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Chemical profiling of Portuguese *Pinus pinea* L. nuts and comparative analysis with *Pinus koraiensis* Sieb. & Zucc. commercial kernels

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Abstract. Being an endemic species to the Mediterranean Basin, stone pine (*Pinus pinea* L.) obtains an extreme economical relevance in Spain, Portugal, Italy, Tunisia and Turkey, where pine nuts are traditionally marketed and consumed. Based on the analysis of 27 different Portuguese populations, pine nuts were characterized by high contents of fat (47.7 g per 100 g dry matter DM), protein (33.9 g per 100 g DM) and phosphorus (1130 mg per 100 g DM) and low contents of moisture (5.9 g per 100 g DM) and starch (3.5 g per 100 g DM). They were also found to be a good source of zinc, iron and manganese. A comparative analysis with *P. koraiensis* nuts showed the latter to have lower protein (14.1 g per 100 g DM) vitamin and mineral contents, and higher fat (68.1 g per 100 g DM) and pinolenic acid contents (14.5% *vs* 0.3% w/w TFA). Protein and pinolenic acid contents could be considered as good parameters to distinguish the edible kernels of these two species.

Keywords. *Pinus pinea* – *Pinus koraiensis* – Proximate analysis – Fatty acids – Mineral analysis.

***Profil chimique du pin pignon portugais* Pinus pinea *L. et'analyse comparée avec* Pinus koraiensis *Sieb, & Zucc*.**

Résumé. *Le pin pignon (*Pinus pinea *L), espèce endémique au bassin méditerranéen, a un poids économique considérable dans quelques régions d'Espagne, du Portugal, d'Italie, de la Tunisie et de la Turquie où, traditionnellement, le pignon de pin est commercialisé et consommé. En se basant sur l'analyse de 27 populations portugaises différentes, le pignon de pin a été caractérisé par des teneurs élevées en graisse (47.7 g pour 100 g de matière sèche MS), en protéines (33.8 g pour 100 g MS) et en phosphore (1130 mg pour 100 g MS) et de faibles teneurs en humidité (5.9 g pour 100 g de MS) et en amidon (3.5 g pour 100g MS). De plus il s'est révélé être une bonne source de zinc, de fer et de manganèse. Une analyse comparative avec le pignon de* P. koraiensis *a montré que celui-ci a des teneurs en protéines (14.1 g MS pour 100 g) et en vitamines et minéraux plus faibles. Par contre, les teneurs en acides gras (68.1 g pour 100 g MS) et en acide pinolénique (14,5% vs 0,3% p/p du total en acides gras) sont plus élevées. Les teneurs en protéines et en acide pinolénique pourraient être considérées comme des paramètres à retenir pour différencier le pignon comestible de ces deux espèces.*

Mots-clés. Pinus pinea – Pinus koraiensis – *Analyse immédiate – Acides gras – Analyse minérale.*

I – Introduction

The Portuguese area of stone pine forest is estimated in 130,000 hectares (IFN5, 2010). Due to the species plasticity, *P. pinea* can be found throughout the country. Among the seven provenance regions delineated in the Portuguese territory (Cardoso and Lobo, 2001), provenance region 5 (South of Tagus) gathers about 62% of the total area due to the particular ecological conditions. Specifically the district of Setúbal, region of Alcácer do Sal, stands out as the most important pine-nut production area due to both yield and quality, concentrating more than 50% of the national production and rendering the region economy highly dependent on pine nut exploitation.

The seeds of *P. pinea* are ancient components of the Mediterranean diet, included as ingredients in confectionery and cooking for its exquisite flavor. Recently, there has been a growing worldwide market for pine nuts. In consequence, a strong competition has been generated by pine nuts of other species, mostly *P. koraiensis* Sieb. & Zucc. from China, which reach the local markets at lower prices and are undistinguishable to the untrained eye of consumers, although they have a different flavor, shape and size.

