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Quartz crystal microbalance gas sensor arrays for the quality control of chocolate

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ABSTRACT

Analysis of chocolate flavour through gas sensor arrays has been carried out. Two different set of sensors have been tested to assess the performance of the different sensor arrays: metallo porphyrins and gold nanoparticles peptide coated quartz crystal microbalances. Two series of chocolate samples containing differently formulated products (dark, white and milk) have been tested: the former made of samples obtained under standard process conditions, the second including samples added with some volatile compounds associated to degradation processes and/or low quality raw materials to obtain artificially off-flavoured samples.

Analysis with both gas sensor arrays resulted in a good discrimination between standard and artificially off flavoured chocolate samples. The best performance was obtained using the gold nanoparticles peptide sensors with over 90% of correctly assigned samples.

1. Introduction

Chocolate, which is a complex emulsion, is nowadays largely consumed throughout the world. Its sensory perception is largely affected by composition and processing techniques. For chocolate products flavour is one of the most important sensory attribute affecting acceptability by consumers. The presence of aroma compounds that varies in quantity depending on cocoa bean genotype and several processes occurring during chocolate production (fermentation, drying, roasting and conching) contribute to the complex volatile fraction of the final product [1,2]. However, non-volatile compounds (including polyphenols) and the characteristics of the continuous fat phase also strongly influence the release of volatiles into the mouth headspace and taste perception [3,4]. The development of a particular flavour is strictly related to the manufacturing process (and in particular all those involving heat, e.g. conching), the fermentation method and the origin of the cocoa. Typical classes of compounds that form the chocolate aroma pattern are alcohols, aldehydes, esters, ketones, furans, pyrans, pyrazines, pyridines, pyrroles, phenols, pyrones and thiozoles. Most of them are products of the Maillard reaction [4–7].

The peculiar flavour of chocolate products is also affected by the presence of other added ingredients (e.g. milk solids, nuts, vanilla). In milk and white chocolate, for example, flavour and aroma is significantly affected the presence of milk derivatives, sugar and cocoa and on they are processing before their addition [8,9]. Undesired aroma compounds that affect the overall typical flavour may be present in chocolate products due to several causes including the use of low quality raw materials (e.g. cocoa beans produced by an incorrect fermentation) and the application of not proper process conditions [1,10]. Moreover, during storage and distribution, physical processes and oxidative reactions, may alter flavour release and/or originate secondary volatile compounds and/or induce the decrease of some typical aromas highly affecting the overall flavour acceptability of the product.

The analysis of the volatile compounds is a main challenge in the food field for the evaluation of their quality, their freshness, and even to control the packaging material [11,12]. Gas-chromatography and mass spectrometry fingerprints matched with proper pattern recognition algorithms are used to detect and identify specific problems. Fingerprints can be also obtained with arrays of chemical sensors. When complemented by a multivariate data analysis technique, such as those used in

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chemometrics, sensor arrays allow for the quantification and the identification of compounds with a performance beyond that of a single device [13].

The aim of this work was to explore the possibility to use the e-nose based approach to assess the quality a food commodity rarely assayed with gas sensors as chocolate, through the detection of off-flavours. For this purpose two different types of gas sensor arrays, carrying different ligands have been used in order to compare their performances. Porphyrins exhibit unique binding properties that are widely exploited in nature to accomplish functions essential for life; the potential mimic of these functions with synthetic counterparts has provided the basis of many kinds of chemical sensors. The porphyrin molecular framework offers a wide range of interaction mechanisms for analyte binding, spanning from the weak Van der Waals forces to hydrogen bond, to $\pi-\pi$ interactions and finally to the coordination to the central metal ion[14]. On the other hand the ability of gold nanoparticles modified with oligopeptides has been recently proposed [15–17]. Encouraging results have been obtained due to the ease of derivatisation, high number of possible configuration and possibility to design via molecular modelling the ligands. The analysis of the headspace of different chocolate samples was optimised and carried out by using the two different arrays while GC-MS was carried out on the same samples in order to obtain a conventional characterisation of the aroma pattern and compare the data. Sensors data were used to define a Partial Least Squares-Discriminant Analysis (PLS-DA) classifier aimed at recognizing the artificially prepared off-flavoured with respect to standard samples.

2. Materials and methods

2.1. Materials

All chocolate samples used in this study were provided by Belcolade, division of Puratos (Industrielaan 16, Industriezone Zuid III, 9320 Erembodegem, Belgium). Two types of samples were analysed, *standard* and *artificially off-flavoured*, of three different kind of chocolate; dark, milk and white.

Standard samples were taken from the conventional chocolate process carried out at industrial scale. All the samples were belonging to batches produced from June 2012 to January 2013. The chocolate samples were provided in drops (weight ~3,20 g each) or as tablet (~250 g each). Upon sampling, they were stored wrapped up in a double-layer aluminium foil to avoid direct exposure to light and oxidation reactions and hermetically packed in high barrier plastic bags until analysis.

