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Review

Review of preparative and analytical procedures for the study of proteins in grape juice and wine

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ABSTRACT

Proteins have a great influence on wine quality as they exhibit a various range of properties. In fact, they are involved among others in white wine turbidity, organoleptic characteristics and foam formation in sparkling wines. These compounds could also be of major interest for varietal differentiation, regarding wine authentication and traceability issues.

To provide a better understanding of the role played by these biomolecules in wine processing and explore their potential applications, there is a manifest need for the quantification and characterization of each individual one in terms of sequence, structure and intrinsic and functional properties.

We thus present an overview of preparative and analytical methods for the study of proteins in grape juices and wines, from routine techniques to dedicated methodologies. They include sample preparation with chromatographic methods for the purification and identification of proteins, quantification protocols and characterization procedures such as electrophoretic techniques, immunological methods, sequencing, mass spectrometry, physico-chemical and structural analyses, and so on. We expose advantages and limits of each technique and focus on the different but complementary information they can provide.

Despite the past years advances in the field proteins identification, the elucidation of the full protein profile for grape juices and wines remains strenuous. Their interactions with other wine compounds make the challenge even harder. We therefore emphasize the requirement of the techniques to be refined and suggest the developments to be expected.

Contents

Abbreviations: PR, pathogenesis-related; SDS-PAGE, sodium dodecyl sulfate polyacrylamide gel electrophoresis; RP-HPLC, reversed phase high performance liquid chromatography; ELISA, enzyme-linked immunosorbent assay; FPLC, Fast Protein Liquid Chromatography; HPSEC, high performance size exclusion chromatography; HIC, hydrophobic interaction chromatography; ConA, concanavalin A; EPE, electroendosmotic preparative electrophoresis; CE, capillary electrophoresis; CGE, capillary gel electrophoresis; MS, mass spectrometry; FFE, free-flow electrophoresis; IEF, isoelectric focusing; ESI-FTICR-MS, electrospray ionization Fourier transform ion cyclotron resonance mass spectrometry; 2D-E, two-dimensional electrophoresis; MS/MS, tandem mass spectrometry; MALDI-TOF, matrix-assisted laser desorption/ionization time-of-flight; 2D-IF-LDS, two-dimensional isoelectric focusing-lithium dodecyl sulfate; PAS, periodic acid-Schiff; PTA, plate trapped antigen; PVDF, polyvinylidene fluoride; NMR, nuclear magnetic resonance; TOCSY, total correlation spectroscopy; HMQC, heteronuclear multiple quantum coherence; EST, expressed sequence tag; iTRAQ, isobaric tag for relative and absolute quantitation.

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1. Introduction

Although winemaking predates recorded History, this biochemical process appears to be a constant changing area. When talking about winemaking, a number of issues arise, linked to grape berry development and wine making and storage. The main concern for winemakers is the wine quality, which is dependent upon its color, clarity and organoleptic properties [1,2]; and in case of sparkling wine, the elegance of its foam and effervescence.

Proteins, ranging from 9 to 65 kDa [3–5] are one of the main grape juice and wine macromolecules along with polysaccharides and polyphenols. The most abundant proteins in grape juice are pathogenesis-related (PR) proteins, including chitinases and thaumatin-like proteins [6], along with invertase [7]. The same proteins are found in wines [8], in addition to mannoproteins coming from yeast during the juice fermentation [1]. They play a leading part in the wine industry concern, despite their relatively low concentration, around ten to hundreds milligrams per litre [9–12]. They are indeed involved in a number of aspects that can impair the acceptance of the product by consumers, such as the haze formation in white wine attributed to the aggregation of some grape proteins, especially PR proteins [13,14] during bottle storage. Accordingly, treatments during the winemaking process, such as bentonite fining, have been used to lower the protein content in wine thereby enhancing wine clarity and stability [1,15,16]. Another negative property of grape proteins comes from their involvement in some rare cases of grape and wine allergy [17–21]. Proteins can however exhibit positive effects such as the stabilization of foam in sparkling wines [22–24]; their interaction with aroma compounds [25–28]; and the protection of wine against tartaric salt precipitation [29–31]; altogether with the interactions they may be involved in with other wine compounds such as ethanol, polyphenols and polysaccharides [32].

The phenomena the protein could be involved in are thus of major interest but their understanding is not that straightforward. The latter implies a full elucidation of grape juice and wine proteins and peptides profile, and the characterization of each individual one.

For that purpose, considerable efforts have been made to develop analytical methods, from routine techniques for the study of proteins to dedicated methodologies for the analysis of grape juice or wine samples. Since the previous reviews by Moreno-Arribas et al. [33] and Curioni et al. [34], improvements have been achieved to obtain protein extracts, from sample preparation to fractionation and purification techniques, and in the one of their characterization, from identification to quantification and structural analysis.

The scope of the present article is to provide an overview of the current trends and recent contributions in grape juice and wine protein sample preparation, purification and characterization fields, giving references to interesting advances as well as future trends. Advantages and limits of each technique are presented, focusing on the different but complementary information they can provide.

2. Obtaining protein fractions from grape juice and wine

The main issue connected to the sample preparation when working on proteins in grape juices and wines follows from their small concentration and the presence of various compounds that may interfere during analyses. However, once the proteins are extracted from their original environment and processed, they may undergo irreversible denaturation, which would impair the following accuracy of their characterization and alter their functional properties. Researchers must then be aware of the impact of the techniques they set up for the study of proteins.

As described by Moreno-Arribas et al. [33] and Curioni et al. [34], different methods allow obtaining concentrated protein extracts from crude grape juice or wine samples and the removal of other macromolecules and potential contaminants: polyphenolic compounds, carbohydrates, salts, and so on.

The most common ways to isolate proteins often involve precipitation or dialysis and ultrafiltration.

Precipitation is a simple and efficient way for obtaining protein fractions, requiring no specific apparatus but it should be considered with caution as it may denature proteins. Considering proteins are less soluble at high salt concentrations, the addition of large amounts of ammonium sulfate can induce the protein precipitation out of the solution [35]. A potassium dodecyl sulfate (KDS) method was developed especially for wine proteins by Vincenzi et al. [36] and currently finds some applications [37]. A following step of centrifugation is generally required to recover the proteins pellets. Other solvents or acids [12,38] can be used to achieve protein precipitation, but they do not seem to be widely used currently.

Dialysis against water is a well-known non-denaturing method for the removal of low molecular mass compounds used by several authors [7,12,39,40].

Ultrafiltration offers the same advantages as simple dialysis, but also allows the sample concentration in a single step. Actually, this technique has been widely used to obtain proteins and peptides extracts from grape juice and wines thanks to its large adaptability (membrane material, filtration surface, cut-off size) [7,41,42]. Its main limitation could arise from its up to medium yield, the poor selectivity of some membranes and their potential clogging when processing unclear samples.

The Table 1 summarizes two different approaches to obtain protein fractions from must and wine, according respectively to the recent works of Jégou et al. [7] and Marangon et al. [35].

Preparative chromatographic methods, such as molecular exclusion or ion exchange chromatography, are now emerging, as a pre-fractionation step of crude grape juice or wine. They could become a very efficient way to isolate the proteins or peptides of interest from a complex starting matrix, provided the chromatographic buffers do not involve protein denaturation and the overall yield is at least equivalent to the other techniques [43].

3. Methods for the study of proteins from grape juice and wine

Proteins exhibit a various range of properties which allows the use of different methods on laboratory scale for their study.

3.1. Quantification

A wide range of techniques are available for protein quantification in general, but three major drawbacks when dealing with wine