Considering that the origin is an important issue for producer and consumer protection, this study aimed to comparatively analyse the chemical profiles of *P. pinea* nuts and Chinese pine nuts (*P. koraiensis*) and specifically find parameters to distinguish them.

II – Material and methods

Local sampling was performed on 27 Portuguese populations from three *Pinus pinea* Provenance Regions and from four external locations. A commercial sample of Chinese pine nuts (*Pinus koraiensis* Sieb. & Zucc.) was also included for comparative analysis (Fig.1A, B). The following chemical analyses were performed: proximate analysis (moisture, crude protein, crude fat, and starch); vitamin analysis (thiamine and riboflavin); fatty acid analysis saturated fatty acids (SFA) palmitic, stearic and arachidic; unsaturated fatty acids USFA oleic, linoleic, α linolenic, pinolenic, gadoleic, eicosadienoic and sciadonic) and mineral analysis (phosphorus, potassium, calcium, magnesium, sulfur, zinc, copper, iron, manganese, and sodium). Chemical analyses were based on composite samples and performed in triplicate, (Evaristo *et al.,* 2010).

Fig.1. A: *Pinus pinea* pine nuts; B: *Pinus koraiensis* pine nuts (commercial sample).

III – Results and discussion

1. Proximate and vitamin analysis

The proximate composition and vitamin content of pine nuts from 27 *P. pinea* populations grown in Portugal (mean value) along with one commercial sample of *P. koraiensis* nuts are shown in Table 1. For the Portuguese samples crude fat was the predominant component (47.71%). Identical values are found on Mediterranean stone pine nuts from Tunisia (Nasri *et al*., 2005a, 2005b) and Spain (Cañellas *et al*., 2000), but lower (44-45%) and higher (50.3%) average concentrations are also reported, for Turkish (Bagci and Kavaagaçli, 2004) and Italian pine nuts from *Pinus pinea* (Ruggeri *et al*., 1998), respectively. In our analysis, *P. koraiensis* presented a higher level of crude fat content (68.07%). Crude protein was the second major component found in the *P. pinea* seeds analysed with an average of 33.85%. Similar results were reported for other samples of Mediterranean stone pine nuts (Nergiz and Donmez, 2004; Ruggeri *et al*., 1998; Cañellas *et al*., 2000). On the contrary, our analyses showed that *P. koraiensis* seeds have a much lower amount of crude protein (14.06%), even lower than that reported previously (17-18%) by Mata (2001). The average moisture for the 27 Portuguese populations was found to be 5.9% while *P. koraiensis* seeds presented 2.5% of moisture, a value far below from that (4.4%) reported by Mata (2001). The present investigation revealed also low levels of starch in Portuguese pine nuts with a mean value of 3.5% DM, when compared with values obtained from chestnuts (43%) (Borges *et al*., 2008; Künsch *et al.,* 1999). Average starch content of *P. koraiensis* seeds was higher (4.9%) but still registered an equivalent low level. Appreciable amounts of vitamins B1 (thiamine) and B2 (riboflavin) were detected in *P. pinea* seeds, reaching mean values of 0.53 mg and 0.19 mg per 100 g DM respectively. These amounts represented approximately 35.3 and 11.9% respectively of the recommended dietary allowance (RDA), which suggests daily ingestion of 1.6 mg of riboflavin and 1.5mg of thiamin for an adult male (Garrow *et al.,* 2000). *P. koraiensis* seeds revealed vitamin poorer levels, presenting 0.19 mg per 100 g DM thiamine and 0.02 mg per 100 g riboflavin.

Table 1. Proximate composition, riboflavine and thiamine of pine nuts from 27 *Pinus pinea* populations and one *P. koraiensis* sample

	Moisture g/100 g	Starch g/100 g	Crude protein g/100 g	Crude fat g/100 g	Riboflavine mg/100 g	Thiamine mg/100 g
P. pinea	5.9±0.8	3.5±1.1	33.85±1.89	47.71±2.03	0.19±0.25	0.53±0.29
P. koraiensis	2.5±0.0	4.9±0.2	14.06±0.12	68.07±1.22	0.02±0.01	0.19±0.03

Values are the mean value±SD.

