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Nuclear Magnetic Resonance Spectroscopy and Chemometrics to Identify Pine Nuts That Cause Taste Disturbance

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Supporting Information

ABSTRACT: Nontargeted 400 MHz ^{13}C and ^{1}H nuclear magnetic resonance (NMR) spectroscopy was used in the context of food surveillance to reveal *Pinus* species whose nuts cause taste disturbance following their consumption, the so-called pine nut syndrome (PNS). Using principal component analysis, three groups of pine nuts were distinguished. PNS-causing products were found in only one of the groups, which however also included some normal products. Sensory analysis was still required to confirm PNS, but NMR allowed the sorting of 53% of 57 samples, which belong to the two groups not containing PNS species. Furthermore, soft independent modeling of class analogy was able to classify the samples between the three groups. NMR spectroscopy was judged as suitable for the screening of pine nuts for PNS. This process may be advantageous as a means of importation control that will allow the identification of samples suitable for direct clearance and those that require further sensory analysis.

KEYWORDS: NMR spectroscopy, *Pinus*, pine nuts, pine mouth, pine nut syndrome, PCA, SIMCA

INTRODUCTION

Pine nuts are the edible seeds found inside the cones of pine trees (*Pinus* spp.).[1] These nuts are universally popular and are used as a snack food or as an ingredient in the manufacture of a variety of food products. The chemical composition of pine seeds varies with the species as well as with geographical and climatic factors.[2,3] Depending on the species, pine nuts contain 10−34% protein and 61% lipid.[4] Linoleic and oleic acids are the predominant fatty acids,[4−6] and the nuts contain only 10% saturated fatty acids as determined by gas−liquid chromatography.[5]

Since 2001,[7] adverse effects of pine nut consumption have been reported that differ from food allergy symptoms[8,9] and the number of complaints has been increasing in recent years.[1,10−12] The effects were characterized as a bitter, metallic taste disturbance, developing 1−3 days after consumption and lasting for days or weeks. Cases typically resolved without treatment and without lasting effects.[13] Some publications have referred to this phenomenon as "pine mouth"[13] or pine nut syndrome (PNS).[12] An association between pine nut ingestion and cacogeusia may exist, but little is known about this condition and a mechanism or specific cause has yet to be identified.[1,13] This phenomenon might be caused by spoilage or rancidity,[11] or it may be unique to nuts imported from China due to harvesting of nontraditional (and probably inedible) species (e.g., *Pinus armandii* and *P. massoniana*).[1,3,7]

Destaillats et al.[1] evaluated the fatty acid profiles of pine species from different countries and proposed an analytical procedure to control the botanic origin of pine nuts imported from Asia. Seeds from Chinese pines have very specific fatty acid profiles. However, evaluation of the total fatty acid composition of Chinese pine nuts revealed no substantial differences from that of species from other countries. Therefore, the authors concluded that the lipid composition of these seeds was not the origin of the taste disturbance linked to their consumption.

Another chromatographic examination of suspect pine nuts revealed novel triglycerides, potentially created by oxidative stress, as a possible cause.[7] The authors suggested that the lipids might undergo some oxidative process that was responsible for the pine mouth syndrome, but the duration of the metallic taste is usually too long to be explained by oxidative reactions.[1] The presence of phenolic acid compounds, which has been associated with astringency and discoloration, is another potential cause.[14] No fungal, heavy metal, or pesticide contamination has yet been revealed for suspect pine nuts.[7] A negligible effect of irradiation was observed on the physical−chemical properties of pine nuts.[15] The effects of toxins or other contaminants have not yet been evaluated.[13]

Möller[12] suggested that PNS may be a result of increased bile production in response to some bioactive compounds in pine nuts. The effect might be compounded by enterohepatic recirculation, which dramatically extends the residence time of many

