Investigation of the aroma compounds aiming to identify species

THESIS

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The applicant met the requirement of the PhD regulations of the Corvinus University of Budapest and the thesis is accepted for the defence process.

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1. INTRODUCTION

On the Department of Food Chemistry and Nutrition several researches have been carried out since the nineties examining the aroma-structure of various spices, wines, spirits, fruits vegetables, honeys and mushrooms. Continuing my former studies to identify spices, I carried out an analysis of tea types.

The tea made from the Camellia sinensis plant's leaves is among the most consumed beverages. Nowadays its agents got to the centre of attention due to the discovery of the beneficial effects of regular tea consumption.

Defining the quality of the tea is very hard; the classification process is partly objective, with instrumental methods, and partly subjective, with sensory methods. The sensory characteristics are determined by the variety, the growing conditions and the processing method of the plant. To the present day the classification of the finished products are primarily done by sensory methods; the basis of that method is the classification of the various flavours in teas. The instrumented physical (colour and conductivity measurements) and the analytical measurements (gas chromatography, liquid chromatography and multi-element analysis) are often used to supplement and confirm the sensory method.

2. OBJECTIVES

The aim of my doctoral thesis was the complex analysis of the flavour of teas from the same type. I wanted to evaluate objectively the quality of the tested teas with instrumental methods. During the execution, the most important tasks were the extraction of the flavourings from the samples, the separation of fragrance components with gas chromatography and the identification of those components with mass spectrometry. The GC-MS analyses were compared with electronic nose and tongue device tests and the results of the sensor profile analysis to decide whether the instrumental or the sensory examination are more suitable to analyse the tea's quality and characteristics.

3. MATERIALS AND METHODS

3.1. Examined samples

The examined samples were provided by Sara Lee Hungary. The packaging and the distribution of teas occur in Hungary, the processing takes place at the location of the cultivation. Analyses were performed with the following samples:

Chinese Keemun black tea, Ceylon black tea, Indian Assam black tea, Indian Darjeeling black tea, Chinese white tea, Chinese green tea, Chinese Oolong tea

Comparative examinations were executed in two groups:

- Black teas from different geographical origins
- Chinese teas with different fermentation degrees

3.2. Methods

The aroma compounds of teas were separated with gas chromatography, using a polar capillary column, the detection and identification of components was carried out with mass selective detector (Hewlett Packard 5890 series II. GC + 5971/A MSD).

The comparison of black teas and Chinese teas with different fermentation degrees was performed based on all constituents and aroma-spectra of the samples. In addition the results of GC-MS analysis were compared with the following examinations:

- Sensor profile analysis
- Electronic nose NST-3320
- Electronic tongue Alpha Astree II.

4. RESULTS

The preparation of the samples plays a crucial role in completing a successful analysis. From the available methods for isolating the volatile components from the tea, I examined the efficiency of steam distillation, simultaneous distillation-extraction and solid-phase microextraction. I found that the modified Likens-Nickerson simultaneous distillation-extraction is the most effective method for extracting the components that are responsible for the fragrance. Though solid-phase microextraction is more sensitive for the aliphatic alcohol, aldehyde and ketone groups, it cannot be used for a suitable extraction of aromatic terpenes. Comparing the distillation processes, with the steam distillation I extracted 54 components, the Likens–Nickerson method resulted 101 components. Most of the 28 common components detected in the sample showed a higher relative intensity value with the Likens – Nickerson method.

The tea extracts were subjected to a detailed mass spectrometric analysis after they were separated with gas chromatography, using a polar capillary column. Every component was identified with a specific method, using the most suitable background compensation. The evaluation of the results was performed with the flavour spectrum method developed by the Department of Food Chemistry and Nutrition on the Corvinus University of Budapest.

The GC-MS analyses were compared with electronic nose and tongue device tests and the results of the sensor profile analysis to decide whether the instrumented or the sensory examination are more suitable to analyse the tea's quality and characteristics. My work had the following conclusions:

- The black teas belong to two subtypes: the Indian Assam and the Ceylon black tea are representatives of the *var. assam* subtype, the Chinese Keemun and the Indian Darjeeling belong to the *var. sinesis* subtype. This classification made possible the comparison of genetically identical cultivated variants and the discovery that whether the different place of cultivation and the climatic conditions are affecting the formation of the flavour compounds. The Indian teas' similarity and richness in flavour become apparent from the spectrum, also the Darjeeling tea's superiority in the relative intensity of some components is revealed.
- The botanical identity of the types suggested that the Ceylon and Assam teas' spectral pattern, and the Darjeeling and Keemun tea's spectral pattern will be similar. However, more obvious similarity showed between the two above

mentioned Indian teas and the Ceylon tea. The resemblance in the patterns appeared mainly in the first half of the flavour spectrum, among the 1000-1700 PTRI components. These components are from the tealeaf's precursors, and their quantities are genetically determined. Therefore the differences are in the flavours formed by the production technology. Based on the spectrum, the Chinese tea differs the most from the other black teas. The profile drawn from the sensory evaluation's rating, showing the samples' odour characteristics, is comparable with the flavour spectra. The Ceylon, the Assam and the Darjeeling teas' profile are very similar to each other, while the Keemun's profile is different from the others.