Artificially off-flavoured samples were prepared at lab scale by adding cocoa butter preliminarily mixed with different off-flavours to melted chocolate. Each off-flavour compound was added in the cocoa butter to achieve a concentration of 125 ppm. Artificially off-flavoured cocoa butter was then added to 400 g of chocolate in order to obtain an estimated final concentration in the sample of ~6 ppm. The chocolate was then tempered, moulded, cooled and demoulded in small square shaped tablets of ~15 g. All the 21 samples (7 for 3 types of chocolate) were then wrapped in a double layer aluminium foil, labelled and stored in plastic boxes.

The volatile compounds used to obtain the off-flavour samples were from Sigma-Aldrich Co (Diegem, Belgium) and chosen as index of uncorrect/unsuitable chocolate processing. In particular the following aromas were taken into account: 3-methylbutanal, phenylacetaldehyde (typically produced during fermentation), acetic acid (produced in conching), tetramethylpyrazine, 2-acetylpyrrole (roasting), 2-nonenal and 2,4-decadienal (fat oxidation). These volatile compounds were added in all the three different types of chocolate under study to obtain the artificially off-flavoured products.

2.2. SPME-GC-MS analysis

Gas-chromatography (GC) combined with mass spectrometer detector (Agilent 5975C Series GC Inert MSD, Agilent Technologies), equipped with an autosampler (Multipurpose Sampler, GERSTEL GmbH & Co. KG) was used to analyse the vapour phase of all the off-flavoured chocolate samples and some of the standard samples. Sampling of the volatile compounds was performed by solid phase micro-extraction (SPME). For each analysis about 5 g of chocolate were cut in small pieces and 1 g was taken and inserted in a 20 ml-vial that was closed with crimp top caps and rubber septa. The sample was kept for 10 min at 40 °C and then exposed to the fiber (StableFlex™, 50/30UM DVB/CARBOXEN-PD, Sigma-Aldrich Co.) for 30 min at fixed temperature. The fiber was inserted in the desorption chamber where GC analysis was carried out with the following temperature gradient: the column was kept 7 min at 40 °C then the temperature was raised up to 220 °C at 16 °C/min and then kept at 220 °C for 8 min. The column used was a Stabilwax® Column (Restek Corporation • 110 Benner Circle • Bellefonte, PA) (lenght 30 m; internal diameter 0.25 mm; film thickness 0.50 μm). Chromatograms obtained were analysed and compounds were assumed to be identified for matching higher than 70% in the mass spectrometer library.

2.3. E-nose analysis

The analyses were carried out using a "Ten 2009" Electronic nose (Tor Vergata Sensors Group, Rome, Italy), equipped with 8 Quartz Crystal Microbalance (QCM) sensor array. Two set of sensors have been used: the first set was coated with a different spray-dried metallo-tetraphenylporphyrin [Me-TPP] (Cu-TPP, Co-TPP, Fe-TPP, Cr-TPP, Sn-TPP, Mn-TPP, Ni-TPP, Mg-TPP), in the second set the sensors were modified using different peptides (Thioglycolic acid, Glutathione, Cys-Ile-His-Asn-Pro, Cys-Ile-Gln-Pro-Val, Cysteinylglycine, Cys-Arg-Gln-Val-Phe, Cysteine) immobilised on gold nanoparticles as reported in [15]. A sensor modified just with gold nanoparticles (GNPs) was also used in the array.

Analysis of the samples was run using 15 g aliquots of chocolate in glass lab bottles (100 mL) heated at 40 °C until full melting. Chocolate was grated or cut in small pieces to favour melting. The sample was then slightly stirred to eliminate possible phase separation of fat and obtain an uniform release of the volatile compounds.

Nitrogen was selected as gas carrier for the experiments and fluxed for 2 min at a flow rate of 4 L/h, measured by a flow meter (DK 800R KROHNE, Germany) through the sample bottle connected to the E-nose system. The purpose of this step was to remove air from the headspace and achieve reproducible measurement conditions. The carrier gas flow was then diverted and the sample was kept for 10 min at 40 °C. This range of time was selected as the optimum time required to reach a steady state by the chocolate volatile compounds in the headspace sample's volume. The N_2 enriched with the volatiles present in the vapour phase of the chocolate sample was then assayed by the E-nose. The file created by the dedicated software allows the operator to compute the Δf and Δf_{Max} from the data recorded. Δf for each sensor was defined as the difference of frequency values between the beginning and the end of the measurement (8 min). The Δf_{Max} represents the difference between the frequency value at the beginning of the measurement and the lowest value reached by the sensor during the measurement.

2.4. Statistical analysis

Matlab (R2009b, Mathworks, Natick, MA, USA) has been used to compute the Δf values of the experiments and to run