Head-space gas chromatography coupled to mass spectrometry for the assessment of the contamination of mayonnaise by yeasts

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ABSTRACT

Head-space (HS) gas chromatography (GC) coupled to mass spectrometry (MS) is proposed for the assessment of the contamination of mayonnaise as an alternative to plate counting, which is the technique commonly used for evaluating microbial contamination. More specifically, this method was applied in the detection of *Candida metapsilosis* and *Zygosaccharomyces bailii*, both of great importance in term of food spoilage since they are resistant to many of the common methods of food preservation. Different chemometric models were investigated using the data obtained by GC-MS (*m/z* profile, area of the chromatographic peaks and entire chromatographic profile), in order to obtain the highest classification success. The best results were obtained using the chromatographic profile (success rate of 92%). Contaminated samples could also be classified according to the concentration of yeast, obtaining a success rate of 87.5%. Finally, a chemometric model was constructed in an attempt to differentiate between strains.

Keywords: gas chromatography; mass spectrometry; mayonnaise; yeast determination; chemometrics

1. Introduction

Microbial contamination, a major problem in the food industry because of associated economic losses, may occur due to the appearance of bacteria, filamentous fungi or yeasts in food. However, because of the great damage they originate, most studies have concentrated on bacteria or filamentous fungi. Although yeast plays a secondary role in food contamination, the environmental conditions of food preservation, which tend to inhibit the growth of bacteria, have favoured the appearance of contaminating yeasts, which are responsible for alterations in the organoleptic properties of foods and, consequently, for food quality.

Among yeasts, the genus *Zygosaccharomyces* is of great importance in food deterioration. Its biochemical characteristics enable it to tolerate high concentrations of sugar, ethanol or acetic acid, and it is resistant to most common preservatives used in food, such as sorbic acid, benzoic acid or SO_2 (Kurtzman, Rogers, & Hesseltine, 1971); it is extremely osmotolerant and is capable of vigorously fermenting hexagonal sugars, with a capacity to grow in media with a pH of around 2.2. *Z. bailii* is one of the most problematic species due to its exceptional tolerance to stressful conditions; for example, it can tolerate up to 70 °C in a high glucose environment. *Z. bailii* can therefore contaminate a wide variety of acidic or high-sugar foods, which would normally be considered stable during storage, such as mayonnaise. Besides its effect on the quality of the product, the alterations of the organoleptic properties of foods caused by this yeast, make them more susceptible to colonization by other microorganisms (Vermeulen, 2008).

For its part, *Candida parapsilosis* is a pathogenic yeast that has been taxonomically classified into 3 groups: *C. parasilopsis*, *C. orthopsilosis* and *C. metapsilosis* (Lo et al., 2017; van Asbeck et al., 2008). This yeast is part of the normal flora of the skin, hands and mucous membranes and can contaminate food in situation of poor hygiene or manufacturing practices. *C. parasilopsis* has been detected and isolated in fruits (Lo et al., 2017), yogurt at concentrations between 10 colony forming units (CFU)/g and 10^6 CFU/g (Rohm, Lechner, & Lehner, 1990), and sauces (Robl et al., 2014), being also very resistant to food preservation treatments. The determination of *Z. bailii* and *C. parasilopsis* is therefore a factor of great importance in the shelf life of certain foods such as sauces.

The most commonly used technique to detect the presence of yeasts is microbial plate counting. However, this methodology requires long periods of time compared to other analytical techniques and so its routine application is not practical, which is one of the reasons why, in the last years, have seen growing interest in the development and application of alternative analytical methods to solve this agrifood problem.

To date, several alternatives have been proposed, such as methods based on direct amplification of the yeast DNA of a sample through the polymerase chain reaction (PCR) (Andorrà, Berradre, Mas, Esteve-Zarzoso, & Guillamón, 2012), or molecular methods based on the analysis of genes encoding ribosomal RNA (Garner, Starr, McDonough, & Altier, 2010). However, these methods are tedious, slow or expensive, and do not distinguish between viable and dead cells (Elizaquível et al., 2013), which complicates their implementation in the food industry. Enzymatic immunoassays (ELISA, enzyme linked immunosorbent assay) have also been proposed, although they have lack of sensitivity when matrices are complex, cross reactions between related antigens, and their automation would also involve a high cost (García et al., 2004). More recent studies describe the use of mass spectrometry (MS) for detecting and identifying yeasts, more specifically, the use of matrix-assisted laser desorption/ionization (MALDI) coupled to a time-of-flight (TOF) analyser (Bizzinia & Greubab, 2010; Quiles-Melero, García-Rodríguez, Gómez-López & Mingorance, 2011; Taverna et al., 2019). This technique allows the identification of microorganisms through an analysis of proteins, associating a specific mass spectrum to a given species. However, it presents drawbacks when used in direct samples due to its low sensitivity, and the need for a considerable amount of protein in order to obtain reliable profiles.