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Table 1. Sample Description, Diagnostic Index (DI), and Botanical Species Assignment of the Pine Nuts under Study

sample number	origin according to labeling information	sensory evaluation	DI[a]	botanical species according to DI
1		normal	3.00	*P. armandii*
2	China	normal	2.97	*P. armandii*
3	Pakistan	normal	0.10	*P. gerardiana*
4	Turkey	normal	0.24	*P. pinea*
5	China	PNS	3.01	*P. armandii*
6		normal	0.10	*P. gerardiana*
7	China	normal	3.42	*P. massoniana*
8		normal	0.11	*P. gerardiana*
9	Mediterranean	normal	0.36	*P. pinea*
10	China	PNS	3.00	*P. armandii*
11	China	PNS	2.80	*P. armandii*
12		normal	0.10	*P. gerardiana*
13	Pakistan	normal	0.11	*P. gerardiana*
14	Pakistan	normal	0.10	*P. gerardiana*
15		normal	0.10	*P. gerardiana*
16	China	PNS	3.09	*P. armandii*
17	Spain	normal	0.30	*P. pinea*
18		normal	0.24	*P. pinea*
19		normal	0.29	*P. pinea*
20		PNS	3.01	*P. armandii*
21	China	PNS	2.94	*P. armandii*
22	China	PNS	3.12	*P. armandii*
23	China	PNS	3.13	*P. armandii*
24	China	normal	3.13	*P. armandii*
25	China	normal	3.38	*P. massoniana*
26		normal	0.24	*P. pinea*
27	Italy	normal	0.25	*P. pinea*
28	China	normal	2.83	*P. armandii*
29		normal	0.12	*P. gerardiana*
30		normal	3.47	*P. massoniana*
31	China	normal	3.04	*P. armandii*
32	Italy	normal	0.25	*P. pinea*
33		normal	0.10	*P. gerardiana*
34		normal	0.09	*P. gerardiana*
35		normal	0.09	*P. gerardiana*
36		normal	0.10	*P. gerardiana*
37		normal	0.10	*P. gerardiana*
38		normal	0.11	*P. gerardiana*
39		normal	0.10	*P. gerardiana*
40	China	normal	2.42	*P. koraiensis*
41		normal	3.16	*P. massoniana*
42		PNS	3.08	*P. armandii*
43		normal	3.42	*P. massoniana*
44		normal	0.26	*P. pinea*
45		PNS	3.00	*P. armandii*
46		PNS	2.97	*P. armandii*
47		PNS	3.08	*P. armandii*
48		normal	0.12	*P. gerardiana*
49		normal	0.10	*P. gerardiana*
50		normal	[b]	
51	Mediterranean	normal	[b]	
52		PNS	[b]	
53	Mediterranean	normal	[b]	
54		normal	[b]	
55		normal	[b]	
56		normal	[b]	
57		normal	[b]	

[a] Diagnostic index (DI) calculated according to Detaillats et al.[1] [b] Not determined due to lack of sample material.

chemicals or drugs present in the digestive tract. In this context, linalool-oxide was suggested as a possible target compound.[10]

At this point in time, no single compound has yet been isolated that might be responsible for the taste disturbance and that might be found exclusively or in high concentrations in suspect samples. The substance could be a naturally occurring component of the seeds or could represent a contaminant or an intentional addition arising during harvesting or processing of the seeds (shell removal, drying, preservation, etc.) or during contact with packaging materials.[1] However, no candidate compounds have yet been identified. The aim of our group, which consists of several governmental food control laboratories in Germany and Belgium, was to develop a nontargeted NMR screening method that would allow for a faster selection of suspect pine nut samples than what is currently possible with sensory analysis.

MATERIALS AND METHODS

Samples, Taste Testing, and Botanical Species Determination. A total of 57 samples from different origins were analyzed using NMR. The samples were either submitted because of PNS effects detected by consumers, or were randomly selected from retail trade by governmental food inspectors in the German Federal State of Baden-Württemberg. Further samples were available from importation control at the Hamburg harbor and from the Belgian market. It must be noted that especially due to the inclusion of samples from consumer complaints, the samples are not representative of the markets in Germany or Belgium.