- During the electronic nose tests the Indian Assam and Darjeeling teas similarity were proven. The Ceylon black tea related to the Indian Assam, both belong to the var. assamica cultivar. Their quality rating in the statistical assessment is close to each other. The discriminant analysis also shows that the separation of the Chinese corresponds with the flavour spectra, thus the obtained results are confirm the gas chromatography measurements.
- Comparing the results of the tea leaf's and beverage's electronic nose device tests it was determined that the leaf's volatile components were more suitable for separation with this device. These components have the lowest boiling point and are the most volatile parts of the flavouring substance; therefore they can be extracted from the tea with head space sample preparation. Further test are needed to prove if these components are enough to show the difference between teas belonging to the same subtype or originating from the same region. In the tea beverage's case, the separation of the samples is less reliable based on fragrances gained from the vapour.
- The results of the sensory examination are corresponding well with the electronic tongue device's results. There's a 99% difference in global taste characteristics between the Ceylon and Assam tea, the Ceylon and Keemun tea and the Darjeeling and Assam based on the ratings given by the reviewers. The results of the electronic tongue device show that the method can be applicable for differentiating teas based on their tastes.

Originally, fermentation was used to enhance the tea's taste and flavours. It is evident that the oxidation, and the degree of the fermentation, can be monitored by measuring the change in the flavouring substances. I attempted using this method, testing Chinese teas with different fermentation degree with gas chromatography.

- The white and green teas, both unfermented, have significantly lower amount of flavours than the half-fermented and fermented teas. Desiccation, fermentation and roasting are increasing many components' quantity, new flavour components are formed. This increase in quantity is significant when the pyrazines and pyrroles are considered. The formation of these components is induced by heat during the Maillard-Strecker reaction, creating a roasted, smoky and nutty taste in the tea. Guaiacol and cresol also create a smoky and burned taste in the tea; I isolated these from the Oolong and the black tea.
- Jasmonates accumulate in the damaged parts of the plants. During the processing of the tea, the breaking of the tea leaf, as a mechanic damage can cause the formation of jasmonates, since the enzymes are still active during the processing. Jasmonic acid (PTRI:1954) was detectable in every sample except the Assam tea; the fermented teas had a bigger amount than the white or the green teas. In the production of black teas longer processing periods result in a higher amount of jasmonic acid. The inactivation of the enzymes during the production of white and green teas causes the lipase enzyme's fast inactivation as well; this explains the lower amount of jasmonic acid. This component can be suitable to indicate the degree of the fermentation. Methyl-jasmonate was only detected in the fermented Oolong and black teas (the Assam tea does not contain this component either).
- Linalool and its oxides are specific to the tea's oxidation rate. The received linalool/linalool-oxide rate of the white – Oolong – black tea corresponds to the data in literatures; as the oxidation progresses, the quantity of the linalool-oxides is rising. The green tea's results are also met the expectations, the linalool/linalool-oxide rate is higher than the half-fermented and fermented samples' and the oxides are present in a lower quantity compared to the Oolong and the black tea. The white tea may have been produced from better quality materials than the green tea, which could be the reason of the unexpected tendency between them.
- The sensory evaluation showed that there was a detectable difference in the odour characteristics of the black and green teas, the black and white teas, the Oolong

and green teas and the Oolong and white teas on a 99% probability level. The reviewers could not tell apart the black and the Oolong teas. Based on the bitter odour characteristics and the smoky, burned smell as well, the black and Oolong teas, the green and Oolong teas and the white and Oolong teas proved to be significantly different. The bitter and the smoky, burned odour are results of the combined effect of the same components. The ratings are reflecting this too; examining the two odours, the Oolong tea got the highest ratings in both cases, followed by the black and the white teas. This effect can be caused by the pyrazines and pyrroles. The pyrazines identified during the examination with gas chromatography showed the highest relative intensity in the Oolong tea; there are several marker components among them. These can be used as type identifiers for the Oolong tea, for example the methylpyrazine, the 2,5-dimethyl pyrazine, the trimethylpyrazine and the 2,5-diethylpyrazine. The guaiacol and cresol components also cause smoky and burned smell; I identified these in the Oolong tea.