Wang et al. (2015) also proposed a metal oxide sensor to detect two osmotolerant yeasts, *Z. rouxii* and *C. tropicalis*, in a high-sugar medium. This sensor array comprises 10 metal oxide semiconductor chemical that allow to detect mainly aromatic compounds, nitrogen oxide, ammonia, hydrogen, alkanes, sulfur compounds, many terpenes and sulphur and alcohols. They demonstrated that sensors sensitive to methane, alcohol, and aromatic compounds might the most important for detection of spoilage caused by *Z. rouxii* and *C. tropicalis*.

In addition, the authors studied the evolution of the volatile organic compounds (VOCs) fingerprint during the growth of these two yeasts using solid-phase microextraction

(SPME) and gas chromatography (GC) coupled to MS. The VOCs produced were mainly composed of alcohols, ketones, aldehydes, acids, and esters. Although the SPME-GC-MS method was not proposed for classifying real contaminated food samples, since the above experiments were only carried out in culture media, it seems that studying the evolution of VOCs by means of GC-MS could be a good alternative for the detection and quantification of yeasts in food.

In fact, it has also been demonstrated that different yeast strains (*Saccharomyces cerevisiae* and *Lachancea thermotolerans*), used during the fermentation of sun-dried must, exercise a great influence on volatile profile of wine (Morales, Fierro-Risco, Ríos-Reina, Ubeda & Paneque, 2019). In this case, volatile composition was determined by dual sequential stir bar sorptive extraction, followed by GC–MS analysis.

The chromatogram obtained from the analysis of microbial volatile compounds can provide information on microbial activity, abundance, community structure, community level and physiological activity (Araki et al., 2012). However, the difficulty of these experiments lies in the large number of metabolites produced, and the diverse nature of their chemical and physical properties, which makes the simultaneous quantification of all metabolites unattainable with current instrumental capacities (García et al., 2004). On the other hand, the limited distribution of molecular weight does not allow for a complete and detailed analysis using only MS, so that it generally needs to be coupled to a separation technique such as GC (Viswanadhan, Rajesh, & Balaji, 2011). This coupling generates a large volume of data, making their processing more complicated.

In this work, head-space (HS) GC-MS is presented as an alternative method for the detection and quantification of two problematic yeasts (*Z. bailii* and *C. parasilopsis*) in sauces, avoiding lengthy times and costs involved in the traditional plate counting technique. Different chemometric models are investigated using all the data obtained by GC-MS (*m/z* profile, peak area of the chromatogram, the entire chromatographic profile or TIC and combination of *m/z* values and TIC), in order to obtain the highest classification success. The results are compared with the chemometric models constructed using other characteristics of the contaminated sauce such as pH or colour.

2. Materials and methods

2.1. Reagents

All the reagents used in this work were of analytical grade and the solvents were of HPLC grade. Hexanal, acetaldehyde, acetone, dimethylsulfide, 2,3-butanodione, ethyl acetate, pentanal, chlorobenzene and methanol (MeOH) were supplied by Sigma Aldrich (St. Louis, MO, USA) and dimethyl sulfoxide by ApplicChem GmbH (Darmstadt, Germany). Tryptic soy broth (TSB), sabouraud dextrose agar with chloramphenicol and peptone water, all from Pronadisa Conda (Madrid, Spain), were used for the preparation of culture media. In addition, chlorobenzene was used as internal standard (IS) in the GC-MS analyses. This was prepared by diluting 2 µL of chlorobenzene in 25 mL of dimethyl sulfoxide to obtain a concentration of 80 µL/L. The solution was stored at -4°C until use. Standard solutions of 1000 mg/L of hexanal, acetaldehyde, acetone, dimethylsulfide, 2,3-butanodione, ethyl acetate and pentanal were prepared in MeOH.