All samples were organoleptically tested for PNS by qualified assessors following ISO 6658:2005 guidelines in order to reach a consensus categorization of the pine nuts into PNS-causing or normal samples. The assessors were confirmed to be able to detect PNS by testing a positive sample. Because of the fast spoilage, the samples were not homogenized. Starting with the tasting of a small amount (5 nuts/tester), typically 10 g of raw material in total were finally tasted per tester if no effect was detected. At least two assessors confirmed each positive sample, and a waiting time of at least 48 h was kept between two negative tests. As a result of the bitter taste caused by the positive samples, the assessors were unable to taste for several days after detecting a positive sample. However, since no special training or sensory skills were required for the detection of the bitter taste, the group of possible panel members was extended to include volunteers from the whole staff of the affiliated institutes. If a divergent result arose between assessors, the panel size was increased to up to five testers to achieve the necessary degree of precision. According to ISO 6658:2005 guidelines, a consensus discussion was initiated if necessary. The taste panels were able to arrive at a group decision in all cases.

To assign the botanical species of the investigated pine nuts, the method of Destaillats et al. was used without modification, which is based on the analysis of fatty acids using gas chromatography (GC) with

a flame-ionization detector (FID).[1] Table 1 gives an overview about the origin, sensory characteristics, and botanical species of all samples.

Sample Preparation. Two grams of pine nuts was crushed and homogenized with 8 g of Na_2SO_4. The fat fraction was extracted overnight with 150 mL of petroleum ether (boiling point 30−50 °C) at room temperature without stirring. The organic phase was then filtered over a fluted filter, the solvent was evaporated at 40 °C and 580 mbar, and the sample was left standing overnight in a fume hood. A 200 mg portion of the fat fraction was dissolved in 0.8 mL of $CDCl_3$ containing 0.1% tetramethylsilane (TMS). A 0.6 mL volume of the mixture was poured into an NMR tube for direct measurement. One replicate was measured per sample.

^{13}C and ^{1}H NMR Measurements at 400 MHz. All NMR measurements were performed on a Bruker Avance 400 Ultrashield spectrometer (Bruker Biospin, Rheinstetten, Germany) equipped with a 5-mm SEI probe with Z-gradient coils, using Bruker Automatic Sample Changer (B-ACS 120). All spectra were acquired at 300.0 K. ^{1}H NMR spectra were acquired using a Bruker standard 1D zg30 pulse sequence with 128 scans and two prior dummy scans. The sweep width was 20.5 ppm, and the time domain of the FID was 132 kB. ^{13}C NMR spectra were acquired using a Bruker zgpg30 pulse sequence with 1024 scans and 4 prior dummy scans. The sweep width was 238.9 ppm, and the time domain of the FID was 132 kB.

The data were acquired automatically under the control of ICON-NMR (Bruker Biospin, Rheinstetten, Germany), requiring about 25 min per sample. All NMR spectra were phased and baseline-corrected.

Chemometrics. The resulting spectra were analyzed using the software Unscrambler X version 10.0.1 (Camo Software AS, Oslo, Norway). The multivariate evaluation included principal component analysis (PCA) and soft independent modeling of class analogy (SIMCA). We examined the 6.2−0.25 (^{1}H NMR) and 200−0.25 (^{13}C NMR) ppm spectral regions with 0.01 ppm bucket width. For details into the nontargeted multivariate data analysis of NMR spectra see refs 16 and 17.

SIMCA was applied for the classification of pine nuts according to their origin. SIMCA is based on building a PCA model for each class in a defined training set. The test-set samples are then compared to the class models and assigned to classes according to their proximity to the training samples.[18] SIMCA is known as a supervised pattern recognition method as the individual PCA models define classification rules. Full cross-validation was applied to determine the optimal number of principal components (PCs) required to obtain robust models. Three (^{1}H NMR) and seven (^{13}C NMR) PCs were found sufficient for an accurate SIMCA classification of pine nuts. The prediction ability of the SIMCA classification models was tested on 12 randomly chosen test-set samples that were not included in the models.

