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Comprehensive lipidome profiling of Sauvignon blanc grape juice

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ABSTRACT

This study presents a comprehensive lipidome analysis of Sauvignon blanc grape juice by combining GC–MS based fatty acid profiling with shotgun lipidomics strategy. We observed that despite grape juice being a water based matrix it contains a diverse range of lipid species, including common saturated and unsaturated free and intact fatty acids as well as odd-numbered and hydroxy fatty acids. Based on GC–MS quantitative data, we found that the total lipid content of grape juice could be as high as 2.80 g/L. The majority of lipids were present in the form of complex lipids with relatively small amount of free fatty acids (<15%). Therefore we concluded that the lipidome should be considered an important component of grape juice with the potential to impact on fermentation processes as well as on the sensorial properties of fermented products. This work serves as a hypothesis generating tool, the results of which justify follow-up studies to explore the influence of the grape juice lipidome and lipid metabolism in yeast on the aroma profile of wine.

1. Introduction

Grape juice is a water-based sugar-rich medium containing different classes of metabolites with concentrations ranging from mg/L (methionine, lysine) (Huang & Ough, 1991) to g/L (malic acid, tartaric acid) (Mato, Suarez-Luque, & Huidobro, 2007), which provides a nutritive environment under stressful anaerobic conditions for yeasts during the alcoholic fermentation process. *Saccharomyces cerevisiae* can grow in the presence of a small spectrum of indispensable organic and inorganic compounds but cell growth under the hypoxic conditions of wine fermentation is limited without an exogenous source of unsaturated fatty acids (UFAs) and ergosterol (Varela, Torrea, Schmidt, Ancin-Azpilicueta, & Henschke, 2012). Anaerobic conditions combined with poor lipid supplementation govern a chain of morphological changes in *S. cerevisiae* cells (Landolfo et al., 2010). This leads to the activation of biochemical pathways requiring NADPH and subsequently the production of high levels of reactive oxygen species (ROS) (Landolfo et al., 2010; Rosenfeld & Beauvoit, 2003), which results in oxidative stress for the yeast cells (Landolfo, Politi, Angelozzi, & Mannazzu, 2008; Landolfo et al., 2010). Biosynthesis of fatty acid molecules is important for the maintenance of structural cell components such as glycerophospholipids, which regulate the physical properties of cell membranes under metabolic stress (Landolfo et al., 2010; Quehenberger, Armando, & Dennis, 2011). It has previously been observed that an increase in unsaturation index in plasma membrane lipids provides yeast cells with higher ethanol tolerance during fermentation (Alexandre, Rousseaux, & Charpentier, 1994; Thomas, Hossack, & Rose, 1978; You, Rosenfield, & Knipple, 2003). However, under hypoxic conditions the biosynthesis of unsaturated fatty acids is repressed, reducing the viability of yeasts (Landolfo et al., 2010; Mannazzu et al., 2008). The inability of *S. cerevisiae* cells to acquire complex lipids (i.e. triacylglycerols) from the extracellular medium makes them highly dependent on exogenous sources of unsaturated fatty acids (Dyer, Chapital, Kuan, Mullen, & Pepperman, 2002). Since *S. cerevisiae* cannot utilize complex lipids (Dyer et al., 2002),

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exogenous phospholipids and glycerolipids can be a potential source of free fatty acids (FFAs) for yeast utilization, which are liberated through lipolytic activity of enzymes supplemented to grape juice or through co-culturing with lipase-secreting microorganisms.

Despite a plethora of studies reporting the amino- and organic acid profile of grape juices and their effect on yeast metabolism in wine fermentation, there is no study that we are aware of characterizing comprehensively the lipid profile of grape juice. This is somewhat surprising since previous studies have shown that the availability of free fatty acids in grape juice affects yeast metabolism, significantly altering the production of varietal aroma compounds, which is clearly pertinent to wine production (Pinu et al., 2014; Varela et al., 2012). Varela et al. (2012) demonstrated that unsaturated fatty acids and ergosterol supplementation stimulate the production of esters, higher alcohols and volatile fatty acids in Chardonnay wines, and Pinu et al. (2014) reported a reduction in the level of acetate esters and 3-mercaptohexyl acetate (passionfruit aroma) in Sauvignon blanc wines with the direct supplementation of linoleic acid to the juice prior to fermentation. Pinu et al. also indicated that the aroma profiles were affected differently depending on the amount of linoleic acid supplemented in the juice (Pinu et al., 2014).

