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ARTICLE

Identification of the Botanical Origin of Pine Nuts Found in Food Products by Gas−Liquid Chromatography Analysis of Fatty Acid Profile

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Pine nuts are traditionally used in various part of the world for the preparation of desserts or sauces or in salads. Local production is not sufficient to cope with the high demand of pine nuts around the world, and countries such as China or Pakistan are exporting much of their production to Western countries. Almost all the nuts that are traditionally consumed belong to the *Pinus* genus, but over the past years, the number of consumer complaints following consumption of commercial pine nuts increased. Some consumers experienced taste disturbance lasting for up to two weeks after consumption. Food safety agencies raised some concerns regarding pine nuts imported from Asia and their association with taste disturbance. However, even though a formal association has not been found to date, the *Pinus* genus comprises species that are not classified as edible and could be eventually used to adulterate edible species. *Pinus* spp. seed lipids are known to contain very specific polyunsaturated fatty acids know as Δ5-olefinic acids. Seed fatty acid profile of conifers had been used in the past as a taxonomic marker, and in the present study to identify the botanical origin of pine nut in nine commercial products. Fast gas−liquid chromatography (GLC) was used to resolve the complete fatty acid profile of *Pinus* spp. samples in less than 5 min. A diagnostic index based on the relative levels of the main fatty acids including distinctive Δ5-olefinic acids was used to identify botanical origins. Results revealed the occurrence of the following *Pinus* spp. in commercial products: *P. pinea*, *P. koraiensis*, *P. gerardiana*, *P. armandii* and *P. massoniana*. The later two species, known as Chinese white pine and Chinese red pine, are only cultivated in China and are not listed as common source of edible pine nuts by the Food and Agriculture Organization (FAO). The present study shows that the botanical origin of pine nuts can be identified in products based on the fatty acid profile.

KEYWORDS: Delta5-olefinic acids; gas−liquid chromatography; identification; pine nut; *Pinus* spp

INTRODUCTION

Pine nuts are traditionally consumed in various parts of the world as such, in desserts, in sauces (e.g., in pesto) or in salads. Almost all traditionally consumed nuts belong to the *Pinus* genus (*1*, *2*) and are listed in **Table 1**. Some of these species are just consumed locally, but others such as *Pinus koraiensis*, *P. gerardiana* or *P. pinea* are worldwide produced and exported (*1*). Local production of pine nuts is not sufficient to satisfy the high demand around the world, and countries such as China and Pakistan became, over the past decade, the main exporting countries of pine nuts. The *Pinus* species cultivated in these geographical regions may differ from the species usually consumed where these products are imported. Especially, some species such as *P. armandii, P. tabuliformis*, *P. yunnanensis* or *P. massoniana* are almost exclusively cultivated in China (*3*). The botanical origins of imported pine nuts are not reported on-pack and therefore are difficult to trace.

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Over the past two years, several cases of taste disturbances following the consumption of pine nuts have been reported to food agencies, in public Web sites and scientific literature (*4*). In most of the cases, a bitter and metallic taste lasting for up to 2 weeks after the consumption of the nuts has been reported by consumers (*5*, *6*). A recent report from the French food safety agency (AFSSA) confirmed the growing number of complaints from consumers in France but failed to identify its causality (*6*). However, the identification of seeds from *Pinus armandii*, also known as Huashan pine or Chinese white pine in Asia, in one of the incriminated products that has been mentioned in this report (*6*). This species is not listed by the FAO as one of the 29 species traditionally used as food item (**Table 1**). It can be hypothesized that the occurrence of *Pinus* spp. that are not are not generally consumed in some markets might be linked with the sensory disturbances notices by some consumers.

The identification of the botanical origin of conifer seeds can be performed based on genetic or chemotaxonomic markers (*7*−*9*). The analysis of seed fatty acid profile is by far one of the easiest ways to identify conifer seeds based on the occurrence and level of specific unsaturated fatty acids having a double bond in position

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Table 1. Name, Geographic Distribution and Use of Pine Nuts from *Pinus* Species Recognized by the FAO To Produce Edible Nuts