RESULTS AND DISCUSSION

Univariate Analysis. The composition of pine nuts and pine nut oil is typically established by some type of chromatography.[2,3,14,19−21] However, chromatographic techniques require extensive sample preparation and the availability of known standards for comparison. Previous studies in food analysis have shown that information regarding food composition and origin can often be obtained more simply and rapidly from NMR spectra.[16,17,22,23] For example, Skakovskii et al. [22] analyzed pine-nut oil (*Pinus sibirica* Du Tour) and oil isolated from seeds of common pine (*P. silvestris L.*) by GC and NMR (^{1}H and ^{13}C) spectroscopy. Identification and quantification of nine fatty acids, including a number not identified by GC, were possible by ^{13}C NMR spectroscopy. However, determination of several compounds was somewhat complicated due to extensive spectral overlapping. The authors did not use multivariate methods nor did they analyze pine nuts known to cause taste disturbances.

The Supporting Information (Figures 1 and 2) shows the entire ^{1}H and ^{13}C NMR spectra as well as detailed magnifications in several spectral regions where the differences between pine nut samples can be observed.

In the ^{1}H NMR spectra, all Chinese species (*P. armandii*, *P. massoniana* and *P. koraiensis*) including the PNS samples, showed signals in the 2.35−2.25, 1.75−1.65, and 2.12−1.96 ppm regions, where samples from other origins (*P. gerardiana, P. pinea*, all non-PNS samples) typically do not have signals. The first two signals are characteristic of $\alpha-$ and $\beta-CH_2$ groups next to carbonyl functional groups, and the third signal region can be attributed to the presence of CH_2 groups next to double bonds.

The ^{13}C NMR spectra were similar to those obtained in ref 22. Differences between the two groups of pine nuts were present in the regions of 173−172, 131−127, 63−62, 34−33, and 27−26 ppm. In the ^{13}C NMR spectra, signals at δC 173.0 revealed the presence of a carbonyl group. Signals at δC 131−127 derive from the presence of double bonds in fatty acids, and the signal at δC 62.5 is due to the glycerol carbons. Signals at δC 33.4 and 26.5 revealed methylene groups. However, no single chemical shift was noted in either ^{13}C or ^{1}H NMR spectra that would allow for the differentiation of PNS pine nuts from normal pine nuts. Our univariate study was therefore unsuccessful at identifying the actual cause of PNS.

Nontargeted Multivariate Analysis. Recent interest in NMR spectroscopy has focused on the possibility of classifying food products without the use of a priori data, through supervised pattern recognition techniques such as principal component analysis (PCA).[16,17,23,24] In general, PCA provides a visual representation of the relationships between samples and variables and uncovers how certain variables cause some samples to be similar to others or reveals how they differ from each other.

Using nontargeted analysis of the full spectral information, the PCA scores (Figure 1) showed that the variance between the spectra of pine nuts is described by the models built from ^{1}H and ^{13}C spectra. The best models explained 93% (^{13}C NMR) and 94% (^{1}H NMR) of the total variance of the data. The Figure shows that the samples can be separated according to their botanical identity (estimated using DI values) into three (^{1}H NMR) or two (^{13}C NMR) groups. Figure 1a shows that *P. armandii*, *P. koraiensis*, and *P. massoniana* botanical species cultivated in Asian countries (China, Japan, and Korea) are in the region of negative PC1, whereas two separate clusters of *P. pinea* (harvested in Mediterranean Europe and Near East) and *P. gerardiana* (common in Afghanistan, Pakistan and India) are in the positive values of PC1. The ^{13}C NMR scatter plot (Figure 1b) revealed one group with *P. armandii*, *P. koraiensis*, and *P. massoniana* species, whereas *P. pinea* and *P. gerardiana* samples are located in the second group.

Next, it is interesting to trace the location of PNS samples on both scatter plots. Figure 1 shows that the PNS pine nuts (belonging to *P. armandii*) are localized in the region of negative values of PC1 for both ^{13}C and ^{1}H data. However, the group also contains as many normal samples as PNS-causing samples (from 27 samples in this group, 14 were normal, and 13 caused PNS). However, only normal samples are situated in the positive value regions of the PC1 of both ^{1}H and ^{13}C NMR (30 samples from 57 samples analyzed in total).

