USE OF AROMATIC PROFILES AS A TOOL FOR DETERMINING THE AUTHENTICITY OF FRUIT JUICES

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Abstract

The purpose of presented work was to develop the analytical and chemometric methodology that will enable authenticity characterization of fruit juices on the base of their aroma profiles. For the experimental part, juices and nectars of Slovenian and foreign producers were selected. Solid phase microextraction (SPME) technique was chosen as the technique for sampling volatile components. Sampling, optimal elution conditions and determination with gas chromatography-mass spectrometry was optimized according to the standard procedures. Peak areas of particular compounds in chromatograms were input parameters for the chemometric analysis (principal component analysis, group analysis and discriminant analysis). The purpose of chemometric part of work was to calculate the model which would be capable to discriminate between the groups of fruit juices. Results of all three chemometric methods were the best in the case of discriminating samples of fruit nectars and in the case when we want to discriminate samples of 100 % orange juices and samples of orange nectars. Worse, but still satisfying results were obtained when different samples of 100 % fruit juices and when samples of 100 % apple fruit juices with samples of apple nectars were compared.

Keywords: aromas, volatile components, fruit juice, SPME, GC-MS, chemometrics

UPORABA AROMATSKIH PROFILOV KOT ORODJA ZA DOLOČANJE PRISTNOSTI SADNIH SOKOV

Izvleček

Namen predstavljenega dela je bil razviti analizne in kemometrijske metode, ki bi karakterizirale pristnost sadnih sokov na osnovi njihovih aromatskih profilov. Za raziskovalno delo smo uporabili sokove in nektarje slovenskih in tujih proizvajalcev. Izbrana tehnika vzorčenja oziroma zajemanja hlapnih komponent arome je bila mikroekstrakcija na trdnem nosilcu - SPME tehnika. Postopke vzorčenja, optimalne pogoje za ločbo in določevanje s plinsko kromatografijo z masno detekcijo smo optimirali po standardnih postopkih za tovrstne analize. Površine vrhov posameznih spojin v kromatogramih so tvorile vstopne parametre za kemometrijsko analizo (metoda glavnih osi, analiza grup in diskriminantna analiza), katere

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namen je bil izdelava modela, ki bo sposoben z dovolj visoko zanesljivostjo razločevati med posameznimi sadnimi sokovi. Rezultati vseh treh kemometrijskih analiz so bili najbolj primerljivi v primeru ločevanja vzorcev sadnih nektarjev in v primeru, ko smo želeli med seboj razlikovati vzorce 100 % sokov pomaranč z vzorci pomarančnih nektarjev. Nekoliko slabše, vendar še vedno dovolj dobre, rezultate pa smo dobili pri medsebojnem ločevanju vzorcev 100 % sadnih sokov in medsebojnem razločevanju med vzorci 100 % sokov jabolk in vzorci jabolčnih nektarjev.

Ključne besede: arome, hlapne komponente, sadni sok, SPME, GC-MS, kemometrijske metode

1 INTRODUCTION

Compounds that contribute to the flavour characteristics of food products are mostly organic compounds belonging in several chemical classes such as esters, terpenoids, poliols, organic acids… Their common characteristic is the volatility [14]. The concentration ranges of a particular compound depend of a plant species, plant variety and of geoclimatic conditions although for a particular fruit juice the characteristic profiles are specific. Determination of the profiles of standard fruit juice samples enables determination of concentration ranges of some, most important compounds, which are usually present at higher concentrations. It is possible to assume that the profiles of volatile fruit juice compounds are similar.

Gas capillary chromatography (GC) with different detectors and gas chromatography coupled to the mass spectrometry (GC-MS) are the methods of choice for the analysis of volatile compounds in food products. One of the analytical approaches to determine volatiles is the analysis of gas phase which is in equilibrium with the sample. For the characterisation of a particular samples the use of multivariate chemometric methods is needed, since with such complex samples only the combination of different variables enable the better view in the nature of a sample. Basic approach in resolving of such questions is the formation of data bank. Such bank consists of a sample data sets belonging to the standard samples which are defined as those with the wanted characteristics. The bank should be extensive enough that it is able to show all ranges of particular compounds that are still in the acceptable limits. On the other hand bank should contain also the samples with evident faults. In most cases that means the higher content of undesirable compounds or the lack of favourable ones. Sufficient number of data in bank enables the construction of models which could be used further on as a tool during the regular quality control or as a tool for finding and evaluation of flavour composition of unknown samples.

Such approaches are already successfully used in many fields of food production. The use of EU isotopic data bank of wine or honey may serve as an example [5,9]. At our Institute we have long term experiences with a similar bank for the composition of hop essential oils which is successfully used in the case of hop variety studies [3,7]. Similarly the use of red wine anthocyanins profiles determined by liquid chromatograpy has been presented in the case of red vine varieties studies. The use of white wines profiles determined with NMR and ICP spectroscopy in the case of determination of the geographical origin and the variety is the another example [1,4,6].