Pinus spp.	natural range	use
P. pinea[a]	Mediterranean Europe and Near East	important in international trade
P. gerardiana	Afghanistan, Pakistan, India	important in international trade
P. koraiensis	China, Japan, Korea	important in international trade
P. ayacahuite	Mexico, Central America	traditional food for indigenous tribe
P. albicaulis	Western Canada and United States	traditional food for indigenous tribe
P. cembra[a]	Europe	locally important
P. flexilis	Western Canada and United States	traditional food for indigenous tribe
P. lambertiana[a]	United States	traditional food for indigenous tribe
P. catarinae	Mexico	traditional food source
P. cembroides	Mexico	traditional food source
P. culminicola	Mexico	
P. discolor	Mexico, United States	
P. edulis[a]	Mexico, United States	locally important
P. quadrifolia[a]	Mexico, United States	locally important
P. juarezensis	Mexico, United States	locally important
P. johannis	Mexico	traditional food source
P. lagunae	Mexico	
P. maximartinezii	Mexico	
P. monphylla	Mexico, United States	locally important
P. nelsonii	Mexico	
P. pinceana	Mexico	traditional food source
P. remota	Mexico, United States	traditional food source
P. pumila	Russia, China, Korea, Japan	locally important
P. sibirica	Russia, China, Kazakhstan, Mongolia	nuts are ground into cooking oil
P. strobiformis	Mexico, United States	traditional food for indigenous tribe
P. coulteri	United States	traditional food for indigenous tribe
P. ponderosa	Canada, United States	traditional food for indigenous tribe
P. sabiniana	United States	traditional food for indigenous tribe
P. roxburghii	India	traditional food source
P. torreyana	United States	traditional food for indigenous tribe

[a] *Pinus* spp. reported within the category TN 0673 by Codex Alimentarius (*2*).

Table 2. Latin and Common Names, Geographical Range and Seed Fatty Acid Composition of the Most Common *Pinus* Species Found in the Global Market for Food Uses

	% of total fatty acids							
	commonly used *Pinus* spp. for edible seed production				*Pinus* spp. found in Asia and exported for food use			
fatty acid	*P. pinea*	*P. gerardiana*	*P. koraiensis*	*P. sibirica*	*P. massoniana*	*P. armandii*	*P. tabuliformis*	*P. yunnanensis*
common name	Italian stone pine	Chilgoza pine	Korean pine	Russian cedar	Masson pine or Chinese red pine	Chinese white pine, Huashan pine or Armand pine	Chinese pine	Yunnan pine
16:0	5.55	5.21	4.91	4.35	3.83	4.49	4.56	4.27
16:1	0.08	0.12	0.08	0.08	0.35	0.13	0.23	0.28
aiso-17:0	0.08	0.09			0.12	0.04	0.10	0.15
17:0	0.05	0.05	0.07		0.05	0.03	0.03	0.03
17:1		0.05				0.02	0.01	
18:0	3.2	1.61	2.22	2.49	1.9	1.90	1.97	1.51
18:1 n-9 + n-7	38.37	37.24	27.47	25.48	16.85	24.33	20.86	18.37
5,9-18:2	0.14	0.42	2.03	1.84	3.82	3.39	4.00	2.19
18:2 n-6	47.19	51.25	45.20	43.45	50.6	46.03	43.44	44.92
5,9,12-18:3	0.35	0.83	14.55	18.29	17	15.98	17.15	20.58
18:3 n-3	0.4	0.46	0.15	0.2	0.41	0.23	0.35	0.41
20:0	0.4	0.43	0.36	0.28	0.54	0.36	0.26	
5,9,12,15-18:4							0.02	0.04
20:1 n-9	0.6	0.64	1.20	1.27	0.29	0.90	0.89	0.58
5,11-20:2	0.14	0.11	0.10	0.11	0.44	0.12	0.4	0.39
20:2 n-6	0.51	0.24	0.57	0.57	0.59	0.57	0.85	0.86
5,11,14-20:3	2.47	0.23	0.84	0.96	3.36	1.31	3.73	4.84
7,11,14-20:3				0.1	0.17	0.06	0.26	0.35
22:0		0.19				0.13	0.07	

5. These fatty acids are almost exclusively present in conifers (Ginkgoatae and Pinatae) and are known as Δ5-olefinic acids. The main Δ5-olefinic acids found in *Pinus* seeds are taxoleic (5,9-18:2), pinolenic (5,9,12-18:3) and sciadonic (5,11,14-20:3) acids. It has been clearly established in several reports from Wolff and co-workers (*7*−*9*) that the fatty acid composition of conifer seeds differs according to the subgenus, section, subsection and genus and that fatty acid profile can be used as a taxonomic marker. The fatty acid composition of 144 species from the *Pinus* genus have been reported in a single review that can be used as a

repository for the identification of the botanical origin of *Pinus* spp. based on fatty acid profile (*7*).