2.2. Instrumentation and software

GC-MS analyses were carried out on a 7890A GC-System gas chromatograph from Agilent Technologies (California, USA), equipped with a temperature-controlled vaporizer (PTV) model CIS4-C506 and an automatic injector (Headspace model Multipurpose Sampler MPS), both from Gerstel (Mülheim an der Ruhr, Germany). The GC system was coupled to a mass spectrometer (5975C inert MSD-triple axis detector from Agilent Technologies). The chromatographic separation was carried out on a DB-624 column with an internal diameter of 0.25 mm, a length of 60 m and a film thickness of 1.40 µm, consisted of 94% dimethylpolysiloxane and 6% by cyanopropylphenyl, also from Agilent Technologies. The injection was made in split mode with a ratio of 1:10. The GC temperature programme was: start temperature 40 °C, hold for 5 min, increase to 150 °C at 10 °C/min and maintain for 2 min; next, the temperature of 220 °C was reached at 25 °C/min and held for 2 min. The mass spectrometer was operated using electron-impact (EI) mode (70 V) and the temperature of the ion source was 230°C. Analyses were carried out using scan mode from 29 to 150.

A SensIONTM pHmeter (Hach, Colorado, USA) was used for pH measurements. Colour determination was carried out using a 962 colorimeter from X-Rite (Michigan, USA) and a Nicolet Evolution 300 spectrophotometer of Thermo Electron Corporation (Massachusetts, USA) was used to prepare the inoculum of *C. metapsilosis* and *Z. bailli*.

Data were processed using Microsoft Office Excel (Microsoft, Washington, USA) and Simca-P (Umetrics, Malmö, Sweden).

2.3. Strains and culture conditions

Two different species of yeast were used: *Z. bailii* and *C. metapsilosis*. The first was obtained from the Spanish Collection of Valencia Type Crops (CBS 680) which was isolated by Lindner and Guilliermond in 1912. *C. metapsilosis* was isolated from a sauce by Productos del Sur S.A., and its identity was verified by comparing the DNA sequencing of the isolated strain with that available in the Spanish Collection of Valencia Type Crops.

The yeast inoculum was prepared by transferring a colony obtained on Sabouraud dextrose with chloramphenicol agar plate to a soybean digested medium (TSB), which was incubated for 24 hours at 25°C. Fresh cultures for the experiments were performed with a sterile seeding loop by incubating a colony of a pure culture for 24 hours in TSB at 25°C. The inoculum was standardized by dilution in TSB to an optical density of 0.9 at 600 nm for *C. metapsilosis* and 0.5 for *Z. bailli*, which is equivalent to a yeast concentration of 10^7 CFU / mL and 10^5 CFU / mL, respectively. Yeast populations were estimated by diffusing suitably diluted aliquots onto plates with agar and chloramphenicol, followed by incubation at 25°C for 48 hours.

2.4. Elaboration and inoculation of mayonnaise

The experiments were carried out using light mayonnaise, i.e. mayonnaise but with lower fat content, and it was prepared using a cooking robot under sterile conditions. The formulation of each mayonnaise consisted of 55.05% soybean oil, 29.00% water, 8.00% yogurt, 3.00% vinegar, 1.80% salt, 1.70% sugar, 0.50% caseinate, 0.80% xanthan gum and 0.15% guar gum. Two different food additives were tested to increase the variability of the samples and the profile of volatiles generated. Specifically, GNS-plus, a synergistic combination of vegetable extracts of citrus fruits (orange, lemon and grapefruit) and dill, and sorbate-benzoate, a mixture (1:1) of potassium sorbate and sodium benzoate. In this way, 21.60 kg of mayonnaise was prepared, which was divided into two batches (10.80 kg each). A batch contained 0.1% of sorbate-benzoate and the other 0.1% of GNS additive.

Aliquots of 50 g of mayonnaise were placed in sterile polyethylene bags. One third of the bags was inoculated with *Z. bailii* and another third with *C. metapsilosis* at concentration of 10^3 CFU/mL. The bags were heat sealed and incubated at 5°C, 25°C and 37°C until sampling. One gram of each bag was sampled weekly for seven weeks, obtaining a sample for each temperature, each strain, and each additive. The remaining bags were not inoculated in order to monitor the evolution of the mayonnaise with no yeast contamination and were incubated and sampled in the same conditions.

Colony forming units (CFU) were determined by plate count, following ISO 6887-1. Mayonnaise samples were diluted in peptone water, and the appropriate dilutions were seeded in sterile plates. Then, sabouraud dextrose agar with chloramphenicol was added and the plates were incubated at 25 °C for 48 hours before counting.

2.5. Sample analysis by GC-MS

For HS-GC-MS analyses, 1 g of mayonnaise was weighed into a 10 mL vial and 50 µL of the chlorobenzene internal standard solution at 80 µL/L was added.

Samples were incubated at 80°C for 20 minutes and injected into the GC. In the PTV, the sample was cooled to 0°C, and heated for 30 seconds at 12°C/s until reaching 180°C, which was maintained for five minutes.

