Scientific paper

Cryotrap/SPME/GC/MS Method for Profiling of Monoterpenes in Cheese and Their Clustering According to Geographic Origin

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Received: 03-08-2012

Abstract

A variant of purge/cryotrap/thaw/static headspace Solid Phase Microextraction (SPME) was developed as a means for preconcentrating Volatile Organic Compounds (VOC) in cheese. An originally designed cryotrap partially filled with glass beads was employed that facilitated efficient flow-through of purging gas and trapping of the volatiles. In stopped-flow mode, thawing was allowed, and the same vessel was used for the exposure of the appropriate SPME fiber, effectively achieving double preconcentration. Gas chromatography/mass spectrometry (GC/MS) was subsequently employed to identify components and assess their relative chromatographic peak areas. Monoterpenes were chosen as a model group of substances, and their relative concentration profiles were evaluated as potential markers for the respective geographic origin. The procedure was tested on samples of five traditional Slovenian cheeses featuring Protected Designation of Origin (PDO): Tolminc, Mohant, Nanoški cheese, together with Bovški cheese and Karst Ewe's cheese. The dataset of the peak areas of nine prominent monoterpenes (α-pinene, camphene, α-phellandrene, β-pinene, 3-carene, 2-carene, limonene, tricyclene, and γ-terpinene) in cheese samples showed clustering that relates the cheeses to the area of production. According to the silhouette metrics, four clusters were identified by partitioning around medoids (PAM) method. The latter packed data for Tolminc and Bovški cheese into a single cluster, closely reflecting the vicinity of their geographic origin, but classified correctly the rest of the data into separate clusters for all other cheeses.

Keywords: Cheese, cryotrapping, solid phase microextraction, monoterpenes, clustering

1. Introduction

Several researchers have tried to identify a possibly tight relation between the volatile organic compounds (VOC) in cheese and its geographic origin.[1–3] Special attention was devoted to monoterpenes, since their presence in milk almost exclusively depends on pastures.[4,5] The highland pastures give rise to a higher abundance of monoterpenes in plants than do the lowland pastures.[6,7] On this basis, the milk and the cheese from different production sites (lowland *vs*. highland) and seasons (winter *vs*. summer) could be differentiated.[8] Based solely on monoterpene content, some judgment can therefore be made about the production conditions of the cheese, although some caution is warranted in drawing detailed conclusions, since a possible microbiological origin of monoterpenes in cheese has also been identified.[9,10]

Gas chromatography (GC) with various types of detectors is the most widely used method for determination of VOC in cheese. When hyphenated with mass spectrometry (MS), it represents a comprehensive tool capable of unambiguous identification and quantification of compounds, since it combines separation with a technique featuring both universal and specific detection. In contrast to this firmly established analytical back-end, which will remain in widespread use in the years to come, the front-end procedures (like extraction and preconcentration) are still the subjects of vigorous research. In this vein, the most efficient combination of techniques is sought for any conceivable type of sample. In VOC analysis, multiple–mostly complementary–choices coexist for preconcentration and enrichment. While covered under a rather general term

»sample preparation«, a plethora of techniques and their numerous variations were developed, including solvent extraction, supercritical fluid extraction, vacuum distillation, simultaneous distillation/extraction, dynamic headspace (Purge & Trap), thermal desorption, and solid phase microextraction (SPME) with static headspace sampling.[11,12]

Due to its simplicity and because it is virtually non-destructive in nature, thus preserving natural characteristics of the sample, the most frequently used approach is to analyze the composition of headspace gases over milk and dairy products.[13,14] Unfortunately, the analytes in this atmosphere are immensely diluted. Taking directly an aliquot of this gas mixture and injecting it into a GC/MS instrument may not produce sufficient signal intensities for all analytes of interest. That is, without a pre-concentration step, some compounds may be present below the detection limit of the method. Quantification of the volatiles present in the cheese samples is seriously burdened by their low concentrations and also by other problems of the dynamic headspace that were already comprehensively covered.[15] One possible path to a reliable quantification of the original concentration is in using a stable isotope labeled internal standard (Isotopic Dilution Analysis).[16]

On the other hand, SPME, as a sampling method, has a lot of advantages, since it is cheap, solvent free, and provides a convenient transfer route to a gas chromatograph, using a splitless universal injector without modification.[17–19] However, one troubling feature of SPME is that there is a competition among the gaseous species for the active binding sites, when adsorption is the predominant binding mechanism, and competition among different phases for analytes in the case of absorption.[20] At prolonged exposure, major components with lower affinities get displaced by those with higher affinities, but with lower partial pressure.[21–23] Consequently, the time of fiber exposure and the temperature till reaching equilibrium values are essential issues. An exact quantification of all analytes is therefore hurdled by their unspecified relative affinities to the SPME fiber coating. To quantify, methods employing isotopically labeled internal standards must be used, which regrettably ruin the spirit of the SPME methods that excel at low cost and simplicity.