Fatty acid analyses are extensively performed in the food industry to identify the quality and nutritional values of products. Recent progress in gas−liquid chromatography (GLC) allows the performance of high-resolution analysis of the fatty acid profile in the so-called "fast" mode (*10*, *11*). Therefore, it appears that, in the context of the identification of botanical origin of imported pine nuts, fatty acid analysis can be an efficient tool to identify the nature of commercial products. In the present study, fast GLC has been used to assess the botanical origin of commercial pine nuts and information allowing identification based on published data are provided. The objective of this study was not to link the lipid composition of the commercially available pine nuts to the taste disturbance reported by consumers but to propose an analytical procedure to identify botanical origin of *Pinus* spp. seeds in commercial products.

MATERIALS AND METHODS

Samples and Reagents. Nine different packed pine nut samples were obtained from different groceries and supermarkets in France and Switzerland. In addition, seeds from *Pinus pinea* were collected in the region of Bordeaux (France). One of the analyzed products was kindly provided by a consumer who experienced taste disturbance after its consumption. All solvents were HPLC grade, and hydrochloric acid in methanol (3 N) was obtained from Supelco (Bellafonte, CA).

Sample Preparation. Pine nuts (about 10 g) were ground in a mortar, and fatty acid methyl esters (FAME) were directly prepared without prior lipid extraction as previously described (*12*). Briefly, ground and dried nuts (100 mg) were mixed with methanol (2 mL), hydrochloric acid in methanol (3 N, 2 mL) and hexane (2 mL) in tightly closed test tubes. The methylation reaction was performed at 100 °C for 1 h. After cooling down to room temperature, water (2 mL) was added and the sample vigorously vortexed for ca. 30 s. After centrifugation for 2 min at 3500 rpm, the hexane phase was diluted with an equal volume of fresh hexane, transferred in vials and analyzed by GLC.

Gas−Liquid Chromatography Analysis. Analysis of FAME was performed on a 7890 Agilent gas chromatograph (Agilent Technologies, Palo Alto, CA), equipped with a fused-silica BPX-70 capillary column (10 m × 0.1 mm i.d., 0.2 μm film thickness; SGE, Melbourne, Australia). Split injector (1800:1) and flame ionization detection (FID) systems were operated at 250 and 300 °C, respectively. Oven temperature programming was 50 °C isothermal for 1 min, increased to 180 at 100 °C/min, isothermal for 1 min at this temperature then increased to 220 at 20 °C/min and then to 250 at 50 °C/min. The carrier gas (H_2) was maintained constant at 0.6 mL/min and the acquisition of the FID signal at 100 Hz.

RESULTS AND DISCUSSION

An important part of the investigation consisted of gathering information regarding *Pinus* spp. known to be consumed locally or globally but as well *Pinus* spp. produced in China or other countries and exported in Western regions. Based on the identified *Pinus* spp. a database containing fatty acid profile reported in the review by Wolff et al. (*7*) was prepared prior to performance of the analytical investigation (**Table 2**).

Fatty Acid Analysis of *Pinus* spp. Seed Lipids. Resolution of the fatty acid profile of conifer seeds containing Δ5-olefinic acids can be obtained by GLC using various stationary phases such as polar cyanoalkylpolysiloxane as in the present study or on polyethylene glycol coated capillary columns (*7*−*9*, *13*−*15*). The main Δ5-olefinic acids found in *Pinus* seeds are taxoleic (5,9-18:2), pinolenic (5,9,12-18:3) and sciadonic (5,11,14-20:3) acids. Traces amount of coniferonic (5,9,12,15-18:4) acid, 5,11-20:2 or 7,11,14-20:3 (the elongation product of pinolenic acid) were also detected in some species in accordance with literature data (*7*). The geometry of the double bonds of the unsaturated fatty acids in these seeds is exclusively *cis*. Typical chromatograms of the two main *Pinus* spp., *Pinus koraiensis* and *P. pinea*, usually consumed in Europe are reported in **Figure 1**. The occurrence of the additional ethylenic double bond in position Δ5 in taxoleic and pinoleic acids confers greater polarity compared to oleic and linoleic acid, respectively. Consequently, taxoleic and pinolenic elute as FAME after their precursors, oleic and linoleic acid when polar columns are used, as shown in **Figure 1**. The resolution obtained on a short highly polar capillary column designed for fast GLC analysis is sufficient to obtain accurate determination of almost all the fatty acids detected in the *Pinus* seed species analyzed (**Figure 1**), the only exception being the lack of resolution between oleic (9-18:1) and *cis*-vaccenic (11-18:1) acid. *cis*-Vaccenic acid can be found in all fats and oils, but its quantification in *Pinus* seed lipids does not provide any information regarding the botanical origin of the seeds.