These data therefore suggest that this separation is not primarily caused by an unknown substance or several substances

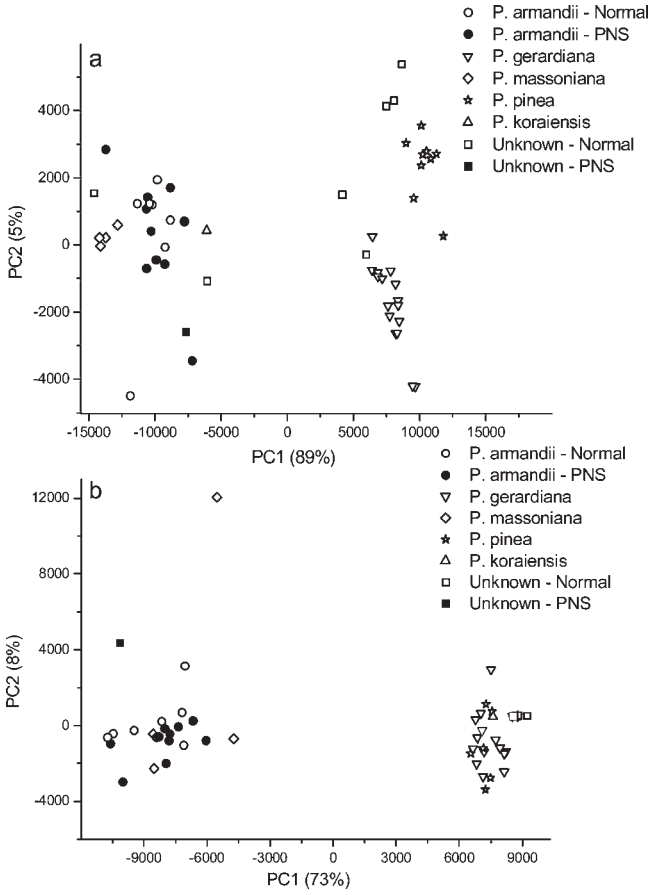

Figure 1. Scatter plot of the PCA scores from ^{1}H (a) and ^{13}C (b) NMR spectra.

that might cause PNS. It derives from the botanical origin of the pine nuts and, similar to our univariate findings and GLC studies,[1] is unable to allow for the definition of a causative compound. The suspect group of samples contains nonedible species (*P. armandii*, *P. massoniana*) from China, whereas edible samples from India, the Mediterranean, Near and Middle Eastern countries (*P. pinea* and *P. gerardiana*) are localized within the other two groups on the ^{1}H NMR PCA score plot. In the ^{13}C NMR scores, the *P. pinea* and *P. gerardiana* samples did not separate. In both cases, all samples that caused taste disturbance were contained in the group containing the samples from China. Our results suggest that PC1 explains the variation between species most commonly harvested in China and *P. pinea*/*P. gerardiana* groups. Furthermore, PC2 carried out from ^{1}H NMR spectra allows for the differentiation between *P. pinea* and *P. gerardiana* species.

The X-loadings of PC1 suggest that the variables around 33.40 (^{13}C NMR) and 1.25 (^{1}H NMR) ppm have an extreme position and that these chemical shifts are the most important for the discrimination of botanical identities of pine nuts (Supporting Figure 3). According to our univariate analysis, these chemical shifts are characteristic of the methylene groups of fatty acids. This also validates the GC findings[1] that the botanical species of pine nuts differ in fatty acid profiles.

In general, ^{1}H NMR alone is judged to be sufficient to conduct a discrimination of pine nuts. As the PNS-causing species were only found in the sample group with negative PC1, we currently propose that samples belonging to the other two groups can be treated as not suspect. In the case of importation controls, these samples could be given direct clearance, whereas samples belonging to the first group (with Chinese species) would require organoleptic testing.

The applicability of the method was also validated by classifying new samples using the SIMCA methodology. With this process, an unknown sample will be recognized as a member of a class if it is similar enough to the other members; otherwise, it will be rejected. The calibration set for SIMCA in the present study consisted of 45 pine samples; the independent test set consisted of 12 randomly selected objects (4 *P. armandii* (2 with PNS, 2 normal) and 1 *P. massoniana*, 5 *P. gerardiana*, and 2 *P. pinea*). First, PCA was performed on each of the three categories (the first group contained the nonedible Chinese species, and the other two groups contained *P. massoniana* and *P. pinea* species). PCA models with minimum prediction error and optimum number of PCs were used to classify the samples from the test set. A 100% correct classification (no false positive and no false negative results) of the test set samples at the 5% significance level was achieved by the SIMCA method.

