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Analytical Methods

Application of a high-throughput process analytical technology metabolomics pipeline to Port wine forced ageing process

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ABSTRACT

Metabolomics aims at gathering the maximum amount of metabolic information for a total interpretation of biological systems. A process analytical technology pipeline, combining gas chromatography–mass spectrometry data preprocessing with multivariate analysis, was applied to a Port wine "forced ageing" process under different oxygen saturation regimes at 60 °C.

It was found that extreme "forced ageing" conditions promote the occurrence of undesirable chemical reactions by production of dioxane and dioxolane isomers, furfural and 5-hydroxymethylfurfural, which affect the quality of the final product through the degradation of the wine aromatic profile, colour and taste. Also, were found high kinetical correlations between these key metabolites with benzaldehyde, sotolon, and many other metabolites that contribute for the final aromatic profile of the Port wine. The use of the kinetical correlations in time-dependent processes as wine ageing can further contribute to biological or chemical systems monitoring, new biomarkers discovery and metabolic network investigations.

1. Introduction

Metabolomics is a recent 'omics' field and is based on biology science devoted to fully identify phenotypes, the connectivity that relates their constituents and the dynamical response to perturbations, as well as the relationships with the rest of the cellular molecular machinery, namely at the regulatory levels such as genomics, transcriptomics and proteomics at the systems biology stage (Feist, Herrgård, Thiele, Reed, & Palsson, 2009).

The study of such complex matrix on a chemical composition perspective can be described as being *multi-scale*, i.e. several orders of magnitude (ppm-ppt) and *multivariate*, i.e. large diversity of chemical substances, and constitutes an extremely challenging problem being the main goal to obtain a comprehensive profile of the matrix. Ideally it would require a true 'omic sensor', i.e. with an unlimited linear zone of response and sensitive to all substances resulting in a global picture of the bioprocess (Jerez, 2008).

A high-throughput metabolomic chromatography systems consists in one part of hardware (analytical equipment) and the other part of bioinformatics (signal processing, data storage and multivariate analysis), which are becoming a trend in modern biotechnology. These methods become more appealing to systems biology and molecular biology sciences, when signal processing is performed which turns chromatography into a high-throughput system. This capacity of peak extraction by automated techniques makes possible the holistic assessment of the metabolism (Villas-Boas, Mas, Akesson, Smedsgaard, & Nielsen, 2005).

Gas chromatography–mass spectrometry (GC–MS) is specially suited for the study of yeast volatile metabolites. These are of great importance in fundamental biological functions, such as signaling and precursors of biochemical pathways, as well as in the formation of aroma from wines that result from fermentation and ageing, either in barrels or bottles. Several hundreds of compounds can be captured by GC–MS, and each compound produces an unique mass spectral fingerprint, which is afterwards used for metabolites recognition and quantification (Dunn et al., 2011). Classical mass spectroscopy is highly laborious and a significant amount of time is necessary for peak analysis, fingerprint recognition, identification and quantification by an analyst (Christensen, Tomasi, & Hansen, 2005). In this context nontargeted methodologies are extremely important, with an unbiased approach, toward understanding the biological system (Castro et al., 2012; Fiehn, 2002; Rodrigues, Barros, Carvalho, Brandão, Gil & Ferreira, 2011). When automatic chromatogram signal processing becomes a robust skill, mass-spectroscopy may be used as a high-throughput technology.

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Chromatography enables physico-chemical deconvolution of the complex matrix components during the analytical process originating a pattern, that is, a chromatogram. Although it allows samples classification based on the different patterns, it is important to relate that there are inherent variations in the chromatographic process, mainly related to the retention time (peak shifts) and baseline drifts. Therefore, in order to use GC–MS data on a high-throughput base, those issues must be focused on the preprocessing data handling prior to multivariate analysis (Christensen et al., 2005). In that regard, several authors have proposed some preprocessing methodologies, including noise filtering, baseline correction, peaks detection, alignment, identification and normalization (Castillo, Gopalacharyulu, Yetukuri, & Oresic, 2009).

In the present study, the aim is to develop a pipeline methodology based on Process Analytical Technology (PAT) multivariate analysis (MVA) and GC–MS data processing in order to provide a holistic view of the impact of the presence of oxygen and higher temperature during the "forced ageing" of a Port wine matrix. This ageing process, is an extremely complex chemical process that has been extensively studied by our research group (Silva Ferreira, Barbe, & Bertrand, 2002; Silva Ferreira, Barbe, & Bertrand, 2003a; Silva Ferreira, Monteiro, Oliveira, & Guedes de Pinho, 2008), where several chemical mechanisms take place and are responsible for differences in sensory perception affecting final product quality.