To quantify the hexanal content a calibration curve was established using standard solutions in dimethyl sulfoxide at the following concentrations: 2, 4, 8, 12 and 16 µg/L, considering the ratio peak area of hexanal/peak area of chlorobenzene (IS) as the analytical signal.

2.6. *Measurement of pH and colour*

Each sample was measured for pH and colour. For the colour determination, the parameters L* (lightness), a* (balance between green and red) and b* (balance between yellow and blue) were determined with an X-Rite 962 spectrophotometer using the D65/10° illumination/observation method. Although the three parameters (L*, a* and b*) were determined, only the L* parameter was considered for the construction of the chemometric models because the other two parameters remained constant throughout the experiment.

2.6. Data treatment

The chemometric analysis consisted of a qualitative analysis of the principal components (PCA) with a confidence interval of 95%, followed by a discriminant analysis of orthogonal partial least squares (OPLS-DA). The OPLS-DA filtered the variation not directly related to the response and maximized the variance among the categories by minimizing it within them. In all the cases, data were randomly divided into two different sets: a classification set (80%) for construction of the models and a validation set (20%). In addition, six different scaling (unit variance (UV), unit variance none (UVN), pareto (Par), pareto none (ParN), centering (Crt) and freeze), as well as the logarithmic transformation of the data, were tested. With UV scaling, the variable j is centered and scaled to "Unit Variance", i.e. the base weight is computed as 1/sdj, where sdj is the standard deviation of variable j computed around the mean. The Par scaling is in between no scaling and UV scaling. The variable j is centered and scaled to Pareto Variance, i.e. the base weight is computed as 1/sqrt(sdj), where sdj is the standard deviation of variable j computed around the mean. UVN and ParN scaling are the same as UV and Par, respectively, but the variable is not centered, i.e. the standard deviation is computed around 0. With Crt, the variable is centered but not scaled and with freeze, the scaling weight of the variable is frozen and will not be re-computed when observations in the workset change or the variable metric is modified after the freezing.

Variable importance in projection (VIP) graphics were also obtained, to check the importance of each variable on the construction of the final model.

3. Results and discussion

3.1. Yeast detection in mayonnaise samples

Initially, all mayonnaises were analyzed using plate counting to assess the microbial contamination level. Based on these results, samples were divided into two groups: contaminated (120 samples) and non-contaminated (130 samples) and were analysed by HS-GC-MS. The pH and colour were also measured. The evolution of log10, pH and colour is shown in Supplemental Figure S1 and as can be seen, no significant differences were obtained between incubation temperatures and sampling time.

Then, chemometric models were constructed using the available data in order to obtain a classification model that allowed the differentiation of contaminated and non-contaminated samples, as an alternative to plate counting.

Four different chemometric models were constructed using: a) the variables of pH, colour and hexanal content obtained by GC-MS analysis; b) the *m/z* profile, simulating that the samples have not been previously separated in the GC; c) area of all the peaks present in the total ion chromatogram (TIC) obtained by GC-MS; and d) the entire chromatographic profile.

3.1.1. Sample classification using pH, colour and hexanal content

The variables of pH, colour and hexanal content (by-product of fat oxidation) have been widely related to microbial food contamination (Huis in't Veld, 1996; Shahidi & Pegg, 2007; Collins & Buick, 1989). For this reason, in a first attempt to obtain a fast and simple analytical method, as an alternative to plate counting, a chemometric model was obtained using these three parameters.

Initially, a PCA model was carried out using the UV scaling. However, separation between the classes was not good and so an OPLS-DA model was therefore implemented. The best validation results were obtained using raw data and UV scaling (Supplemental Table S1). However, the separation between contaminated and non-contaminated samples was not effective using these three parameters (Figure 1), which produced a success rate of 60.0%. While 76.9% of non-contaminated samples were correctly classified, only 41.7% of contaminated samples were well identified (Supplemental Table S2).

Alterations in pH, colour and hexanal content seemed to be mainly related to the additive used, in this case sorbate-benzoate or GNS, since both group of samples appear well separated in the OPLS-DA chart.

3.1.2. Sample classification using m/z profile

The monitored *m/z* ranged from 29 to 150 and the *m/z* profile was obtained using GC software. Data were processed by summing each *m/z* throughout the entire analysis time, i.e. it simulates the sample not being previously separated in the chromatographic column.

Data differed considerably between samples and, in order to equalize the data matrices so that they were comparable, *m/z* were rounded to the nearest whole number, summing all the intensity values for the same whole number.