In our study an attempt was made at establishing an appropriate method for obtaining VOC chromatographic profiles of different cheeses, which can later be related to their geographic origin. We developed and evaluated a purge/cryotrap/thaw/static headspace SPME technique followed by GC/MS analysis. Our final goal was to identify a candidate set of monoterpene compounds, and demonstrate that they can serve as chemical markers specific to Protected Designation of Origin (PDO) cheeses, so that by a suitable clustering method, the original products could be properly classified, in contrast to similar or counterfeit ones.

2. Experimental

2. 1. Sample Preparation

Five Slovenian PDO cheeses were used as an example to show the applicability of newly developed analytical as well as clustering techniques. Six samples of both Tolminc cheese (produced from raw cow's milk) and Bovški cheese (produced from raw ewe's milk), 13 samples of Karst Ewe's cheese (all three types of cheese were full-fat hard cheeses ripened between ten and twelve months), 14 samples of Nanoški cheese (full-fat hard cheese produced from thermized cow's milk), and two samples of Mohant cheese (full-fat soft cheese produced from raw cow's milk, ripened for two months in traditional wooden containers), were obtained from different local producers. Except for Mohant cheese, which was placed in glass beakers, all cheese samples were wrapped in aluminium foil, and stored at 4–6 °C. They were conditioned to ambient temperature for 10 minutes before analysis. The rinds of the hard cheese samples were discarded to a thickness of approximately 1 cm and the inner part of the cheese body was finely grated.

2. 2. Cryotrapping/SPME

Twenty grams of grated cheese were weighed and placed into a purging flask. The volatiles were purged at room temperature through a frit with helium flow of 50 mL/min and preconcentrated in a specially designed liquid nitrogen cooled cryotrap for one hour. The in-house made glass trap had a total volume of 60 mL, and was topped with a standard size rolled flange and a rubber crimp cap (Figure 1).

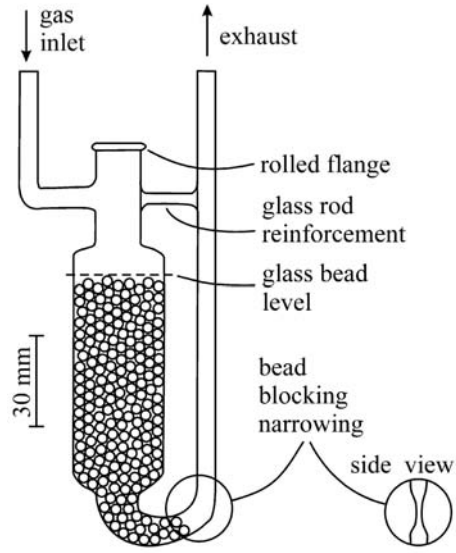

Figure 1. Cryotrap design. Note that glass beads, which filled the body up to ¾ of the trap's height, are not to scale.

The gas passed through the 6 mm outer diameter inlet pipe that was attached from the side to the 30 mm diameter body (partially filled with 4 mm diameter glass beads that consumed some 30 mL of free volume), immersed in liquid nitrogen. The volatiles froze while helium passed down through the interstices around the chilled packed beads. The stream proceeded to the bottom, and flowed through a parallel narrowing that blocked any possible escaping of the beads, but allowed free passage of the fluids. Passing through a 6 mm outlet pipe, so dimensioned for easy connection with external tubing, the gas flow entered the controlling flow meter and finally left the apparatus for an exhaust. At the end of the collecting stage, the helium flow was discontinued, the trap was disconnected from the external tubing, and both the inlet and the outlet tap were sealed with Parafilm® (Pechiney Plastic Packaging Company Inc., Chicago, IL, USA) to prevent contamination and losses. In the case of possible trap malfunction (excessive pressure development due to the presence of ample amount of volatiles, e.g. CO_2) this breakable diaphragm would also act as a safety valve. The trap was then taken out of the liquid nitrogen bath and allowed to thaw, upon which no visible liquid phase was observed. Therefore only gas phase losses were possible through the septum or Parafilm seal, which was deemed inconsequential, since we only determined relative values and not absolute concentrations. Additionally, surface-to-volume ratiois low enough that wall processes do not influence the bulk atmosphere in the pre-concentration trap. After equilibrating to ambient temperature, the SPME needle was inserted through the rubber cap into the headspace of the thawed cryotrap, where the fiber (polydimethylsiloxane (PDMS), 100 μm film thickness, Supelco, Bellefonte, PA, USA) was exposed to the vapors for 30 minutes. Since monoterpenes were already suggested in the literature as possible indicators of geographic origin and to assure high concentration factors, we have selected non-polar fiber with thick stationary phase. Aging effects were prevented by using the same fiber just for 50 injections. Following the exposure, the SPME device was then transferred to the injection port of GC where thermally desorbed compounds were analyzed for targeted substances by Ion Trap Mass Spectrometer in full scan mode.

