorous, iron and magnesium. The fatty acid profile gives these seeds a potential value as a health-promoting food ingredient. Regarding valorisation as a food, future research should focus on amino acid, vitamin, and polyphenolic profiles, and evaluation of other lipid compounds such as sterols. A toxicological evaluation could also be beneficial to safeguard consumers. Cultivation/collection practices to maximize efficiency and reduce harvest costs would be highly relevant.

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CRediT authorship Contribution Statement

Frazão DF, Peres F, Paulo L, Moitinho A and Delgado F wrote the original manuscript, and all authors reviewed the manuscript. Frazão DF, Peres F, Delgado F, Espírito Santo C, Paulo L and Moitinho A conceptualized the work. Frazão DF, Peres F, Barroca C and Resende M performed the practical work. Frazão DF and Peres F analysed and discussed data.

Conflict of interests

The authors declare that there are not conflict of interests.

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