In the literature a lot of examples of use of different chemometric methods PCA, HCA, LDA in combination with different analytical techniques which enable determination of different parameters in connections with the determination of the authenticity of a wide range of food products as wine, olive oil, beer, fruit juices could be find [2,8,10-13].

2   MATERIAL AND METHODS

The work was divided into several parts as optimization of the process of binding the volatile flavors of fruit juices on the selected fiber by varying the temperature, the ratio of volume between liquid and gas phase and the time of balancing/bonding. The objective of optimization was to determine the bonding time, temperature, volume and an acceptable amount of certain compounds related to the fiber. These parameters were further used for a complete analysis of all juice samples. We analyzed 53 different fruit juices of Slovenian and foreign producers, available on the domestic Slovenian market. Of these, 26 fruit juices with 100% fruit content and 27 nectars. Fruit juices are juices made from different fruits and have 100 % fruit base, while nectars are essentially the same except they are diluted with water that is usually followed by the addition of sugar and aromas of natural or artificial sources. The second part of presented work was getting aromatic profiles using SPME and subsequent determination by GC-MS.

25 mL of samples were placed in a 40 mL vials and thermostated at 50 °C for 60 min. A 100 μm polydimethylsiloxane (PDMS) SPME fiber was inserted through the septum and exposed for 50 min at 50 °C. The compounds adsorbed on the fiber were analyzed by GC-MS with a Varian STAR 3400 CX GC system coupled to an ion trap mass selective detector (Varian SATURN 2000); transfer line 260 °C, source 180 °C, ionization potential 70 eV. The fiber was inserted manually into the injector port (180 °C), desorbed and chromatographed on a nonpolar column (5% diphenyl and 95% dimethylsiloxane) (HP–5MS, 30 m, 0.25 mm, 0.25 μm; Agilent Technologies). Helium at a constant pressure of 240 kPa was used as carrier gas. After fiber insertion, the column temperature was increased from 60 to 190 °C at a rate of 2.5 °C/min followed by increasing to 240°C at the rate of 50 °C/min and ended with the final stage of 10 min at 240 °C. Detector temperature was set to 280 °C.

Chemometric evaluation of obtained results was the last part of our work. All the calculations where done by SCAN statistical program (Minitab Inc., USA).

3   RESULTS AND DISCUSSION

3.1   Qualitative evaluation of chromatographic peaks

First, we recorded the chromatograms of samples of different kinds of fruit juices (orange, apple, pineapple, pear, grapefruit and lemon) under the semi-quantitative conditions and identified the individual chromatographic peaks (Fig. 1). For the identification of compounds, we used the mass spectra of standard solutions, a library of mass spectra and literature data. These chromatograms are of great value to continue the work as they served as standards. For reliable identification, we identified and confirmed compounds, for which we had available