All glassware, including the beads, was thoroughly cleaned between the runs. A detergent wash was followed by a rinse with distilled water, and then by methanol. Drying was accomplished in an oven at 200 °C. The vessel and the beads were then allowed to cool in an active silica gel desiccator, where they were stored until use.

2. 3. GC/MS Analysis

A Varian Star 3600 CX AirGC gas chromatograph equipped with RTX-5MS fused silica capillary column (60 m × 0.25 mm i.d. × 0.5 μm phase thickness by Restek Corporation, Bellefonte, PA, USA) and coupled to the Varian Saturn 2000 mass spectrometer was used for determination of volatiles in the samples. The injector was heated to 250 °C, while the mobile phase helium flow was set at 1 mL/min. After SPME needle injection, the ramp was set at 2 °C/min from 40–90 °C, continued by 20 °C/min from 90–250 °C, where the temperature was held for 12 minutes, until the program stopped. Mass spectra were recorded using electron impact ionization at 70 eV energy. Mass scans at 1 scan/s were performed in the full scan range of m/z = 40–400. With the baseline peak width of approximately 1 min featuring about 60 measuring points, the chromatographic peak areas were thus quite adequately integrated. For most of monoterpene hydrocarbons, the m/z = 93 fragment is a prominent peak in their mass spectrum,[24] hence Extracted Ion Chromatograms (EIC) at m/z = 93 were obtained by subsequent data processing using Varian STAR software.

Chromatographic peaks were identified by comparing corresponding mass spectra and retention times to those of standard compounds when available. Otherwise, a tentative identification was made by matching unknowns against the mass spectral data from the NIST 92 spectral library (National Institute of Standards and Technology, Gaithersburg, MD, USA) and manually picking the most plausible target, judged by a critical comparison of the unknown and the likely candidates. The two monoterpenes, that is α-phellandrene and 2-carene, for which we did not have any standards, were considered just tentatively identified.

2. 4. Data Pruning

Our raw data consisted of the EIC, which were integrated using Varian STAR software, and were initially entered as a unified data frame. The data for an individual run were normalized to the maximum peak within the sample group, which was mostly α-pinene (Bovški, Karst Ewe's, and Nanoški cheese), but also β-pinene (Tolminc) and limonene (Mohant). Before subsequent clustering, all lines (corresponding to chromatogram runs) in the initial data frame that contained a datum exceeding two standardized MAD (median absolute deviation from the median) within the same column, and pertained to the same type of cheese were discarded as outliers. Thus, eight samples of Nanoški and Karst Ewe's cheese, three of the Tolminc and Bovški cheese, and two Mohant cheese samples remained in our dataset. There was no regularity in eliminating the outliers that could be explained by seasonal changes or differences among the producers.

2. 5. Cluster Analysis

Cluster analysis was performed within the classic framework of Kaufman and Rouseeuw,[25] as implemented in the "Cluster" package of the R software, which can be obtained at any Comprehensive R Archive Network repository. The main objective was to partition a set of data into k clusters that contain objects with the highest simila-

rity, while objects belonging to different clusters are as dissimilar as possible. To this end, a dissimilarity matrix was computed using DAISY procedure with a data frame formed of rows representing samples and columns representing relative abundances of selected monoterpenes. Since log ratio metrics proved to be most useful in calculating the dissimilarity matrix, all missing data indicating relative abundances below the limit of detection were assigned an arbitrary value of 10^{-3} to avoid division by zero. Partitioning Around Medoids (PAM) was used as a classification procedure because of its higher resistance and robustness over the more classical *k*-means. In order to obtain *k* clusters using this procedure, the method selects *k* representative objects in the dataset. The clusters are then formed by assigning each remaining object to the nearest representative object. The best selection of the representative object is obtained when these objects are located in the center of the individual cluster, that is, when the average distance of the representative object to all other objects in the same cluster is minimized. Graphic representation of the clusters was performed using CLUSPLOT procedure where objects were plotted using the first two components obtained by Principal Component Analysis.