As in the previous section, a PCA model did not provide good separation between groups. Therefore, an OPLS-DA was performed using the 80% of samples (Figure 2). In this case, different scaling data transformations were also tested, obtaining the best results with data transformed to the logarithm ParN scaling (Supplemental Table S3). This scaling reduces the impact of noise and artefacts on models (Wiklund et al., 2008), dividing each variable by the square root of its standard deviation.

OPLS-DA was validated using the remaining 20% of samples and a success rate of 78.0% was obtained (84.6% of non-contaminated and 70.8% of contaminated samples were correctly classified, Supplemental Table S4).

The contribution plots (Supplemental Figure S2) showed that *m/z* values of between 121 and 150 contributed significantly to the correct classification of non-contaminated samples, while *m/z* values of between 29 and 74 were suitable for classifying contaminated samples. It could be associated to the decomposition of the mayonnaise components in volatile compounds of smaller size by the action of the yeasts, although it would be studied in more detail.

3.1.3. Classification of samples using area of all the peaks present in the TIC obtained by HS-GC-MS

As mentioned above, the limited distribution of molecular weight does not allow for a complete and detailed analysis using only MS, and so the chromatogram obtained by GC-MS was studied.

Initially, the peaks present in the TIC were integrated manually (Figure 3). These peaks were normalized with respect to the area of the IS. Of the 13 peaks integrated, seven of them could be identified as acetaldehyde, acetone, dimethyl sulphide, 2,3-butanodione, ethyl acetate, pentanal and hexanal, while the remaining 6 were processed as unknown compounds. The seven compound were identified using the GC-MS library and were confirmed by injecting fine sauce spiked with each compound at 1 μg/g.

OPLS-DA models were also constructed using different scaling and data transformation. In this case, the highest classification success was obtained using raw data without transformation and an UVN scaling (Supplemental Figure S3, Supplemental Table S5). However, the use of peak area was also insufficient for the totally successful classification of samples, since contaminated and non-contaminated samples appeared intermixed. The application of the OPLS-DA models to the validation set gave a classification success of 76.0%, classifying correctly 88.4% of non-contaminated samples but only 62.5% of contaminated samples (Supplemental Table S6). Therefore, this method would result in a high number of false negatives. The peaks that contributed most to the classification corresponded to acetaldehyde, ethyl acetate, hexanal, 2,3-butanodione and one non-identified compound (unknown compound 5) (Supplemental Figure S4). These compounds tended to show higher intensity in the contaminated samples, but due to the great variability between samples, a clear relationship could not be established (Supplemental Figure S5).

3.1.4. Classification of samples using the total ion chromatogram

The TIC is the sum of the intensities of all the mass spectral peaks belonging to the same scan, so it contains all the information concerning a sample and can be used as a fingerprint. OPLS-DA models were therefore constructed using TIC data.

Baseline correction was not necessary, since the TICs were stable throughout the experiment. In this case, the best models were obtained using logarithmic transformation and the UV scaling (Figure 4, Supplemental Table S7), which provided a success rate of 92.0%. The 92.3% of the non-contaminated samples and 91.7% of the contaminated samples were correctly classified (Table 1).

3.1.5. Classification of samples by combining the m/z values with the chromatographic profile

The best classification results were obtained using TIC (92.0 %) and *m/z* data (78.0%) separately. Then, new OPLS-DA models were obtained combining both data in order to enhance the classification success. The best results were also obtained using logarithmic transformation and UV scaling (Supplemental Table S8). However, the classification success was the same as when only the TIC data were used.

3.2. Classification of contaminated samples according to microbial concentration

The chemometric models constructed using the TIC data obtained by HS-GC-MS demonstrated high success in classifying samples contaminated and non-contaminated by yeast. Therefore, the potential of TIC data to quantify the yeast level was investigated.

First, contaminated samples were divided into seven groups with different concentration levels of yeast: group 1 (between 10^1 and 10^2 CFU/g), group 2 (between 10^2 and 10^3 CFU/g), group 3 (between 10^3 and 10^4 CFU/g), group 4 (between 10^4 and 10^5 CFU/g), group 5 (between 10^5 and 10^6 CFU/g), group 6 (between 10^6 and 10^7 CFU/g) and group 7 (between 10^7 and 10^8 CFU/g). OPLS-DA models were also constructed with 80% of samples (calibration set) using both the raw data matrix and the logarithmically transformed matrix, and different scaling. The optimal model was obtained using the raw matrix again adjusted to a UV scaling. However, this model was not sufficient for differentiating between the seven groups of samples and a success rate of 54.17% was obtained when it was applied to classify the validation set (20% of remaining samples).

