Rancidity control of nut mixtures using an Electronic Nose

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ABSTRACT: Nuts are rich in polyunsaturated fats, which are particularly sensitive to lipid oxidation. A Flash GC based Electronic Nose was used to identify the causes of rancidity in nut mixes and to monitor global sensory quality. Five appetizers of different sensory qualities, all composed of the same nut types, were considered. It was shown that peanuts were the most critical ingredients in the development of rancidity off-flavours. Pecans and cashew nuts also presented a relatively high concentration of off-odours, but, their relatively low proportion in the final mix made them less critical towards rancidity. As for Brazil nuts, almonds and hazelnuts, containing lower amounts of volatile compounds, they proved to have a low impact on the overall mix aroma.

INTRODUCTION

The aroma profile and volatile profile of food products can be analysed by GC/MS. This technique allows to identify the chemical compounds after separation. Another technique consists of using an Electronic Nose, which performs a global analysis of the volatile profile of products, as the human nose, and can deliver both chemical and sensory information. This instrument has already been used to analyse the volatile profile of various food products such as fruits (1), vegetables (2), cheese (3), fish (4) or oils (5). For many food products it is important to reliably assess and monitor sensory features that play an important role in consumers’ acceptance. Nuts are rich in polyunsaturated fats, which are particularly sensitive to lipid oxidation that generates characteristic off-odours. In appetizer mixes, each type of nuts has a different contribution to potential overall rancidity. Therefore, it is important to identify the causes of possible rancidity to monitor the global sensory quality of these appetizers. After having received a claim from a consumer about an unpleasant off-flavour in their product, a manufacturer needed to explain the origin of the defect. The analysis of the overall aroma profile of 5 mixes containing 6 types of nuts was conducted using HERACLES Flash GC based E-Nose. The objective was to determine which nuts most contributed to bad quality.

MATERIALS AND METHOD

Equipment
Over the last decade, “electronic sensing” or “e-sensing” technologies have undergone important developments from a technical and commercial point of view. Among them, electronic noses have been designed to mimic the human sense of olfaction in order detect and recognize odours and flavours. Basically, an electronic nose consists of a sample delivery system, a detection system and a computing system for pattern recognition (Figure 1).

- When headspace injection mode is used, the sample delivery system enables the generation of the headspace (volatile compounds) by heating the sample at a constant temperature under agitation. The system then injects this headspace into the detection system of the electronic nose. The sample delivery system is essential to guarantee constant operating conditions.

- The detection system is the “reactive” part of the instrument. Various technologies can be employed to detect volatile compounds: gas sensor arrays, mass spectrometry or gas chromatography. In the case of gas sensors, a change of electrical properties is recorded when in contact with volatile compounds, and these data constitute the input for further data processing. Each sensor is sensitive to all volatile molecules but each in their specific way. Therefore the array of several different sensors allows to characterize the overall volatile and odour profile of the samples in a unique way. In mass spectrometry instruments the raw data taken into account for data processing are the fragments mass to charge ratio and abundance, and for gas chromatography e-noses, the raw data consist of retention times and peak areas.

- The computing system works to combine the multiple raw data, which represents the input for the multivariate statistical processing. This part of the instrument performs global fingerprint analysis and provides results and representations that can be easily interpreted. Moreover, the electronic nose results can be correlated to those obtained from other techniques (sensory panel, GC, GC/MS).

Assessing and monitoring rancidity development is crucial to guarantee a good sensory quality.
In this study, the electronic nose used, the HERACLES (Alpha MOS, France), is based on the technology of ultra-fast gas chromatography with liquid or headspace injection modes. In order to increase analysis sensitivity, it includes an embedded Tenax trap located before the columns for pre-concentration of the injected fraction prior to rapid thermo desorption.

The instrument features two short columns of different polarities (DB5 apolar and DB1701 slightly polar), coupled to 2 Flame Ionization Detectors (FID). Therefore, two chromatograms are obtained simultaneously. The Electronic Nose was operated through its specific AlphaSoft software for instrument monitoring and statistical data processing. The AlphaSoft was used with AroChembase module consisting of a library of chemical compounds with name, formula, CAS number, molecular weight, Kovats retention index, sensory attributes and related bibliography. This will allow to characterize the chemical compounds detected on the chromatograms with both chemical and sensory information.

From the early days of these instruments, they have been used to analyse oily food products such as edible oils, to follow up shelf life (6), control the sensory quality (7) or compare sensory profiles (8). In this study, an HERACLES instrument was employed to perform headspace analysis of the nut appetizers with an aim to compare the odour profile of various nuts and get information on the behaviour towards oxidation and sensory ageing.

**Analytical conditions**

Headspace method was set up to analyse the volatile profiles of the appetizers. This method consists of heating the sample (in this case a solid sample) for a determined time period in order to homogenize the headspace phase i.e. the volatile compounds generated by the sample. Then a fraction of the headspace is collected and injected into the electronic nose for analysis.

For this study, the use of large vials (100mL) was particularly suitable to analyse entire nuts (easier sample preparation with no cutting) and larger quantities of product therefore more representative of the samples. Table 2 describes the analytical parameters applied for the analysis.

### RESULTS

**Chromatograms**

The comparison of chromatograms showed significant differences in volatile compounds profile between the different nut mixes (Figure 4). Bad products clearly show...
higher contents in volatile compounds. The characterization of these volatile compounds will help find and explain their origin and the possible causes of defects.

Characterization of volatile compounds

The nature of the main volatile compounds detected in the headspace of nut mix samples was investigated using their Kovats retention indices (Table 5) and the AroChemBase library. The main volatile compounds mostly correspond to secondary oxidation molecules: aldehydes, ketones and alkanes, which confirm that the presence of volatile compounds is strongly related to an oxidation phenomenon.