In a complementary approach, the same dissimilarity matrix was fed to DIANA procedure, implementing divisive clustering. That is a hierarchic technique that proceeds in inverse order.[25] At each step, a cluster is split into two smaller ones until finally all clusters contain only a single element. Thus, the hierarchy of the dataset with *n* objects is obtained in *n*-1 steps.

3. Results and Discussion

3. 1. Cryotrapping/SPME

VOC are present in cheese in fairly low concentrations of a few μg/kg.[26] Therefore, to improve the sensitivity of chemical analysis, a distinctive sampling procedure, which generally favors volatiles over semi-volatiles, needs to be used for preconcentration. Additionally, since the water content of the cheese is up to 50 %, most water must somehow be removed from the stream of volatiles in one of the well-documented ways,[27] if larger amounts of sample were to be used, and GC/MS were intended as a back-end instrument. The major drawback of all procedures probing the gas phase above cheese samples is partitioning of the VOC between the solid and the gas phase, which substantially hinders VOC quantification in cheese. One way to alleviate this problem in our case was to use an exhaustive purge that would transfer virtually all volatiles to another vessel, devoid of solid state matter that would reabsorb the volatiles. The cryotrap thus featured as a first preconcentration vessel that stored volatiles including water vapour. When the second preconcentrating step was performed by exposing the SPME fiber to the atmosphere of the cryotrap vessel equilibrated to the room temperature, the water was essentially removed from the analyte path, due to fiber's hydrophobicity. The absorption of VOC in PDMS coating of SPME fiber is an equilibrium process, so a higher amount of organic compounds could be deposited on the same fiber only by increasing their partial vapour pressure. Related problems were already mentioned in the introductory section, and were thoroughly discussed in the literature.[23] The equilibration time was a multiple of exposure time for the fiber, and the equilibrium in the gas phase reached reflected a stationary state where all possible losses of VOC (dissolution in rubber cap and Parafilm sealing, permeation through Parafilm) were already included. In a previous study with this type of trap tackling ambient air samples, a series of successive 10 minute exposures of the fiber to the same trapped VOC atmosphere produced for each specific compound a sequence of decreasing amounts featuring the *geometric series*.[28] With each exposure, approximately one third of each compound was withdrawn. On the other hand, with only 1 minute successive fiber exposure (and full chromatographic run in-between), the amounts desorbed from the fiber were essentially the same, and with no consistent trend. The major part of the depletion of the compounds in the trap's headspace was evidently due to sequestration by the SPME fiber and not due to other losses of any kind. The fiber was solely exposed to the gas phase in our case, so possible matrix effects like selective suppression and/or enhancement that would originate from solutions, were essentially averted, especially since no visible separate liquid phase was present in the sampling vessel. Additional difficulties stem from matrix effect during absorption, aging of fibers and formation of artifacts during evaporation. All those issues should be taken into consideration, and should be properly addressed during the method development, and closely monitored thereafter.

The described method was optimized for the monoterpene fingerprinting in various cheeses. However, if one is to perform analysis on a larger group of VOC present in the cheese, an additional selection of SPME stationary phases is warranted for different groups of compounds.[29] With these limitations in mind, we suggest that our device adequately responded to the demand for an inexpensive preconcentration procedure that would subsequently still produce at least a qualitative fingerprint of an individual cheese using simple chromatographic peak areas.

3. 2. GC/MS Analysis

VOC were preconcentrated in the trap, so their vapour pressure was higher than during hypothetical ordinary static headspace conditions. Effectively, by using the cryotrap/SPME procedure, it was possible to obtain characteristic chemical profiles in terms of GC/MS data, which enabled us to identify and assess (on a relative basis) many compounds in a single run. Several classes of VOC were identified, including aldehydes, ketones, alcohols, fatty acids, and monoterpenes. It was suggested in the literature that