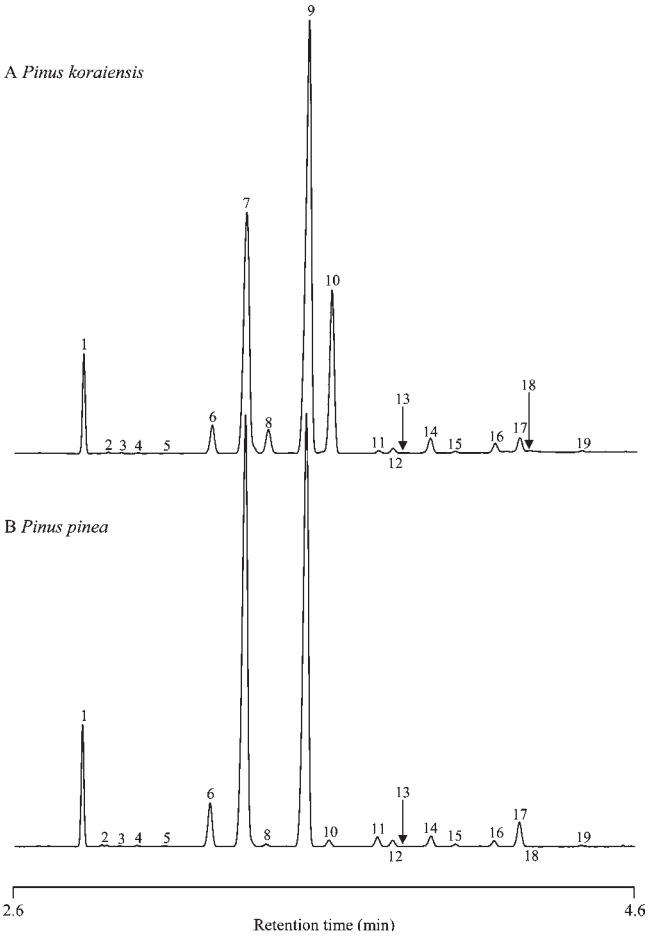

Figure 1. Typical chromatograms of fatty acid methyl esters prepared from (**A**) *Pinus koraiensis* and (**B**) *Pinus pinea* obtained by fast gas−liquid chromatography. Identification of peaks: (1) 16:0; (2) 16:1 n-7 and n-9, (3) aiso-17:0, (4) 17:0, (5) 17:1, (6) 18:0, (7) 9-18:1 and 11-18:2, (8) 5,9-18:2, (9) 9,12-18:2, (10) 5,9,12-18:3, (11) 9,12,15-18:3, (12) 20:0, (13) 5,9,12,15-18:4, (14) 20:1 n-9, (15) 5,11-20:2, (16) 11,14-20:2, (17) 5,11,14-20:3, (18) 7,11,14-20:3 and (19) 22:0.

Analysis of Commercial Pine Nuts. Analyses of the commercial samples gave very consistent results compared to the literature; the analysis of authentic *Pinus pinea* (sample F) seeds helps for peak identification based on previous reports (*7*). As a first observation, the analyses confirmed that all the pine nuts analyzed have been collected from conifers since Δ5-olefinic acids were detected in all samples. Obvious similarities among the analyzed products such as between products A and D, B and H or F, G and J were easy to notice (**Table 3**).