Table 5. Characterization of nut mix volatile compounds based on their Kovats Index.

<table>
<thead>
<tr>
<th>Retention time</th>
<th>Kovats Index</th>
<th>Possible matching compounds</th>
</tr>
</thead>
<tbody>
<tr>
<td>DB5</td>
<td>D81701</td>
<td></td>
</tr>
<tr>
<td>4.8</td>
<td>4.7</td>
<td>Pentane</td>
</tr>
<tr>
<td>6.3</td>
<td>7.4</td>
<td>2-butenal</td>
</tr>
<tr>
<td>8.9</td>
<td>8.4</td>
<td>2-pentenal</td>
</tr>
<tr>
<td>9.7</td>
<td>11.7</td>
<td>Hexanal</td>
</tr>
<tr>
<td>12.9</td>
<td>15.3</td>
<td>Heptanal</td>
</tr>
<tr>
<td>13.3</td>
<td>19.3</td>
<td>Octanal</td>
</tr>
</tbody>
</table>

The electronic nose allows to measure and characterize off-odors in oily products such as nuts.

Odour map

To globally compare products’ aroma, a general odour map based on Principal Component Analysis (PCA) was generated using all chromatograms peaks (Figure 5). Principal Component Analysis (PCA) is used to explore the data and to assess discrimination performance (i.e. the capability to determine which of the differences are important and to which degree). Very often, a PCA is presented as a two-dimension graphic that gives the plan showing the best discrimination of samples. A percentage is attached to each axis, which represents the part of the information brought by this axis. The discrimination index is calculated by dividing the surface of groups by the surface inter-groups. It gives the discrimination quality based on an indication of the surface between groups. When groups overlap each other, the discrimination index is negative. When groups are distinct, the discrimination index is positive; and the higher this value, the better the discrimination.

For the Electronic Nose, the PCA can be used to assess:
- the discrimination and similarities between different samples and groups
- the repeatability of the analytical method
- the detection of outliers

Odour map

It can be used for qualitative analysis, correlation with sensory panel or other technology as a preliminary study but also to perform product matching (i.e. comparison between different companies and competitive brands or formulations). In this study, E-Nose analysis confirmed the sensory panel results, since samples were clearly differentiated based on the 3 odour qualities linked to rancidity (Figure 6). The next step would consist of evaluating each type of nut individually to determine which ones mainly contribute to the global rancidity defect.

Individual aroma analysis of each nut type

In each mix, the nuts were separated by type (peanuts, almonds, cashew nuts, Brazil nuts, pecan nuts, hazelnuts). Then each type of nuts was analysed individually with the E-Nose and their aroma profiles compared with the corresponding mix. The analytical conditions were the same as the ones previously applied for the mixes analysis (Table 1). Figure 7 represents the odour map of bad B3 mix and its different nut components. The same odour maps built for the 2 other bad samples (B4, B5) and the medium quality sample (M2) showed a similar distribution of the nuts. In bad and medium quality samples, peanuts were the nuts having the volatile profile closest to nut mix aroma. To precisely evaluate the volatile compounds content and thus their propensity to oxidize, the Euclidean distance between a blank and each mix, then between the same blank and each type of nuts isolated, was calculated based on HERACLES E-Nose measurement (Figure 8). The Euclidean distance is calculated as follows:

$$D (X, Y) = \sqrt{\sum_{i=1}^{n} (X_i - Y_i)^2}$$

Where $X_i$ (respectively $Y_i$) is the surface of the $i$th peak among the $n$ selected peaks of the mix [or nut] (resp. the blank).
of the mix mass, the quality of this constituent must be carefully and strictly monitored. Pecans and cashew nuts also present a relatively high concentration of rancidity off-odours. However, the relatively low proportion of these nuts in the final mix (especially for pecan nuts) makes them less critical towards rancidity.

As for Brazil nuts, almonds and hazelnuts, that contain relatively lower amounts of volatile compounds, they have a low impact on the overall mix aroma. The same classification of odour intensities was observed between the different nuts in the various mixes. Every nut seems to undergo lipid oxidation with proportional rates. This could be explained by an oxidation kinetics dependent upon the fatty acid and tocopherol contents and the nut geometry. For example, the relatively higher level of γ-tocopherol observed in brazil nuts compared to peanuts could compensate the higher proportion of polyunsaturated fatty acids in the former. Furthermore, as the size of brazil nuts is relatively important compared with peanuts, the deep lipid content of these nuts may be better protected against oxygen and light, factors that accelerate oxidation. Globally, in order to guarantee the quality of nuts mixes, a quality control of each ingredient should be achieved before blending. This quality control should be conducted in priority on peanuts and cashew nuts, but the analysis of other minority nuts should not be neglected either.

The distance, the higher the volatile compounds content in the mix (or nut). The distance for hazelnuts, which was very low, is not presented in the graph. Peanuts and cashew nuts are the most concentrated in volatile compounds, which are linked with lipid oxidation and rancidity. Thus, peanuts are by far the most responsible for the off-odour in bad mixes. Cashew nuts could also have an important impact on final flavour of nut mix. Even in the good mix (G1), the different nuts show the same ranking based on volatile compounds content, but with much lower concentrations. This may indicate that the oxidation rate is the same in the various mixes.

CONCLUSION

The global aroma analysis performed thanks to HERACLES E-Nose showed that peanuts are the most critical ingredients in the development of rancidity off-flavours in nuts mixes. Indeed, among the 6 types of nuts contained in the appetizer, peanuts contain the highest level of volatile compounds. Moreover, as this nut accounts for approximately 60 percent...