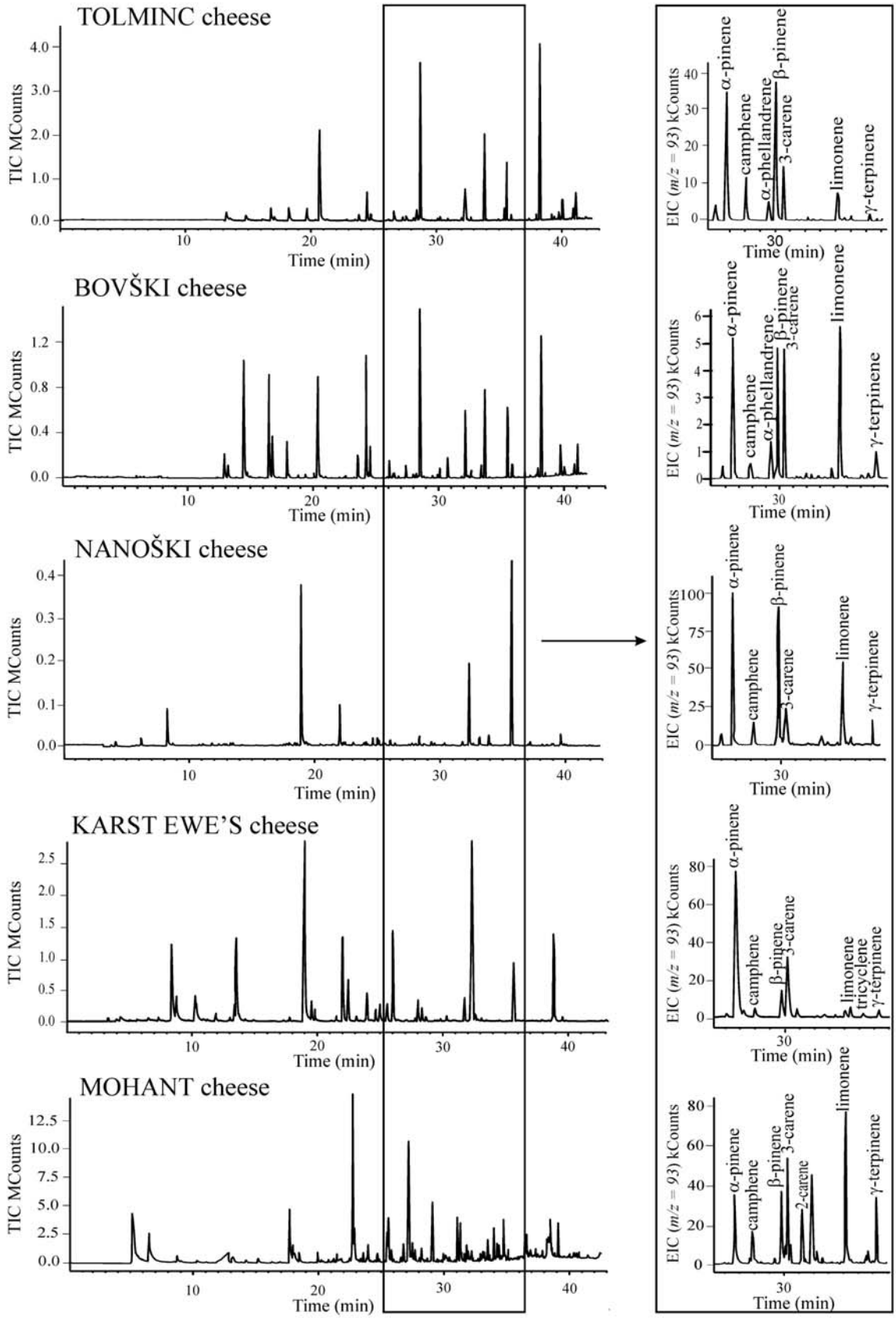

Figure 2. Total ion VOC profiles of five Slovenian PDO cheeses; the chromatograms on the right are magnifications of the marked regions on the left, however only ion intensity at $m/z = 93$ is shown as extracted ion chromatogram (EIC).

monoterpenes could be used as chemical markers for the determination of the origin of cheese,[18,19] so we focused only on this group of compounds in our subsequent studies.

Representative Total Ion Chromatograms (TIC) and corresponding EIC with expanded region of interest for each type of cheese are displayed in Figure 2. The qualitative compositions of monoterpenes in all three cow's cheeses were different. The largest diversity of monoterpenes in terms of qualitative composition was found in Tolminc cheese, an Alpine cheese produced in Upper Posočje with a pronounced Mediterranean influence and Mohant cheese produced in Bohinjska češnjica (Central Julian Alps). We were able to obtain just two samples of Mohant cheese in one vegetation period, because it is only produced in a limited season and in low volume. Having used only two samples of Mohant, its data should not be regarded as conclusive. A lower number of monoterpenes was detected in Nanoški cheese (mixture of Mediterranean and continental climate). For a comparison, we have also analyzed two ewe's cheeses. Bovški cheese showed a different VOC profile, but had similar monoterpene composition as Tolminc cheese produced from cow's milk. This was an expected result, since they were produced in the same geographic region and climate regime. The Karst Ewe's cheese showed the closest similarity to Nanoški cheese which was again expected due to the similar climate regimes.