Therefore, other concentration ranges were selected, and the calibration set was divided into three balanced groups: group 1 comprised the concentration range from 10^1 to 10^3 CFU/g (31 samples), group 2 from 10^3 to $10^{5.5}$ CFU/g (32 samples) and group 3 from $10^{5.5}$ to 10^8 CFU/g (32 samples). In this case, good separation was achieved between groups (Figure 5) and the best model was also obtained applying a logarithmic transformation and UV scaling (Supplemental Table S9). The validated model had a classification success of 87.50%.

3.3. Classification of contaminated samples according to yeast strain

Given the success of HS-GC-MS to classify contaminated sauce samples according to the yeast concentration, the suitability of the method for differentiating between different types of yeast was studied.

For that, contaminated samples were divided into two groups: samples contaminated with *Z. bailii* and samples contaminated with *C. metapsilosis*. Most of the contaminated samples belonged to the *Z. bailii* group, since this yeast grows faster than *C. metapsilosis*. So, in order to obtain balanced group a number of 31 samples was included in each group.

The best chemometric model was obtained with data transformed logarithmically and adjusted to a UV scaling. Both groups appeared completely separate (Supplemental Figure S6). However, when this model was applied to the validation sample set, only 62.50% success was achieved. This model should be improved, e.g. by increasing the number of samples during the calibration, for it be used successfully.

4. Conclusions

HS-GC-MS analysis is proven to be a viable alternative to classical microbial counting methods for the identification and quantification of yeasts in sauces, reducing time and costs.

The use of MS without previous separation in GC does not allow the optimal classification of samples, and it needs to be coupled to GC. Most authors opt to monitor some VOCs by GC-MS to detect samples contaminated by microorganisms. However, it was demonstrated that the use of the entire chromatographic profile (TIC), and therefore of the complete VOC profile, achieves better results when classifying sauces contaminated by yeasts, since a validation success of 92.0% was achieved. Other parameters such as pH and colour were not suitable for the correct classification of the samples.

Moreover, the complete VOCs profile obtained by HS-GC-MS allowed the contaminated samples to be classified according to yeast concentration level, with a validation success rate of 87.50%.

Chemometric modelling using these data allows different type of yeast to be differentiated, in the present case *C. metapsilosis* and *Z. bailii*. However, although two well-differentiated groups were obtained, when the model was applied to the validation set only 62.50% of samples were correctly classified.

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Figures

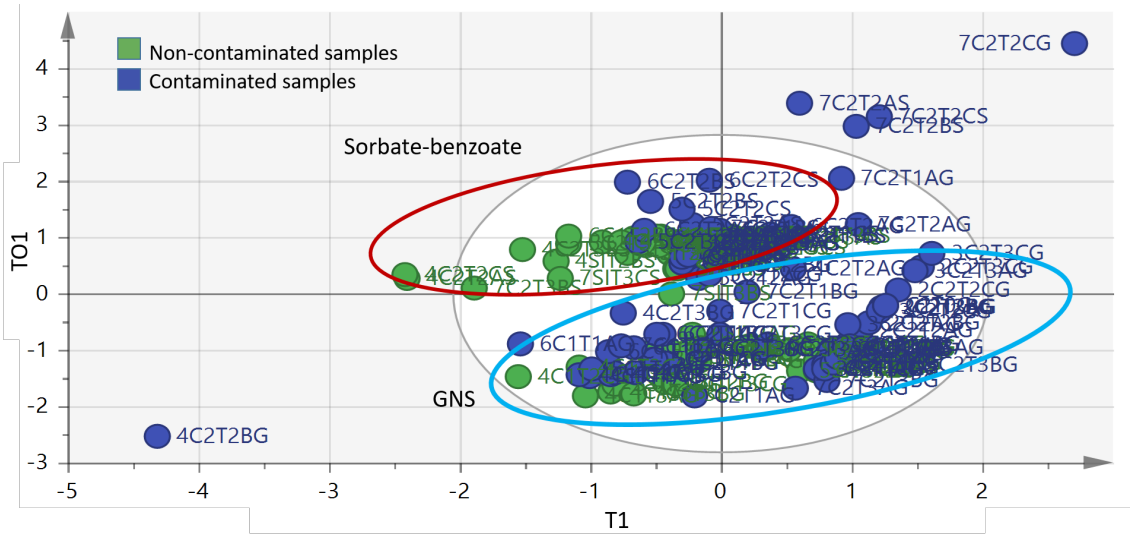


Figure 1. OPLS-DA model obtained using variables of pH, colour and hexanal content. The additive sorbate-benzoate and GNS are indicated with an "S" or "G" at the end of the sample code, respectively.