In conclusion, our study is the largest evaluation of pine nut samples by NMR carried out so far. However, similar to the studies mentioned in the Introduction, we also were unable to identify the actual cause of PNS. We agree with Destaillats et al. [1] that the compound responsible is probably not contained in the lipid fraction, and we will proceed with investigations of the nonlipid fractions in the future. For now, our method is still applicable for food testing and would be especially valuable as a form of food importation control. The PNS species were only found in the sample group with a Chinese origin (*P. armandii*, *P. koraiensis*, and *P. massoniana* species). A larger set of authentic samples of known origin and species is clearly required to confirm the causative factor for the clustering observed in our samples.

Our SIMCA models allow for the exclusion of about half of the samples (in our case, 30 of the 57 samples (53%)) from further and time-consuming organoleptic testing. Our method is a good alternative to macroscopic analysis, for which a detailed methodology has not been published or validated so far, or to time-consuming GC analysis.[1] While macroscopic evaluation might be faster and simpler, an instrumental method to confirm such observations is often preferred by the control institutions, especially in light of liability issues if batches are taken from the market. As a screening method for importation control, this approach is extremely advantageous as about half of the samples received can be given immediate clearance after undergoing the NMR test. Only the other half has to be deferred from clearance while organoleptic analyses are ongoing. From a legal standpoint, samples that cause PNS can be removed from the market in the European Union on the basis of the general food safety requirement stipulated in regulation (EC) No. 178/2002, or they could be removed on the basis of the novel food regulation (EC) No. 258/97 if it can be proved that nontraditional species have been used.

■ ASSOCIATED CONTENT

Supporting Information. ^{1}H NMR spectra of pine nuts from different origins; detailed ^{13}C NMR spectra of pine nut samples; X-loadings plots of the PCs of ^{1}H NMR (a) and ^{13}C NMR (b) spectra. This material is available free of charge via the Internet at http://pubs.acs.org.

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REFERENCES

(1) Destaillats, F.; Cruz-Hernandez, C.; Giuffrida, F.; Dionisi, F. Identification of the botanical origin of pine nuts found in food products by gas-liquid chromatography analysis of fatty acid profile. *J. Agric. Food Chem.* **2010**, *58*, 2082–2087.

(2) Zadernowski, R.; Naczk, M.; Czaplicki, S. Chemical composition of *Pinus sibirica* nut oils. *Eur. J. Lipid Sci. Technol.* **2009**, *111*, 698–704.

(3) Destaillats, F.; Cruz-Hernandez, C.; Giuffrida, F.; Dionisi, F.; Mostin, M.; Verstegen, G. Identification of the botanical origin of commercial pine nuts responsible for dysgeusia by gas-liquid chromatography of fatty acid profile. *J. Toxicol.* Vol. **2011**, Article ID 316789. (doi: 10.1155/2011/316789).

(4) Miraliakbari, H.; Shahidi, F. Oxidative stability of tree nut oils. *J. Agric. Food Chem.* **2008**, *56*, 4751–4759.

(5) Wolff, R. L.; Marpeau, A. M. Delta 5-olefinic acids in the edible seeds of nut pines (*Pinus cembroides edulis*) from the United States. *J. Am. Oil Chem. Soc.* **1997**, *74*, 613–614.

(6) Ryan, E.; Galvin, K.; O'Connor, T. P.; Maguire, A. R.; O'Brien, N. M. Fatty acid profile, tocopherol, squalene and phytosterol content of brazil, pecan, pine, pistachio and cashew nuts. *Int. J. Food Sci. Nutr.* **2006**, *57*, 219–228.

(7) Mostin, M. Taste disturbances after pine nut ingestion. *Eur. J. Emerg. Med.* **2001**, *8*, 76.