3. 3. Data Pruning

The reproducibility of the SPME method was evaluated by measuring monoterpene composition in Nanoški cheese seven times. We found that the relative standard deviation of normalized areas ranged from 16% to 24% for limonene and β-pinene, respectively. Nevertheless, during experiments we experienced nonuniform day-to-day response, so a need arised for the data to be normalized. This was done by dividing all peak areas by the area of the maximum peak *within the group.* In case the compound giving maximum peak switched from run to run, the one that gave rise to the maximum peak most times had to be chosen. Still, a lot of variability remained among the peaks within different runs for the same type of cheese, so we decided to prune the data by rejecting as outliners all those runs where at least one relative peak area exceeded two standardized MADs for corresponding data within the group. The rationale for this rather drastic measure was in nonuniformity of the datasets pertaining to the same type of the sampled cheese. Peak areas were determined in the EIC mode at $m/z = 93$, since the peaks were poorly resolved in the TIC mode. After implementing this cleansing, the data were biased toward the center by excluding extremes within the group. Thus, data burdened by gross instrumental and sampling errors were rejected, so this protocol primarily guards against flawed conclusions. On the other hand, it also rejects data which is extreme, but still possibly viable. In this way we decided to deal with the central tendencies within the group, and, by applying strictly robust statistical estimators, we did not presuppose any kind of distribution (Gaussian or non-Gaussian).

As seen in Figure 3, one obvious observation regarding different types of cheese is that not all probed components are equally spread. The two minute components,

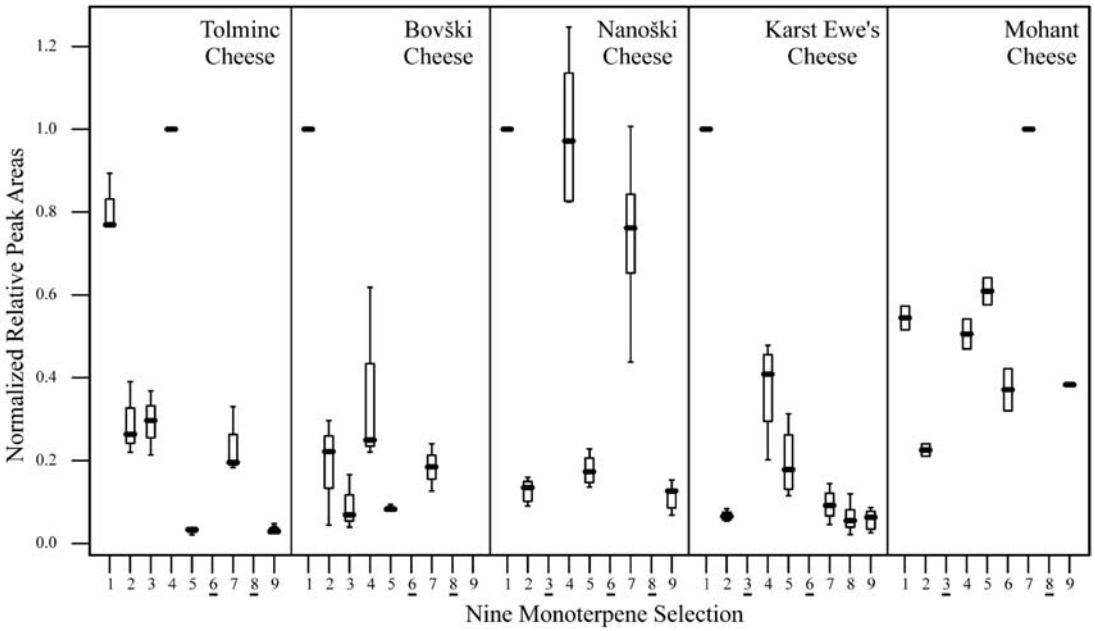

Figure 3. Boxplots of normalized relative peak areas pertaining to nine monoterpenes: α-pinene (1), camphene (2), α-phellandrene (3), β-pinene (4), 3-carene (5), 2-carene (6), limonene (7), tricyclene (8), and γ-terpinene (9), for five groups of cheese samples. The number with an underscore denotes a monoterpene that was below the limit of detection.

like 3-carene and γ-terpinene (peaks numbered 5 and 9) in Tolminc, showed little variability, mostly due to their low relative peak areas. On the other side, with Mohant, we only probed two samples, so there is little ground for enthusiasm over supposedly good reproducibility. With Nanoški cheese, a major variability in β-pinene should be properly conceptualized, since it shows that the prevailing number of samples contained more α-pinene than β-pinene. Nevertheless, in many cases the rank of α-pinene and β-pinene was reversed, so the latter might still have been the dominant monoterpene, if a larger number of samples had been used. At this point, we can conclude all five cheese types showed quite distinct overall pattern of relative peak areas, noticeable even by mere inspection.