Table 3. Fatty Acid Profile of Commercial Pine Nuts and a Sample Collected from *Pinus pinea* (sample F) Determined by Fast Gas−Liquid Chromatography. Results of Duplicate Analysis[a]

	% of total fatty acids																			
	A		B		C		D		E		F		G		H		I		J	
fatty acid	MV	SD	MV	SD	MV	SD	MV	SD	MV	SD	MV	SD	MV	SD	MV	SD	MV	SD	MV	SD
16:0	5.00	0.13	4.62	0.08	4.60	0.38	4.81	0.02	4.70	0.09	6.24	0.17	5.99	0.21	4.63	0.07	5.43	0.37	5.99	0.01
16:1 n-9	0.02	0.00	0.05	0.00	0.05	0.00	0.02	0.01	0.03	0.01	0.09	0.00	0.08	0.00	0.06	0.01	0.04	0.00	0.08	0.00
16:1 n-7	0.07	0.01	0.08	0.02	0.07	0.03	0.07	0.00	0.08	0.01	0.09	0.01	0.10	0.01	0.09	0.01	0.06	0.02	0.11	0.00
aiso-17:0	0.07	0.03	0.02	0.01	0.08	0.05	0.06	0.01	0.05	0.00	0.04	0.01	0.03	0.00	0.05	0.01	0.07	0.03	0.05	0.00
17:0	0.05	0.00	0.05	0.00	0.05	0.00	0.06	0.00	0.05	0.00	0.08	0.00	0.08	0.00	0.06	0.00	0.06	0.01	0.09	0.01
17:1	0.03	0.00	0.05	0.00	0.03	0.01	0.03	0.00	0.03	0.00	0.04	0.00	0.04	0.00	0.03	0.00	0.05	0.01	0.05	0.00
18:0	2.27	0.06	2.07	0.04	1.80	0.01	2.28	0.01	2.13	0.12	3.57	0.10	3.96	0.01	2.06	0.08	2.07	0.01	3.94	0.03
18:1 n-9 + n-7	26.26	0.03	23.45	0.07	20.73	0.02	25.78	0.04	25.61	0.08	40.99	1.06	39.57	0.09	23.00	0.09	38.03	0.50	39.01	0.05
5,9-18:2	2.27	0.05	3.78	0.09	2.51	0.07	2.31	0.00	2.74	0.14	0.20	0.02	0.13	0.00	3.75	0.02	0.23	0.01	0.12	0.00
18:2 n-6	44.96	0.03	46.03	0.12	47.44	0.06	45.35	0.06	45.41	0.11	42.82	0.57	44.61	0.20	46.30	0.27	50.99	1.16	45.20	0.02
5,9,12-18:3	14.50	0.32	15.45	0.08	17.57	0.58	14.70	0.04	14.98	0.31	0.52	0.00	0.33	0.00	15.46	0.02	0.38	0.02	0.32	0.01
18:3 n-3	0.18	0.03	0.25	0.01	0.30	0.06	0.19	0.01	0.20	0.03	0.81	0.02	0.68	0.00	0.38	0.13	0.38	0.08	0.66	0.02
20:0	0.39	0.01	0.41	0.02	0.35	0.01	0.39	0.01	0.38	0.01	0.51	0.01	0.62	0.01	0.40	0.03	0.50	0.02	0.63	0.01
5,9,12,15-18:4	0.01	0.01	0.03	0.00	0.05	0.00	0.02	0.00	0.03	0.00	0.00	0.00	0.00	0.00	0.03	0.00	0.00	0.00	0.00	0.00
20:1 n-9	1.33	0.02	0.98	0.00	1.11	0.02	1.31	0.01	1.18	0.00	0.96	0.02	0.85	0.02	1.00	0.01	0.76	0.03	0.86	0.01
5,11-20:2	0.15	0.00	0.20	0.01	0.21	0.00	0.15	0.00	0.15	0.01	0.18	0.00	0.14	0.01	0.21	0.01	0.07	0.00	0.16	0.01
20:2 n-6	0.69	0.03	0.59	0.01	0.70	0.01	0.68	0.00	0.61	0.02	0.48	0.02	0.57	0.02	0.61	0.00	0.25	0.00	0.54	0.04
5,11,14-20:3	1.18	0.02	1.33	0.02	1.78	0.01	1.27	0.01	1.17	0.08	2.06	0.05	1.74	0.10	1.40	0.01	0.24	0.02	1.88	0.00
7,11,14-20:3	0.10	0.00	0.10	0.01	0.14	0.00	0.10	0.00	0.09	0.01	0.00	0.00	0.00	0.00	0.11	0.02	0.00	0.00	0.00	0.00
22:0	0.12	0.01	0.14	0.01	0.13	0.01	0.12	0.00	0.12	0.00	0.12	0.00	0.14	0.00	0.14	0.00	0.15	0.01	0.14	0.00
other FA	0.35	0.01	0.32	0.03	0.31	0.02	0.30	0.01	0.26	0.01	0.20	0.09	0.34	0.01	0.23	0.16	0.20	0.08	0.17	0.00

[a] Seeds collected in a botanic garden. Preparation of dry fruits and seeds for salads, pine nuts were separated from other materials before analysis.