- The discriminant analysis of the electronic nose device's results is can reliably separate the four sample groups. The discriminant plots show, that the relative positions of the groups related to the odour, formed by the examinations on 25 and 55°C, changed. The samples are probably emitting different odours into the sampling vapour in different rates when examined on different temperatures. The head space sampling method's dependence on temperature is proving my observation on sample preparation; this states, that the use of simultaneous distillation-extraction is appropriate if the aim is to get repeatable, representative picture of the flavours in the tea.
- The electronic tongue device's results shows that the unfermented teas are close to each other. Based on the first variable, the half-fermented Oolong tea is close to the unfermented teas; the fully fermented black tea is the farthest away. This differentiation led to better results than the electronic tongue device, the samples' relative position reflects the rate of the fermentation better. Comparing the sensory evaluation's ratings with the electronic tongue device's results it can be stated, the subjective examination can be substituted with the objective method. The reviewers could differentiate the black and green teas and the black and white teas based on the bitter taste. The electronic tongue device can also perform this with high confidence, using statistical analyses. It is also able to detect other differences

between the variously fermented teas. The electrochemical sensors are proven to be suitable for differentiate teas based on flavours.

- The teas can be separated by performing statistical analyses on the electronic nose and tongue devices' results. This method is more reliable than the subjective sensory examination. However, the background of this difference can only be explored with chromatographic methods.

5. NEW SCIENTIFIC ACHIEVEMENTS

The aim of my work was the complex analysis of the flavour of tea. The GC-MS analyses were compared with electronic nose and tongue device tests and the results of the sensor profile analysis to decide whether the instrumented or the sensory examination are more suitable to analyse the tea's quality and characteristics.

The new scientific and methodological results are the following:

1. Development of a Likens-Nickerson simultaneous distillation-extraction method using undecanol-1 internal standard for the extraction of the volatile compounds of teas. The tea extracts were separated with gas chromatography, using a polar capillary column, then they were subjected to a detailed mass spectrometric analysis, searching specific compound that characterises each of the tea types.
2. Using the aroma-spectra method developed to gain a more illustrative interpretation of the results, I found that the aroma-spectra of the samples are resemble each other if compared by the place of the cultivation, the climatic conditions and the different processing method.
3. The discriminant plots of the electronic nose device's results showed, that the relative positions of the groups related to the odour, formed by the examinations on 25 and 55°C, changed. The samples were emitting different odours into the sampling vapour in different rates when examined on different temperatures. The head space sampling method's dependence on temperature was proving my observation on sample preparation; this states, that the use of simultaneous distillation-extraction is appropriate if the aim is to get repeatable, representative picture of the flavours in the tea.
4. Correlations were established between identified components' fragrance and the sensory scores of teas. The results of electronic nose device were confirmed with the results of analytical investigations.
5. Comparing the sensory evaluation's ratings with the electronic tongue device's results it can be stated, that the subjective examination can be substituted with the objective method. The teas fermented on different degrees can be separated by performing statistical analysis on the electronic tongue devices' results. The electrochemical sensors are proven to be suitable for differentiate teas based on flavours.

6. RECOMMENDATION FOR FURTHER RESEARCH

The health-conscious nutrition has a significant effect on the customs of food consumption in developed countries, thus in Hungary as well. Attention is now focused on teas and its beverages, similarly to other functional foods. According to statistical data, tea consumption is growing in Hungary. A few years earlier the black teas were the most popular, and even though they are still leading the market, the consumption of green and white teas, herbal teas and fruit teas is increasing dynamically.

In consequence of the growing consumption, the quantity of imported tea has increased in Hungary as well. That is the reason why the quality control of imported products, the elimination of adulteration is of great importance.

The goal of my doctoral thesis was the investigation of the aroma-composition of teas from the same type. I wanted to evaluate the reliability of the tested teas' quality with instrumental methods. The GC-MS analysis was compared with electronic nose and tongue device tests and with the results of the sensor profile analysis to decide whether the instrumental or the sensory examinations are more suitable to analyse the tea's quality and characteristics. These investigations would help to ensure the excellent quality of teas and the quick detection of possible adulterations.

The analysis of aroma-compounds in teas and the discussion of the results provided the substantial part of the thesis. Similar investigations have not been carried out in Hungary yet, therefore my results were compared with the data found in international scientific literature.

The analyses performed with electronic nose and tongue devices look promising; these make possible the fast inspection of teas: the electronic devices are able to detect differences between the black teas as well as the variously fermented teas more reliably than the subjective sensory examination. The fast and simple sample preparation and the short-time examination allow this method to be used as an objective quality control in the trade to check the origin of the teas. If the electronic sensors detect any difference, the background of this divergence can be explored with GC-MS investigations, and justify the difference between the samples. This also can be confirmed with the marker compound characteristics of the cultivars, which were identified during my examinations as well.

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