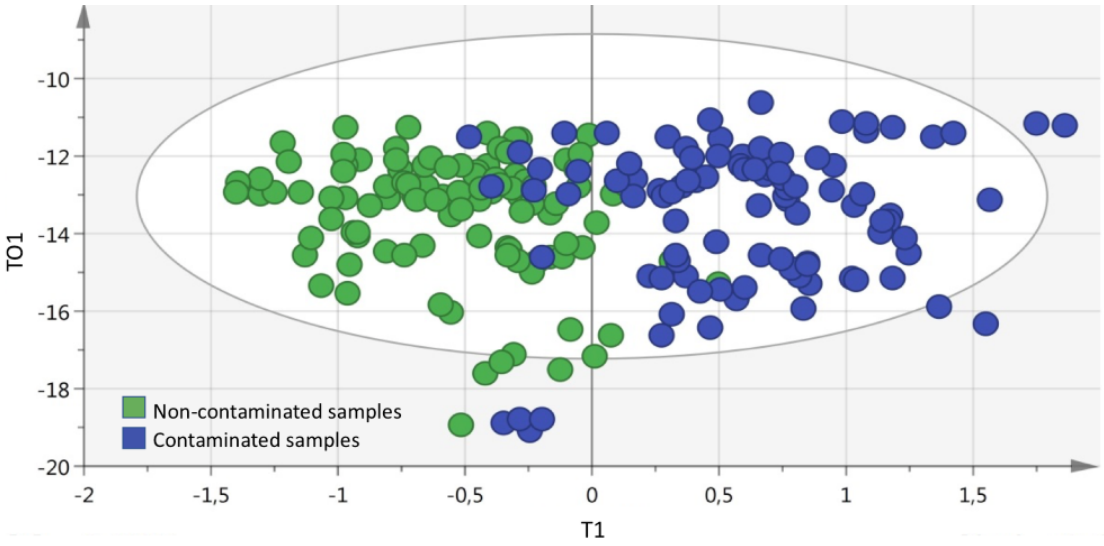


Figure 2. OPLS-DA model obtained with *m/z* profile data

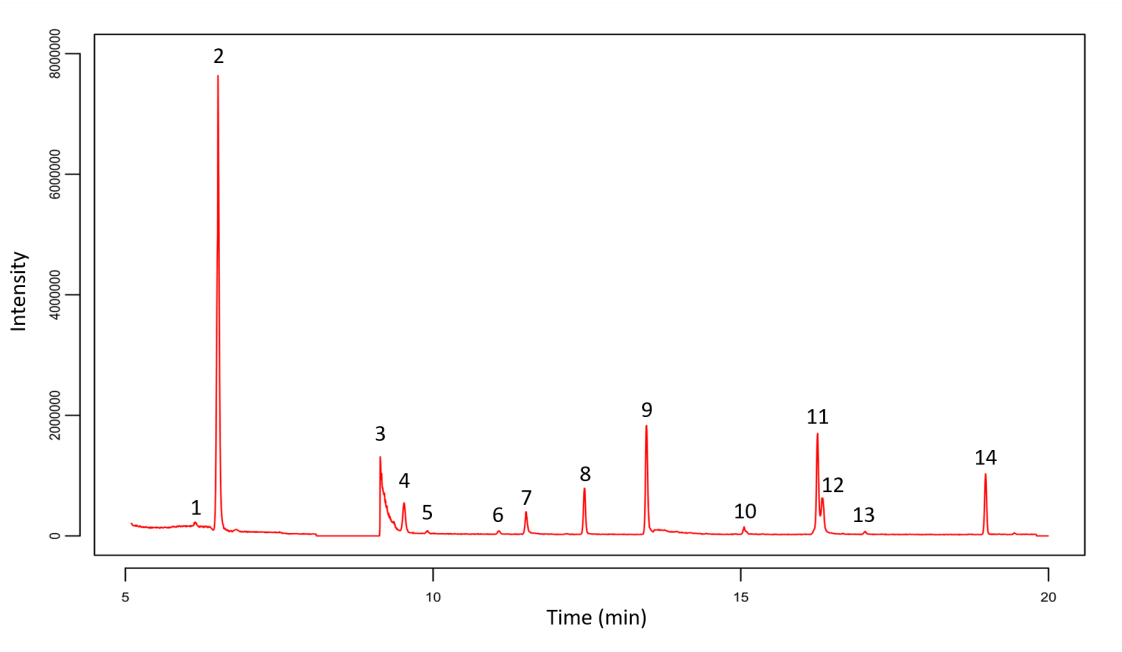


Figure 3. Chromatogram of a sauce sample obtained by HS-GC-MS. 1: Unknown 1, 2: Acetaldehyde, 3: Acetone, 4: Dimethyl sulphide, 5: Unknown 2, 6: Unknown 3, 7: 2,3-Butanodione, 8: Ethyl acetate, 9: Unknown 4, 10: Pentanal, 11: Hexanal, 12: Unknown 5, 13: Unknown 6, 14: Chlorobenzene (IS).