The fatty acid profile of products A and D is typical of *P. koraiensis* seeds, a pine commonly found in Asia and globally consumed (*1*). The fatty acid profile of *P. koraiensis* seeds has been reported several times in the literature, and averaged values calculated from the data compiled in the review by Wolff et al. (*7*) are reported in **Table 2**. The main characteristics of the composition of *P. koraiensis* seeds fatty acid profile are the levels of taxoleic acid (1.9 to 2.3%), oleic + *cis*-vaccenic (25 to 29%), pinolenic (0.7 to 1.3%) and linoleic acids (about 45% of the total fatty acids, **Tables 2** and **3**).

Seeds from *P. pinea* (Italian stone pine) collected in a botanic garden (sample F) were analyzed, and results were in agreement with literature data (**Table 2**). The main Δ5-olefinic acid in *P. pinea* is sciadonic acid while the other *Pinus* spp. commonly found in commercial products contain mainly taxoleic and pinolenic acids (**Tables 2** and **3**). Based on the fatty acid composition found for samples G and J (**Table 3**), it was easy to conclude that the seeds in these products were collected from *P. pinea*. The very low level of taxoleic and pinolenic acids in *P. pinea* is a very distinct feature of this species and is shared with *P. gerardiana* (**Table 2**). However, *P. gerardiana* seeds do not contain sciadonic acid (**Table 2**) as observed for sample I (**Table 3**) that was imported from Pakistan (**Table 4**) where this pine is commonly found and cultivated.

In the report from the French Food Safety Authorities (AFSAA), it was mentioned that some products contain pine nuts from *P. armandii* (*6*). The analyses revealed that two of the samples (B and H) had fatty acid composition very close to the fatty acid composition of *P. armandii* (**Table 2** and **3**). The distinct feature of *P. armandii* seed fatty acid composition is the lower level of octadecenoic acids and the higher level of taxoleic acid compared to other *Pinus* spp. such as *P. koraiensis* (*7*).

Determination of a Diagnostic Index. In order to assign the botanical origin, a diagnostic index based on the level of the main Δ5-olefinic acids and their respective metabolic precursors was used (**Figure 2**). The formula of the diagnostic index (DI) used was the following:

$$\mathrm{DI} = [(5,9\text{-}18:2 + 5,9,12\text{-}18:3 + 5,11,14\text{-}20:3)/(18:1\ \text{n-9 and n-7} + 18{:}2\ \text{n-6} + 20:2\ \text{n-6})] \times 10$$

The use of a single index might simplify the identification of pine nut origin. The index proposed takes into account the biosynthetic pathway of the formation of Δ5-olefinic acids (*13*). Evaluation of the values obtained for the main "edible" species revealed that each *Pinus* sp. tested had virtually different diagnostic index values (see **Figure 2**). Calculation of the diagnostic index value for samples A, B, D, F, G, H, I and J were in accordance with reference value obtained from literature data (**Table 4**).

The use of the proposed diagnostic index value was very helpful in the case of sample E. Indeed, the seed fatty acid composition of sample E (**Table 3**) was not in agreement with any of the referenced *Pinus* spp. (**Table 2**). Further visual examination of the sample E revealed the putative occurrence of different types of seeds. Two independent skilled technicians were asked to separate pools of morphological identical seeds from this sample, and the fatty acid profiles were determined. The seed fatty acid composition of the different pools revealed the occurrence of two types of *Pinus* spp. in the sample having diagnostic index values of 2.40 ± 0.013 and 2.91 ± 0.027. The diagnostic index value of the sample E was 2.64 ± 0.016 (**Figure 2**), which is intermediate between the diagnostic values of *P. koraiensis* (2.38) and *P. armandii* (2.92). It was therefore possible to conclude that sample E consisted of a mixture of seeds from *P. koraiensis* (53%) and *P. armandii* (47%).