3. 4. Cluster Analysis

Cluster analysis was finally applied to the measured experimental data of nine monoterpenes, namely α-pinene, camphene, α-phellandrene, β-pinene, 3-carene, 2-carene, limonene, tricyclene, and γ-terpinene in order to test the possibility for these compounds to be used as markers of geographic origin. As described in Experimental, missing data were replaced with an arbitrary value of 10^{-3}, which also influenced the perceived quality of clustering. Those values surely show zero deviation, which again apparently enhance separation from datasets pertaining to type of cheese that produced a measurable amount of the monoterpene in question. By setting an even lower value, the separation among clusters would be enhanced. We adopted a conservative value that was roughly one order of magnitude below lowest normalized peak area of approximately 0.021 for 3-carene in Tolminc dataset, which remained after pruning, but just below of relative peak area of approximately 0.004, pertaining to tricyclene in one of the samples of Karst Ewe's cheese, which was rejected as outlier.

Using the described clustering procedure, the best clustering performance, as judged by the average silhouette (AS),[25] was obtained when four clusters were formed (Figure 4a). The final confirmation for the selection of monoterpenes included in clustering came after we had performed a total combinatorial survey of classifications. We computed all combinations of 3–9 monoterpenes in an exhaustive enumeration and obtained the best score only when data for all nine monoterpenes were included. Namely, the best results were obtained for the number of clusters of 4 (AS = 0.764), following by the number of clusters of 5 (AS = 0.726), which coincides with our nominal number of different cheeses. As proposed by Rousseeuw,[30] the AS above 0.75 may be considered as an indicator that data itself show clustering, so that AS of 0.764 indicates clearly four clusters. Judging by the same measure, the AS of 0.726 falls into "moderate clustering" case, which is properly visualized both on the dendrogram in Figure 4b as well as in cluster plot in Figure 4a. Again it could be seen that Tolminc and Bovški cheeses showed the closest similarity and are even present in the same cluster, while all other cheeses are well separated from each other. Overall, with this limited dataset, the observed clustering is quite satisfactory, and coincides well with the geographical origin of particular cheese. It is plausible that with im-

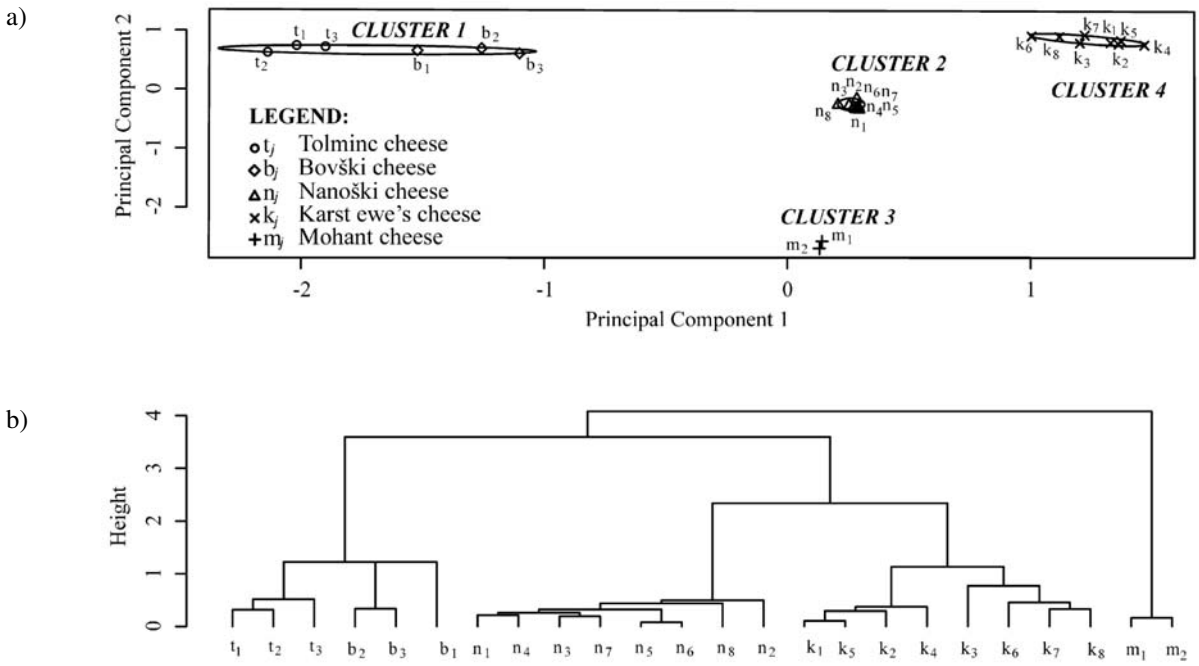

Figure 4. The results of cluster analysis for the determination of geographic origin of Slovenian PDO cheeses: **a)** Cluster plot produced mainly by CLUSPLOT procedure with some additional annotation; **b)** Dendrogram obtained by DIANA procedure.

provements, particularly in the chromatographic part, an overall dynamic range of the observed peak areas would increase and more detailed clustering would be observed.