standard solutions as hexyl alcohol, α - pinene, hexyl acetate, limonene, linalool, methyl caprilate, β - caryophyllene, α - humulene.
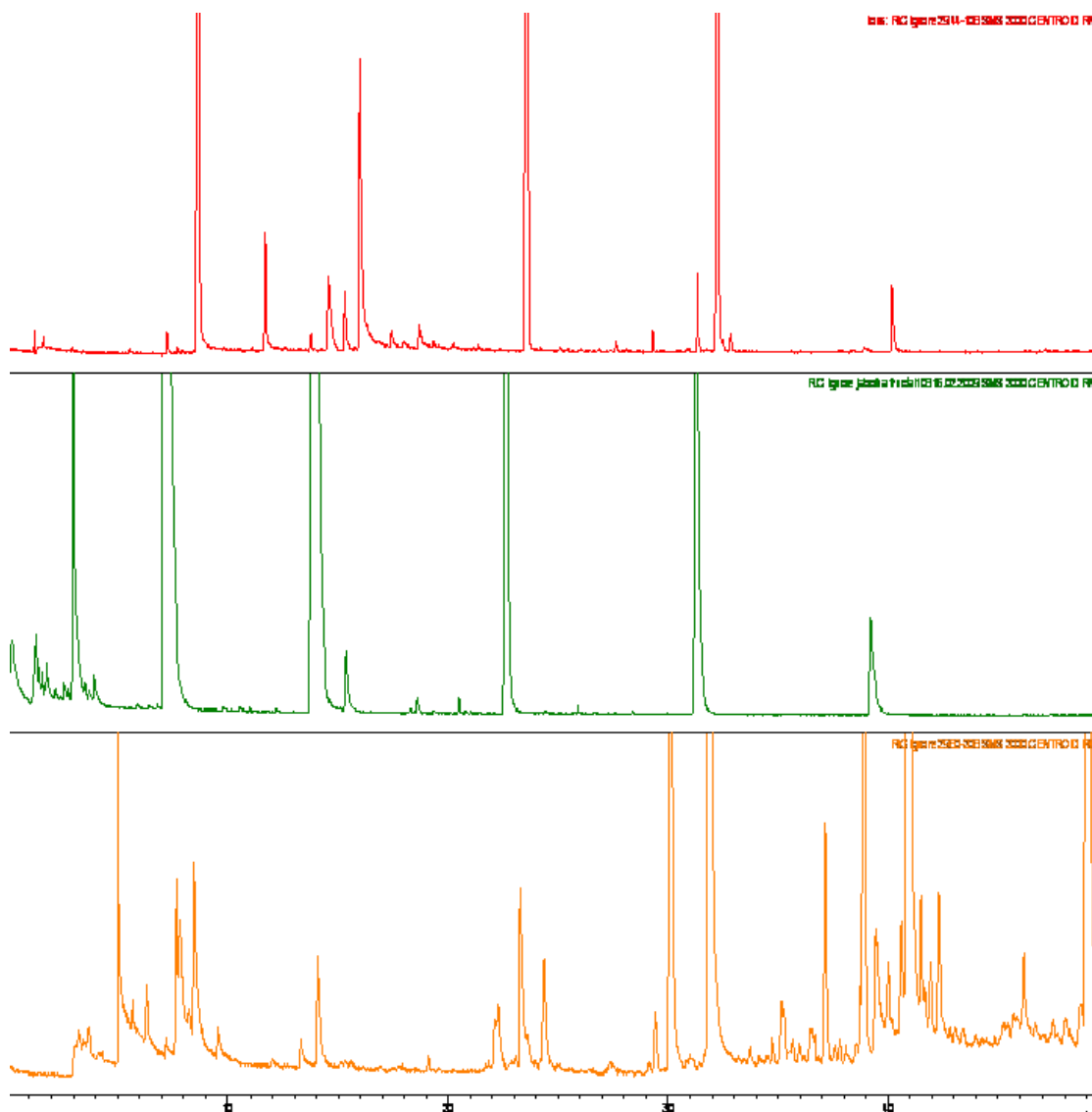

Figure 1: Comparison of GC chromatograms of 100% fruit juices of different fruits. From the top to the bottom chromatograms of juices or oranges, apples and pinepple are presented as an example.
Slika 1:   Primerjava GC kromatogramov 100 % sadnih sokov različnega sadja. Od vrha proti dnu si kot primeri sledijo kromatogrami sokov pomaranč, jabolk in ananasa.

3.2   Chemometric analysis

In the second part microextraction of volatile compounds on solid medium was performed and 53 samples of fruit juices were analyzed under the semiquantitative GC/MS method. 26 samples, representing 100% juices and 27 samples of nectars of different kinds of fruits

(orange, apple, pineapple, pear, grapefruit and lemon). This was followed by chemometric analysis (principal component analysis, hierarchical clustering and discriminant analysis of groups) through which we created a model that was able with sufficient confidence to distinguish between different fruit juices.

With the use of principal component analysis basic information about similarities and differences of 25 samples of 100% juices and 26 samples of nectars was searching. First, we were interested in the similarity or difference between the 25 samples of 100% juices. 100% pear juices formed their own group. Other 100% juices (orange, apple, pineapple and grapefruit) are close to each other, but groups of certain types of juices are indicated (Fig. 2). It is also evident that one orange juice and pineapple juice are outliers of their groups.
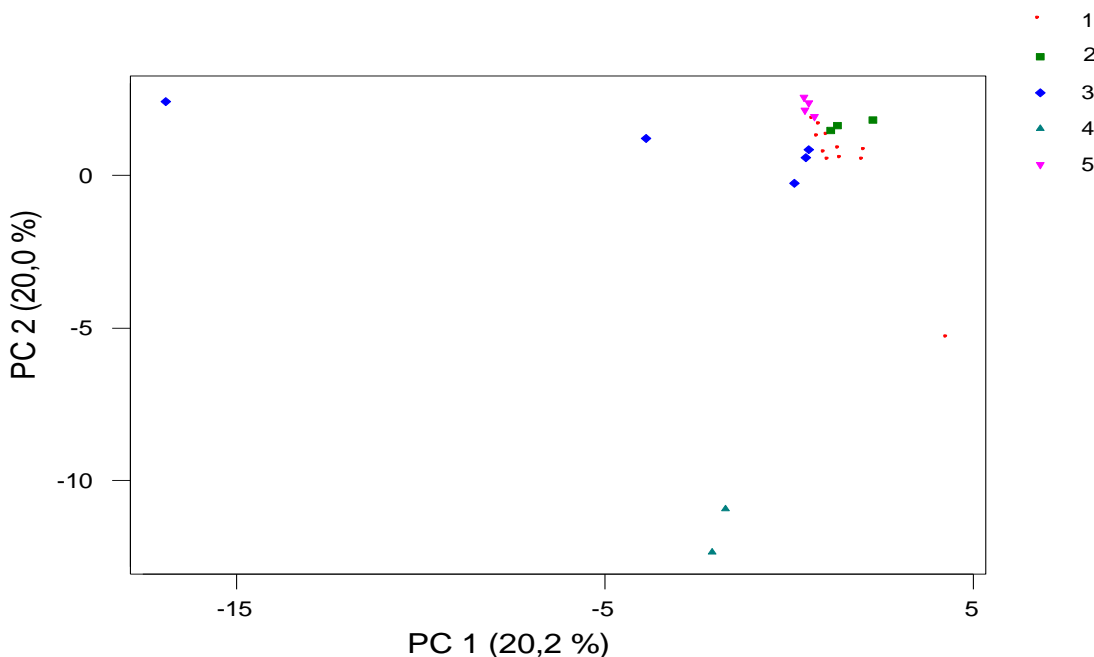