(8) Hilper, U.; Spoo, J.; Elsner, P.; Bauer, A. Anaphylaxis after ingestion of pine nuts. *Allergologie* **2004**, *27*, 462–467.

(9) Roux, K. H.; Teuber, S. S.; Sathe, K. Tree nut allergens. *Int. Arch. Allergy Immunol.* **2003**, *131*, 234–244.

(10) Nesbitt, J. Pine mouth cases leave a bad taste. *The Irish Times*, Nov 2, **2010**, p 14.

(11) Weise, E. 'Pine mouth': hard to crack; rare syndrome linked to nuts. *USA Today*, Mar 16, **2010**, p 7D.

(12) Möller, G. The curious case of the epicurean nut (Online Exclusive). *Food Technol.* **2010**, *64* (5).

(13) Munk, M.-D. "Pine Mouth" syndrome: cacogeusia following ingestion of pine nuts (Genus: *Pinus*). An emerging problem? *J. Med. Toxicol.* **2010**, *6*, 158–159.

(14) Senter, S. D.; Horvat, R. J.; Forbus, W. R. Comparative Glc-Ms analysis of phenolic-acids of selected tree nuts. *J. Food Sci.* **1983**, *48*, 798–799.

(15) Golge, E.; Ova, G. The effects of food irradiation on quality of pine nut kernels. *Radiat. Phys. Chem.* **2008**, *77*, 365–369.

(16) Lachenmeier, D. W.; Frank, W.; Humpfer, E.; Schäfer, H.; Keller, S.; Mörtter, M.; Spraul, M. Quality control of beer using high-resolution nuclear magnetic resonance spectroscopy and multivariate analysis. *Eur. Food Res. Technol.* **2005**, *220*, 215–221.

(17) Lachenmeier, D. W.; Humpfer, E.; Fang, F.; Schütz, B.; Dvortsak, P.; Sproll, C.; Spraul, M. NMR-spectroscopy for nontargeted screening and simultaneous quantification of health-relevant compounds in foods: the example of melamine. *J. Agric. Food Chem.* **2009**, *57*, 7194–7199.

(18) Wold, S. Pattern-recognition by means of disjoint principal components models. *Pattern Recogn.* **1976**, *8*, 127–139.

(19) Evaristo, I.; Batista, D.; Correia, I.; Correia, P.; Costa, R. Chemical profiling of Portuguese *Pinus pinea* L. nuts. *J. Sci. Food Agric.* **2010**, *90*, 1041–1049.

(20) Lantto, T. A.; Dorman, H. J. D.; Shikov, A. N.; Pozharitskaya, O. N.; Makarov, V. G.; Tikhonov, V. P.; Hiltunen, R.; Raasmaja, A. Chemical composition, antioxidative activity and cell viability effects of a Siberian pine (*Pinus sibirica* Du Tour) extract. *Food Chem.* **2009**, *112*, 936–943.

(21) Venkatachalam, M.; Sathe, S. K. Chemical composition of selected edible nut seeds. *J. Agric. Food Chem.* **2006**, *54*, 4705–4714.

(22) Skakovskii, E.; Tychinskaya, L.; Gaidukevich, O.; Klyuev, A.; Kulakova, A.; Petlitskaya, N.; Rykov, S. V. NMR analysis of oils from pine nuts (*Pinus sibirica*) and seeds of common pine (*Pinus silvestris* L.). *J. Appl. Spectrosc.* **2007**, *74*, 584–588.

(23) Belton, P. S.; Colquhoun, I. J.; Kemsley, E. K.; Delgadillo, I.; Roma, P.; Dennis, M. J.; Sharman, M.; Holmes, E.; Nicholson, J. K.; Spraul, M. Application of chemometrics to the H-1 NMR spectra of apple juices: discrimination between apple varieties. *Food Chem.* **1998**, *61*, 207–213.

(24) Forveille, L.; Vercauteren, J.; Rutledge, D. N. Multivariate statistical analysis of two-dimensional NMR data to differentiate grapevine cultivars and clones. *Food Chem.* **1996**, *57*, 441–450.