The metabolomics pipeline methodology here provided is applied to a "forced ageing" Port wine data set and includes both chromatographic signal preprocessing and MVA methodologies, together with the exploration of candidate metabolites expression and co-expression within the overall ageing process (see Fig. 1). Candidate metabolites correspond to MVA model variables, candidate to explain specific pathways of the metabolism that have chemical meaning and can be used for understanding the overall process. The co-expression of a candidate metabolite constitutes a powerful feature, enabling the study of potential interconnections between the candidates formation and consequently allowing further research for overlapping/connections between chemical and/or biochemical mechanisms. In this context, the global high-throughput pipeline methodology provides increased rate of metabolites identification involved in the chemical process contributing to further build the metabolic network of the overall process.

2. Materials and methods

2.1. Wine material and treatments

Young Port wine characterized by a pH = 3.4, 2.5 mg/L of dissolved oxygen, 17 mg/L of free SO_2, 150 g/L of reducing sugars and 20.5% (v/v) alcohol made on the year of the experiment (without any oak contact) was used in this experiment.

Different oxygen treatments were introduced: 0 (P#_NoO2), 1 (P#_1inj), 2 (P#_2inj) and 5 (P#_5inj) saturations in glass vessels filled with 500 mL of Port wine. Oxygen saturation was obtained by stirring each sample vigorously for about 1 h until an oxygen concentration of about 8–9 mg/L was reached. This was performed in a laminar flow chamber under UV light to prevent microbial contamination. Oxygen injections were measured with a WTW 340 Oxygen Probe (Silva Ferreira, Hogg, & Guedes de Pinho, 2003b), during 18 weeks ('P1' to 'P18') of storage at 60 °C in a temperature controlled incubator, and discrete samples were obtained heuristically for each oxygen regime and further analyzed by GC–MS (see Section 2.4 GC–MS analysis, for specifications).

The forced ageing experiment was implemented to simulate the typical oxidation aroma of Port wine by promoting chemical changes on wine composition. Samples were supplemented with different oxygen regimes and kept at high temperatures (60 °C). Although those extreme conditions are not representative of real ageing process, they were selected in order to be able to reproduce in the laboratory on a reasonable time the ageing process, in spite of the risk of promoting other chemical reactions which would not occur in the normal process. The length of the duration for the forced aged protocol was sensory-driven. In fact, at each sampling point, samples were submitted to sensory analysis in order to validate that the product was still perceived as Port wine and it was observed that after 18 weeks it not accepted as such.

2.2. Chemicals

All chemicals employed were of analytical grade: anhydrous sodium sulphate (HPLC grade) (Merck, Darmstadt, Germany), dichloromethane (Lab Scan, Sowinskiego, Gliwice), 3-octanol (97%)

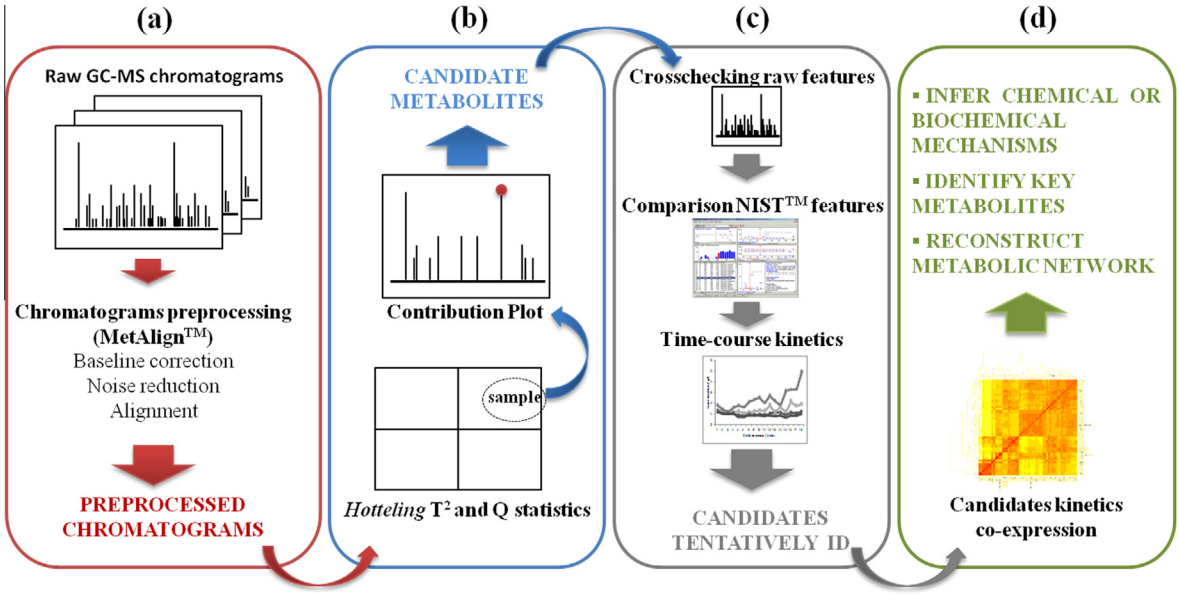

Fig. 1. High-throughput pipeline methodology for GC-MS data processing: (a) GC-MS data preprocessing; (b) Multivariate Analysis; (c) Candidate metabolites identification; (d) Temporal relationships of candidate metabolites for process contextualization.