The important physiological role of lipid molecules in yeast cells and recent discoveries of their influence in the wine aroma profile provide convincing reasons to explore the lipidome of grape juice. Although a number of methods have been described for fatty acid profiling (Abdulkadir & Tsuchiya, 2008; Akoto, Vreuls, Irth, Pel, & Stellaard, 2008; Armstrong, Metherel, & Stark, 2008; Cantellops, Reid, Eitenmiller, & Long, 1999; Connerth, Grillitsch, Kofeler, & Daum, 2009; Glaser, Demmelmair, & Koletzko, 2010; Mazalli & Bragagnolo, 2007; Paterson & Amado, 1997; Quehenberger et al., 2011; Rodriguez-Palmero, Lopez-Sabater, Castellote-Bargallo, Dela Torre-Boronat, & Rivero-Urgell, 1997; Ulberth & Henninger, 1995; Wiesman & Chapagain, 2009), they often involve laborious procedures with several extraction steps and a combination of different solvents, anhydrous conditions and freezing cycles, which make sample preparation very time consuming. Moreover, the high sugar content in grape juice poses a significant problem to profile and quantify lipids at their trace levels due to the matrix effect. These factors have encouraged the optimization of a high-throughput and robust fatty acid profiling method. The principle of this method is based on GC–MS analysis of fatty acid methyl esters (FAMEs) or their analogs generated by the derivatization of FFAs after the saponification of complex lipids. An in-house R package *MetabQ* was developed in order to simplify the extraction of quantitative data from GC–MS generated data files. *MetabQ* is a user-friendly software allowing high-throughput data extraction and could be applied in various metabolomics studies that include sample analysis employing GC–MS.

While GC–MS platform, traditionally, was widely employed for analysis of certain classes of lipids (e.g. fatty acids, sterols) (Sjovall, Lausmaa, & Johansson, 2004), the development of the state-of-the-art high resolution MS instrumentation allowed for the detailed investigation into all major classes of cellular lipidome (Shevchenko & Simons, 2010). Thus, shotgun lipidomics has become a powerful tool aiming to characterize the system through the identification of major lipid classes (Han & Gross, 2005).

In this work, a comprehensive Sauvignon blanc grape juice lipidome study is presented that quantifies and differentiates free fatty acids from total fatty acids available in a wide range of Sauvignon blanc grape juices harvested in different geographical locations in New Zealand over three consecutive vintages as well as shotgun lipidomics data of selected juices which together provide a resource for wine science research.

2. Experimental section

2.1. Chemicals

Methanol, chloroform, pyridine, potassium hydroxide, 2,6-bis(1,1-dimethylethyl)-4-methylphenol (butylated hydroxytoluene, BHT), anhydrous sodium sulfate, nonadecanoic acid, 2,3,3,3-d_4-alanine and methyl chloroformate (MCF) were purchased from Sigma–Aldrich (St. Louis, MO, USA). Standard mixtures of fatty acid methyl esters (FAME37) and both GLC-458 and GLC-455 standard mixtures were purchased from Sigma–Aldrich and Nu-Check Prep, Inc. (Elysian, MN, USA), respectively. Internal standard trinonadecanoin (TAG 19:0/19:0/19:0) was purchased from Nu-Check Prep, Inc. Internal standard triethanolamine trimyristate (TEM) was purchased from Omics Biochemicals Limited (Auckland, New Zealand).

2.2. Grape juice samples

In total 217 Sauvignon blanc grape juices were used in this study including 10 juices from 2010, 105 juices from 2011 and 102 juices from 2012 vintages. Selected wineries from across New Zealand, with an emphasis on Marlborough region, were invited to contribute juices from their vineyard blocks to participate in the Juice Index project. This project was part of the Sauvignon blanc II Programme (C11X1005) which started in 2010–2011. That year approximately 180 different juices were analyzed and made into wine. For the 2011–2012 season, mostly juices from the same vineyard blocks as the previous year were used.

Commercial fruit was harvested and processed by each company in its own particular way with its own sulfur additions. For each vineyard block juice, three new 1 L bottles were filled with clear juice (after cold stabilization, just before fermentation) with each juice clearly coded. The juices were kept at 4 °C at the wineries until collection in the same day.

After overnight cold storage in the laboratory, the three bottles of juice were consolidated into one sample before sub-sampling for the individual analyzes and winemaking. For the chemical analyzes of the juice, exactly 60 mL of juice was decanted in new 70 mL bottles to which 0.48 mL of 50 mM solution of internal standard d_4-alanine was added. All juice samples were frozen at −20 °C, until processing. Juice samples were thawed at 4 °C for 8 h prior to sample preparation.

2.3. Quantitation of free fatty acids

1 mL aliquot of each grape juice sample was prepared in triplicates and internal standard nonadecanoic acid was added to each sample. The juice sample was then freeze-dried using a BenchTop K manifold freeze dryer (VirTis, SP Scientific, Warminster, PA, USA) and derivatized using methyl chloroformate (MCF) as described previously (Smart, Aggio, Houtte, & Villas-Boas, 2010), followed by GC–MS analysis.

2.4. Quantitation of total fatty acids

2.4.1. Lipid extraction

A modified Bligh and Dyer method (Bligh & Dyer, 1959) was applied for lipid extraction from grape juice samples. In summary, 3 mL aliquot of each grape juice sample were mixed in triplicates with 3 mL of chloroform–methanol mixture (1:2, v/v), 50 μg of BHT to prevent lipid oxidation and 20 μL of internal standard (5 mM trinonadecanoin solution in chloroform). The mixture was homogenized for 3 min using a vortex mixer. Thereafter, 1 mL of chloroform was added to the mixture and vortex mixed for another