The case of sample C was the most challenging since the seed fatty acid composition and diagnostic index value (3.17 ± 0.094) were close to those of both *P. sibirica* (3.03) and *P. massoniana* (3.55). The example shows the limitation of the principle of the determination of the botanical origin based on the seed fatty acid profile. However, the level of octadecenoic acids (18:1 n-9 and n-7, 20.73 ± 0.02 of the total fatty acid) found in the sample was

Table 4. Information and Identification of the Botanical Origins of 9 Different Commercial Products and One Known Species Collected in a Botanic Garden Based on Diagnostic Index (DI)[a] Value

sample	purchased country	mention on pack regarding the origin of the pine nuts	botanical identity determined from fatty acid profile	DI value
A	France	imported product	*P. koraiensis*	2.50 ± 0.059 (reference value 2.38)
B	France	China	*P. armandii*	2.93 ± 0.026 (reference value 2.92)
C	France	China	*P. massoniana*	3.17 ± 0.094 (reference value 3.55)[b]
D	France	imported product	*P. koraiensis*	2.55 ± 0.007 (reference value 2.38)
E	France	China	Mixture of *P. koraiensis* (53%) and *P. armandii* (47%)	2.40 ± 0.013 for *P. koraiensis* (reference value 2.38) 2.91 ± 0.027 for *P. armandii* (reference value 2.92)
F	France	not applicable[c]	*P. pinea*	0.33 ± 0.005 (reference value 0.34)
G	Switzerland	none	*P. pinea*	0.26 ± 0.010 (reference value 0.34)
H	France	imported product	*P. armandii*	2.95 ± 0.001 (reference value 2.92)
I	France	Pakistan	*P. gerardiana*	0.10 ± 0.007 (reference value 0.17)
J	Switzerland	Italy	*P. pinea*	0.27 ± 0.001 (reference value 0.34)

[a] DI = [(5,9-18:2 + 5,9,12-18:3 + 5,11,14-20:3)/(18:1 n-9&n-7 + 18:2 n-6 + 20:2 n-6)] × 10. [b] The calculated index value is also close to the reference for *P. sibirica*, but the level of octadecenoic acids (18:1 n-9 and n-7) found in the sample is closer to the level usually found in *P. massoniana*. [c] Seeds collected in a botanic garden.