4. Conclusions

A new variation of a double preconcentrating sampling procedure for GC/MS profiling of VOC in dairy products was developed and its usefulness demonstrated. The specially designed flow-through vessel, partly filled with glass beads that was used for cryotrapping the volatiles from the matrices showed quite promising performance in terms of enrichment efficiency. The proposed method of preconcentration is not only suitable for dairy products, but can be used for preconcentrating VOC from any solid, liquid and gaseous samples. It shares both the advantages and the weaknesses of SPME – on the one hand, it is simple, involves no solvents, and facilitates implicit water removal, but on the other hand we must accept limited reproducibility, limited quantification and possible compounds partitioning due to evaporation/absorption processes. If higher accuracy is sought, further refinements will be needed, like the use of isotopic labeled internal standard to accurately quantify compounds during preconcentration step and a tighter temperature control during fiber exposure.

The main disadvantage of the described procedure is partitioning of the volatiles that occurs during sample preparation. Such partitioning prevents the possible quantification of the volatiles in cheese unless we resort to using an expensive stable isotope labeling technique. As we were more interested in qualitative classification of cheeses and their connection to geographic origin, we have used chromatographic peak areas as indicators of the VOC concentrations in the individual samples. Although no quantification was performed, such peak area distribution can still be used as a fingerprint of an individual cheese by using otherwise strictly defined experimental conditions. We also found a candidate group of monoterpenes that would enable an assignment of milk products to specific animal feeds in order to serve as markers for food origin. The extent of usefulness of the developed analytical procedures together with ensuing clustering was demonstrated on five traditional Slovenian cheeses. In all of them a group of nine monoterpenes followed a characteristic fingerprint pattern that adequately suited our goals. Again, the developed VOC fingerprinting procedure is not limited just to characterization of cheeses but can be used for any food product to find a possible correlation between VOC profile of the food product and environmental conditions where the raw material was produced or grown.

In conclusion, it should be stressed that a low number of cheese samples was included in our clustering exercise. Also, that they were obtained during a single vegetation season. The results of the described study were therefore only indicative and were used as an illustration of the applicability of the developed analytical procedure. In order to develop a robust clustering technique for the assignment of geographic origin to particular cheeses, more experimental data will have to be obtained which will make it possible to account for seasonal and producers' differences between cheeses from the same region.

5. Acknowledgements

The authors wish to thank the Ministry of Education, Science and Sport of the Republic of Slovenia for financial support through the grants P1-0153 and V4-0748.

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Povzetek

Razvili smo različico metode mikroekstrakcije na trdni fazi (SPME), za predkoncentriranje hlapnih organskih spojin iz sirov. V ta namen izdelana hladna past, delno napolnjena s steklenimi kroglicami in potopljena v tekoči dušik, je omogočila učinkovit pretok prepihovalnega plina in lovljenje hlapnih spojin. Po prepihovanju smo past odtalili in v prostoru nad kroglicami izpostavili SPME vlakno, s čimer smo uspešno dosegli dvakratno predkoncentriranje. S pomočjo plinske kromatografije sklopljene z masno spektrometrijo smo hlapne spojine identificirali in določili ploščine njihovih kromatografskih vrhov. Za modelno skupino spojin smo izbrali monoterpene, ki so služili kot potencialni markerji geografskega porekla. Metodologijo smo preizkusili na vzorcih petih tradicionalnih slovenskih sirov z zaščiteno označbo porekla (PDO): Tolmincu, Mohantu, Nanoškem siru ter Bovškem in Kraškem ovčjem siru. Zbrani podatki o ploščinah kromatografskih vrhov devetih značilnih monoterpenov (β-pinen, kamfen, α-felandren, β-pinen, 3-karen, 2-karen, limonen, triciklen in γ-terpinen) so pokazali grupiranje, ki nakazuje povezavo med siri in njihovim geografskim poreklom. Po kriterijih metrike s silhuetami se skupine podatkov za naše sire pri metodi porazdelitve okrog medoid (PAM) razbijejo v 4 gruče. Uporabljena statistična metoda je podatke za Tolminc in Bovški sir razporedila v eno gručo, kar jasno nakazuje podobnost geografskega porekla, medtem ko so bili podatki za ostale sire porazdeljeni v ločene gruče.