Figure 2: Projection of the 25 samples of 100% fruit juice in the plane defined by the first two principal components PC1 and PC2, which together represent 40.20% of the variance. The numeric codes are: 1 oranges, 2 apples, 3 pineapple, 4 pears, 5 grapefruit.

Slika 2: Projekcija 25 vzorcev 100 % sadnih sokov v ravnini definirana s prvima dvema glavnima osema PC1 in PC2, ki skupaj predstavljata 40,20 % variance. Številčne oznake so: 1 pomaranče, 2 jabolka, 3 ananas, 4 hruške, 5 grenivke.

Further on we were interested in the similarity or difference among the 26 nectar samples of different kinds of fruit (orange, apple, pineapple and pear). In this case, we have used principal component analysis on the basis of 25 variables. Fruit nectars are classified into three groups. The first group consisted of apple nectars (with the exception of one nectar - outlier), which was close to other groups. The second group is consisted of orange and three pear nectars. The third group is formed by pineapple nectars and two pear nectars.

In the third case, we were interested in the similarity or difference between 100% orange juices and samples of orange nectars. Juices and nectars were divided into three distinct

groups. The first group qualified for all orange nectars including three 100% orange juices. The second group consisted of three 100% orange juices and a third group are the remaining five samples of 100% orange juice. In this case we get three 100% fruit juices, which classified in the group of nectars and it was anticipated that these juices were actually not 100% juices but nectars.

In the fourth case, we were interested in the similarity or difference among samples of 100% apple juice and apple nectar samples. Juices and nectars were divided into two separate groups with the exception of one nectar sample that is placed close to the 100% apple juices.

The results of the analysis by hierarchical clustering were virtually the same as in the case of the use of the principal component analysis. Therefore in this paper we will not discuss these results in details. Results showed the same classification and found the same patterns, which represented outliers as described above.

Figure 3:   Classification of 25 samples of 100% fruit juices on the basis of the calculated model with RDA (A) and the validation of the model done by cross validation (B). The numeric codes are: 1 oranges, 2 apples, 3 pineapple, 4 pears, 5 grapefruit.
Slika 3:   Klasifikacija 25 vzorcev 100 % sadnih sokov na osnovi izračunanega modela z RDA (A) in prikaz rezultatov validacije modela, ki je bil narejen z navzkrižno validacijo (B). Številčne oznake so: 1 = pomaranče, 2 = jabolka, 3 = ananas, 4 = hruške, 5 = grenivke.

The last step of chemometric analysis was the use of regularised discriminant analysis (RDA). Within the 25 samples of 100% juice, we got 92% correct classification results, since only two of 25 samples ranked outside of their group. One outlier (pineapple) was the same as in the two previous methods, while another one (grapefruit) by this method was otherwise classified.

Among 26 nectar samples 92.3% correct classification results were obtained, because in this case, only two of 26 samples of nectars were classified wrong.

In the last case within 100% of apple juice and apple nectar samples we obtained 100% correct classification results. In this case, we got slightly different results, as with the earlier methods, where in both cases, one of the nectars ranked among the 100% juices.

The results of all three chemometric analysis were the most comparable in the case of separation of 27 samples of nectars and where we wanted to distinguish among 100% orange juices and nectars. Slightly less, but still enough good results were achieved in the separation of 26 samples of 100% fruit juices (Fig. 3).

4   CONCLUSION

With the chemometric analysis very good results were achieved. Even if some differences among the results is noticed, the values where regularised discriminant analysis was used were high (about 90%), which assures that we have created a model that is able to distinguish among different fruit juices with sufficient confidence.

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