2. Mineral analysis

In the present study, the contents of ten minerals were determined (Table 2) in the studied 27 Portuguese populations and the commercial composite sample of Chinese pine nut. Phosphorus (P) (1130 mg per 100 g DM) was the most abundant element in the seeds of *P. pinea* followed by potassium (K) (892 mg per 100 g DM), magnesium (Mg) (533 mg per 100 g DM) and sulfur (S) (485 mg per 100 g DM). The other elements were, in descending order by quantity, calcium (Ca), manganese (Mn), zinc (Zn), iron (Fe), copper (Cu), and sodium (Na). Those results compare favorably with those obtained by Gómez-Ariza *et al*. (2006).

Table 2. Mineral composition of pine nuts from 27 *Pinus pinea* populations and 1 *P. koraiensis* sample

	Macronutrients				
	P (mg/100 g)	K (mg/100 g)	Ca (mg/100 g)	Mg (mg/100 g)	S (mg/100 g)
P. pinea	1130±110.7	892±68.6	31.9±3.0	533.2±38.4	485.4±32.5
P. koraiensis	539±7.51	596±0.2	10.3±0.0	246.3±0.1	201±7.4
	Micronutrients				
	Na (mg/100 g)	Fe (mg/100 g)	Mn (mg/100 g)	Cu (mg/100 g)	Zn (mg/100 g)
P. pinea	1.01±1.25	11.12±1.52	16.05±8.45	3.43±0.29	11.12±0.85
P. koraiensis	0.24±0.05	5.54±0.00	7.34±0.22	1.28±0.08	6.16±0.11

Values are the mean value±SD.

In contrast, our analysis revealed that *P. koraiensis* seeds had a lower mineral content, frequently less than half the mean of *P. pinea* seeds, except for K and Zn. These minerals have many functions in human basal metabolism and are of interest due to their pro-oxidant activity and health benefits (Ozcan, 2006). The mean ratio of Na:K and Ca:P was considered very poor, as it was less than the unity, indicating that mineral supplementation of human dietary is needed (Iqual *et al.,* 2006).

3. Fatty acid analysis

Fatty acid composition of the oil extracted from the seeds is shown in Tables 3 and 4. Nuts are generally recognized for having a healthy fatty acid profile richer in unsaturated fatty acids (USFA). As expected, both *P. pinea* and *P. koraiensis* nut samples contained a predominant fraction of USFA (89.01 and 89.58% w/w TFA), consisting of 39.24 and 29.32% of monounsaturated fatty acids (MUFA) and 49.77 and 60.26% of polyunsaturated fatty acids (PUFA), respectively (data not showed). Saturated fatty acids (SFA) accounted for 10.93 and 7.75% of total fatty acids for *P. pinea* and *P. koraiensis* seeds, respectively. The main contributing SFA was palmitic acid (C16:0) followed by stearic acid (C18:0) in similar proportions relative to the FA total amount for both species.