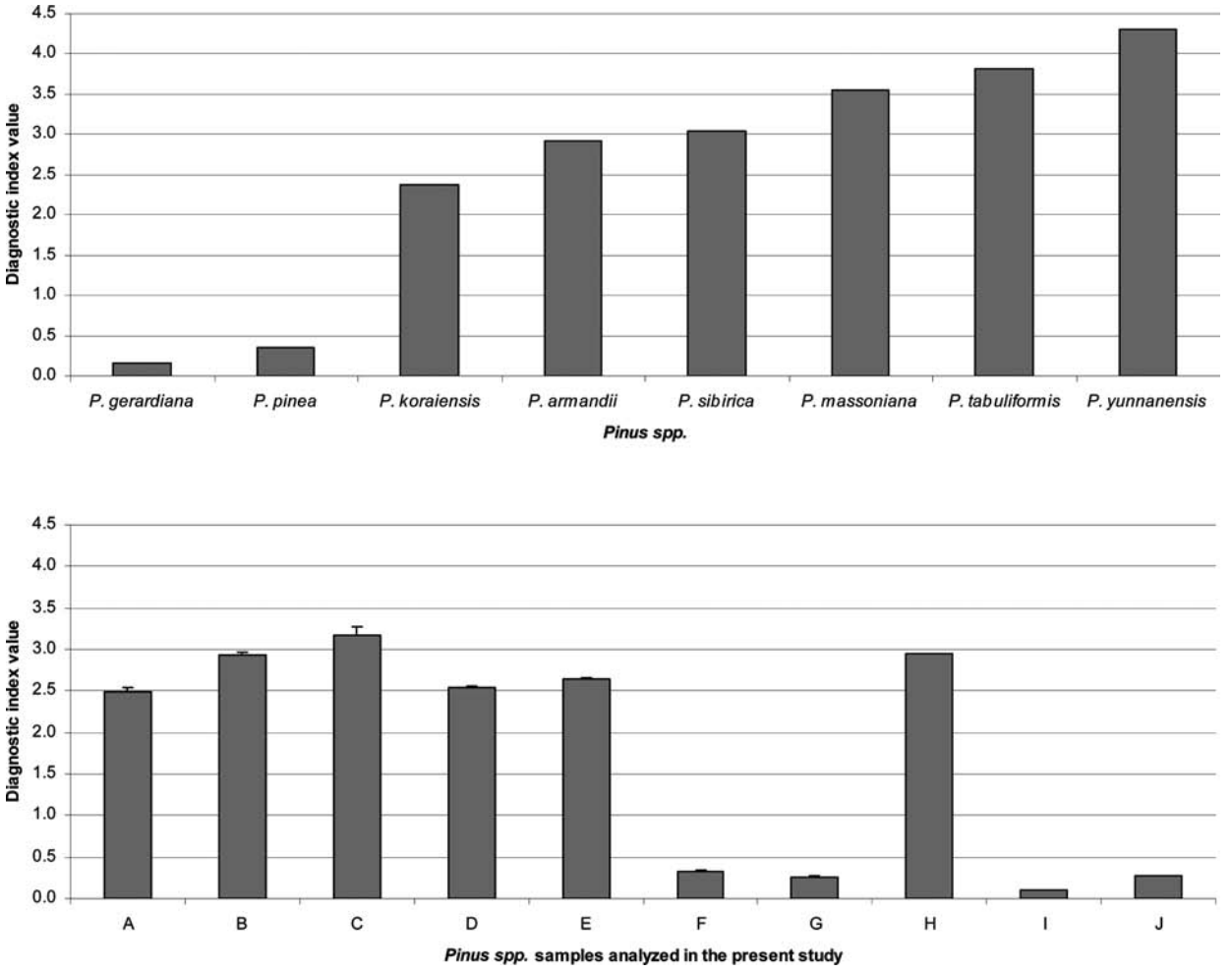

Figure 2. Diagnostic index values calculated as [(5,9-18:2 + 5,9,12-18:3 + 5,11,14-20:3)/(9-18:1 and 11-18:1 + 18:2 n-6 + 20:2 n-6)] × 10 of the *Pinus* spp. most frequently found in food products (top panel (**A**)) and samples analyzed in the present study (bottom panel (**B**)).

closer to the level reported for *P. massoniana* in the literature (16.85% of total fatty acids). In addition, the geographical origin of the product was clearly indicated on-pack (China, **Table 3**) and *P. sibirica* is mainly cultivated in Russia.

***Pinus* spp. Authenticity and History of Consumption in Western Countries.** The analysis of the products analyzed in the present study confirmed the occurrence of *Pinus* spp. usually not reported as edible source of pine nuts according to FAO (**Table 1**) (*1*). This is particularly the case for samples B, C, E and H in which seeds from *P. armandii* and *P. massoniana* were found (**Table 4**). Of particular interest is the case of sample E that was kindly provided by a consumer who experienced taste disturbance for about 2 weeks. The analysis of this sample and the use of the proposed DI allowed the determination that this product consisted of a nearly equal mixture of *P. koraiensis* and *P. armandii* (**Table 3**). This species also known as Chinese white pine is used extensively in China for wood and pine nut production. However, in Europe and North America *P. armandii* is only used as an ornamental tree in parks and gardens. It is also the case for *P. massoniana* (Chinese red pine), that is exclusively found in China and used by the paper

industry. There is therefore no history of use of the seeds from *P. armandii* and *P. massoniana* for food consumption in Western countries.

A recent report from the French food safety agency (*6*) confirmed that no traces of residual contaminants know to be associated with bitter or metallic perception were found in commercial products. Fatty acid compositions of Chinese red and white pine seeds are not substantially different from the composition of the edible species such as *P. koraiensis* or *P. sibirica* (**Table 2**). The lipid composition of these seeds cannot be therefore the origin of the taste disturbance linked to their consumption and reported by consumers. However, it has been suggested that the oxidation level in some pine nut samples was elevated and therefore that some oxidation products might be responsible for the taste disturbance issue (*4*). This hypothesis is interesting but speculative since bitter or metallic taste that can last for up to 2 weeks is not usually experienced with lipid oxidation products. One can hypothesize that the compound(s) responsible for the taste disturbance is found specifically or in higher concentration in Chinese red and white pine nuts compared to other *Pinus* spp. seeds. This compound can be present naturally in the seeds but as well produced after harvesting or during the process of seed shell removal, drying, preservation or by contact with some packaging materials. A thorough identification of the chemical identity of compounds present in seeds from Chinese pines and in particular from *P. armandii* and not found in more common species such as *P. koraiensis* may help to identify the candidate molecule. However, as a first step, the analytical procedure presented in this study may help to control the botanical origin of pine nuts imported from Asia.

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