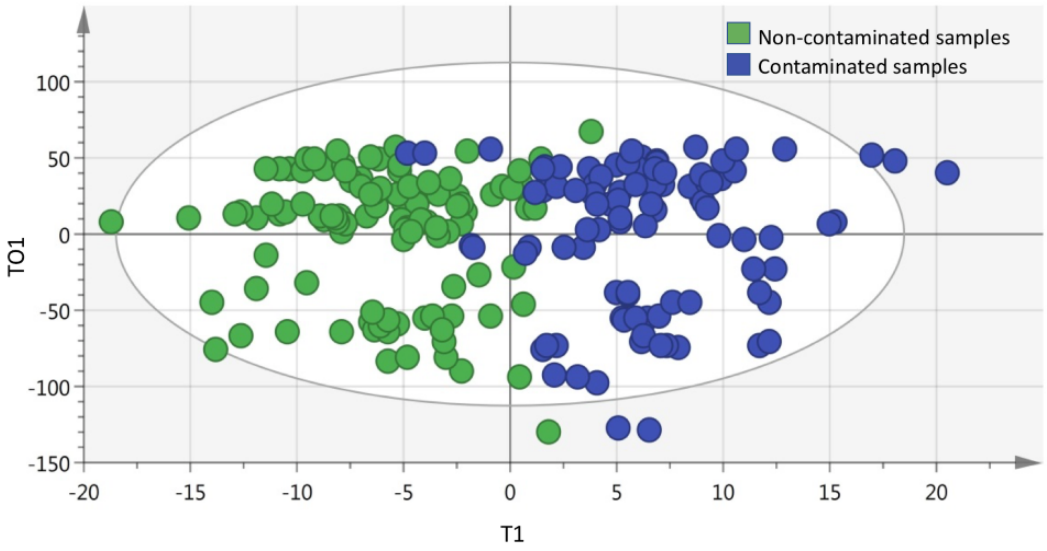
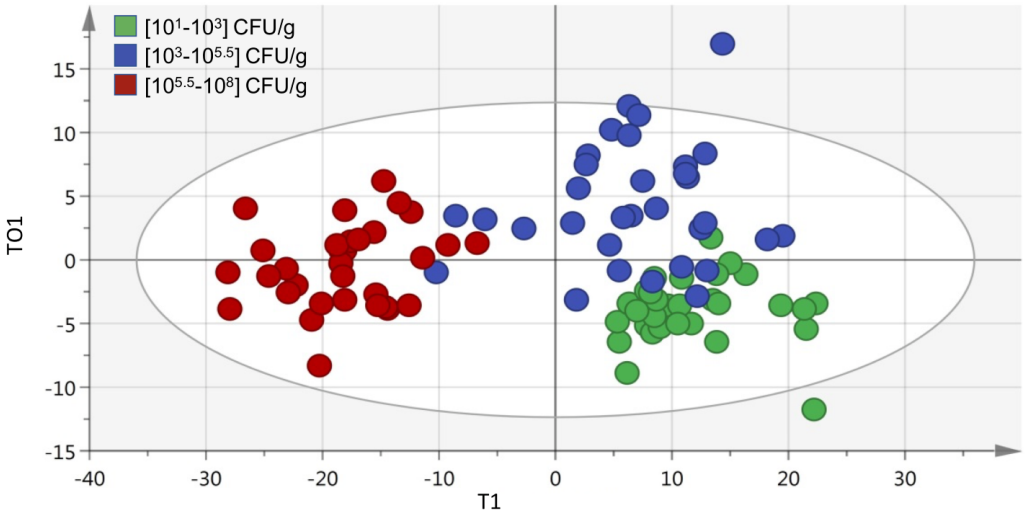


Figure 4. OPLS-DA model obtained using total ion chromatogram data



475

476 Figure 5. OPLS-DA model constructed using TIC data to classify contaminated samples
477 into three groups of yeast concentrations.

478

479 Table 1. Validation matrix for non-contaminated/contaminated sauce samples of the
480 OPLS-DA model built using the TIC

PREDICTION / ACTUAL	Contaminated samples	Non-contaminated samples
Contaminated samples	22	2
Non-contaminated samples	2	24
Total	24	26
% Correct	91.7%	92.3%

481

482