In agreement with the typical composition of the *Pinaceae* nut oil, the linoleic acid (C18:2) was the most abundant fatty acid in *P. pinea* and *P. koraiensis*. Together with MUFA oleic acid (C18:1n-9), seeds comprised respectively in average 84.76 and 72.2% w/w of total USFA. Although no large differences were found between the two pine species on the linoleic acid content, predominance of PUFA over MUFA was considerably higher (PUFA/MUFA ratio, 2.06 *vs* 1.275) and the oleic acid concentration distinctively lower on *P. koraiensis* seeds (28.05 *vs* 38.40%). *P. pinea* and *P. koraiensis* seed oils were also characterized by several minor fatty acids contributing to their composition, such as acids C20:1 *cis*-11 n-9 (gadoleic), C20:2 *cis*-11,14 n-6 (eicosadienoic) and C18:3 *cis*-9,12,15 n-3 (α-linolenic acid, ALA). All studied pine nut oils contained also two particular minor fatty acids of the unusual *cis*-5-unsaturated polymethylene interruped fatty acid family, namely *cis*-5,9,12 C18:3 (pinolenic acid) and cis-5,11,14-C20:3 (sciadonic acid). While sciadonic acid occurs in the seed lipids of all Gymnosperm species containing Δ5-UPIFA, pinolenic acid is restricted to certain families and presents a higher interspecific variation in the genus *Pinus* (Bagçi and Karaagaçli, 2004). A large discrepancy in pinolenic acid concentration was detected between *P. koraiensis* and *P. pinea* (14.47% *vs* 0.30%), accounting for its much higher PUFA predominance. On the other hand, *P. pinea* nuts show a minimal level fluctuation in pinolenic acid concentration among all Mediterranean populations analysed so far, from European to Moroccan, Turkish and Tunisian (Nasri *et al*., 2005 a, b). The same high standards of pinolenic acid in *P. koraiensis* nut oil were reported in other studies suggesting a positive effect on LDL-lowering by enhancing hepatic LDL uptake (Lee, Lee, Lee, Kim and Rhee, 2004), and on appetite reduction by inducing satiety hormones (Pasman *et al*., 2008).

These results suggest that pinolenic acid is a stable component that could be used to specifically distinguish *P. pinea* from *P. koraiensis.*

Table 3. Saturated fatty acid composition of pine nuts from 27 *Pinus pinea* populations and one *P. koraiensis* sample

	C16:0 %	C18:0 %	C20:0 %	ΣSFA %
P. pinea	6.22±0.17	4.03±0.21	0.68±0.04	10.93±0.27
P. koraiensis	4.98±0.05	2.38±0.04	0.39±0.01	7.75±0.10

Values are the mean value±SD.

Table 4. Unsaturated fatty acid composition of pine nuts from 27 *Pinus pinea* populations and one *P. koraiensis* sample

	C18:1 %	C20:1 %	C18:2 %	C18:3 *cis*5,9,2 %	C18:3 *cis*9,12,15 %	C20:2 %	C20:3 %
P. pinea	38.36±1.25	0.88±0.07	46.40±1.24	0.30±0.03	0.71±0.06	0.57±0.06	1.79±0.19
P. koraiensis	28.05±0.24	1.28±0.01	44.19±0.00	14.47±0.11	0.00±0.00	0.61±0.00	0.99±0.00

Values are the mean value±SD.

IV – Conclusions

The chemical composition data of Portuguese pine nuts gathered in our study for proximate components, vitamins and fatty acids, is very consistent with the corresponding results of all Mediterranean *P. pinea* populations reported so far, in spite of the geographical differences. We confirmed that pine nuts have high protein, unsaturated fat (mainly linoleic acid) and vitamin (B1 and B2) contents and are a rich source of P, Fe and Zn. As the only exception, mineral composition of Portuguese *versus* Turkish pine nuts showed substantial variations which, upon confirmation by further studies on different producing years and mineral characterization of the remaining origins of *P. pinea* seeds, might prove useful to discriminate Mediterranean populations.

P. koraiensis seeds were characterized in our study by comparatively lower contents of crude protein, moisture and minerals, and higher contents of total fat, in particular PUFA with a particular higher and distinctive fraction of pinolenic acid. Supporting the use of seed FA composition as a *Pinus* chemotaxonomic marker, as suggested by Bagci and Karaagaçli (2004), our results suggest that pinolenic acid content is a good biochemical indicator and could be used to clearly distinguish *P. koraiensis* from *P. pinea*. Establishment of tools for kernel identification is particularly important to control the processing procedures, protect producers and avoid unfair competition.

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