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Chemical affinity and binding capacity between pre-purified Cabernet-Sauvignon/Merlot anthocyanins and salivary proteins monitored by UHPLC Q-ToF MS analysis

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Abstract. As dominant phenolic compounds in red wine and grape skin, anthocyanins are expected to influence sensory consequences due to their interaction with salivary proteins during gustation. Recent studies have demonstrated the interaction between anthocyanins and salivary proteins and their effect on mouthfeel properties. This study investigates the chemical affinity and binding capacity between anthocyanins from pre-purified Cabernet Sauvignon/Merlot grape skin and human salivary proteins using ultra-high-performance liquid chromatography coupled with quadrupole time-of-flight mass spectrometry (UHPLC-Q-ToF-MS). Results demonstrated selective bindings of certain anthocyanin compounds with specific salivary proteins, offering insights into the molecular mechanisms behind wine perception and its impact on taste. The findings contribute to the understanding of how anthocyanins might modulate astringency in wine and may help in tailoring winemaking practices to optimize flavor profiles. This study provides valuable knowledge for wine chemists and sensory scientists seeking to improve wine quality and consumer experience.

1. Introduction

Anthocyanins, naturally occurring pigments in grapes, play a crucial role in determining colour [1] and influencing the flavour and mouthfeel of red wines [2]. These phenolic compounds interact with salivary proteins during wine consumption, influencing sensory attributes such as astringency and bitterness [3]. Understanding the binding mechanisms between anthocyanins and salivary proteins is essential to deciphering how these interactions affect wine's organoleptic properties.

In this study, we investigate the chemical affinity and binding capacity between pre-purified anthocyanins from Cabernet Sauvignon and Merlot grape skin and human salivary proteins. We employ ultra-high-performance liquid chromatography coupled with quadruple time-of-flight mass spectrometry (UHPLC-Q-ToF MS), to monitor and quantify these interactions. By identifying the specific binding complexes formed, we aim to enhance the understanding of anthocyanin-protein interactions, ultimately contributing to the improvement of wine quality and sensory characteristics.

2. Materials and methods

2.1. Cabernet Sauvignon/Merlot grape skin anthocyanins pre-purification

The material used in this study consists of grapes from Cabernet Sauvignon and Merlot varieties, harvested from the Saint-Emilion appellation, vintage 2021. Berries were separated from the stems and stored at -24°C until its use. For preparation 4.4 kg of berries were utilized. The skins were removed from the frozen berries, thoroughly washed, freeze-dried, and ground into the fine powder. The powder was then extracted using a liquid-solid method in 0.1% TFA methanolic solution.

Further method implied pre-purification on the Amberlite XAD-16 resin, using an open column. After the pre-purification process, the solvent was evaporated, and the remaining extract was dissolved, frozen, and freeze-dried for further analysis.

2.2. Saliva collection and preparation of salivary protein/grape skin extract samples

2.2.1. Saliva collection

Saliva collection involved 10 volunteers (5 males and 5 females, aged 24 to 47 years) between 11 a.m. and 12 p.m. to account for circadian rhythms, following Ethical Permission [3]. Volunteers were instructed to refrain from eating or drinking for at least one hour before collection.

Saliva has been gathered in 15 ml Eppendorf tubes, pooled, frozen at -20 °C, and freeze-dried. Prior to testing, the freeze-dried saliva was reconstituted in phosphate buffer, vortexed thoroughly, and refrigerated at 4 °C for 1 hour. The solution was then centrifuged at 4000*g* for 10 minutes, and the supernatant was used as the salivary protein sample.

2.2.2. Preparation of salivary protein/grape skin extract samples

The binding test of grape skin phenolics with salivary proteins was conducted following the methods [3-4], with slight modifications to the protocol. Freeze-dried grape skin samples were prepared at a concentration of 1 mg/mL in a wine-like solution (12 %vol ethanol, 4 g/L tartaric acid, pH 3.5), thoroughly mixed for 1 hour, and centrifuged at 3000*g* for 5 minutes to remove any insoluble particles. Next, 4 mL of the sample solution was combined with 1 mL of whole saliva protein solution, or phosphate buffer (pH 6.8) as a control, and incubated at 37 °C for 5 minutes. Following incubation, the mixtures were centrifuged at 17000*g* for 5 minutes, and the supernatants were filtered through 0.22 µm nylon syringe filters. The filtrates, along with the control were processed through SPE cartridges, eluted with acidified methanol, and analyzed on UHPLC Q-ToF MS.

2.3. UHPLC Q-ToF MS analysis

The analysis of phenolic compounds, including identification, separation, and quantification, was performed using an Agilent 1290 Infinity UHPLC system, coupled with a 6530C Q-ToF mass spectrometer (Agilent Technologies, Inc., CA, USA), following the detailed method [5]. Chromatographic separation was carried out at 40 °C on a Zorbax C18 column (2.1 x 50 mm, 1.8 µm) from Agilent technologies. The mobile phases consisted of (A) ultrapure water and (B) acetonitrile (both MS grade), with 0.1% formic acid (MS) added to both. The flow rate was set at a constant 0.3 mL/min, with an injection volume of 5 µL. The QToF-MS system utilized a Dual Agilent Jet Stream electrospray ionization (ESI) source, operating in both positive (ESI+) and negative (ESI-) ionization modes. For suspect screening, data-dependent acquisition (DDA) was employed using Auto MS/MS mode with a collision energy of 30 eV. The Auto MS/MS parameters included an m/z range of 100-1700 and a scan rate of 1 spectrum/sec. Data evaluation and analysis were performed using Agilent Mass Hunter software. Phenolic compounds were identified based on their monoisotopic mass, MS fragmentation patterns, and previously published data [6-10]. Quantification of individual phenolics was performed using available standards, with results expressed as mg of standard equvalents per kg of lyophilized sample. Accurate masses of components were calculated using ChemDraw software (version 12.0, CambridgeSoft, Cambridge, MA, USA). The percentage of grape skin phenolics bound to salivary proteins was determined by comparing the area ratios of identified compounds in control samples and filtrates.

2.4. Data analysis

The data obtained has been analyzed via the RStudio program, version 2024.04.02.

3. Results

The most predominant class of anthocyanins identified were malvidin derivatives, accounting for approximately 46%, followed by peonidin and petunidin derivatives. Among the individual anthocyanins, both glucoside forms as well as acetylated and coumaroylated anthocyanins were detected.

The binding affinity of anthocyanin glucosides with salivary proteins ranged from 44% to 67%, while acetylated anthocyanins exhibited a binding capacity between 35% and 68%, and coumaroylated anthocyanins ranged from 47% to 64%. These findings indicate that the strength and nature of anthocyanin interactions with salivary proteins are closely dependent on the anthocyanin's type, structure, and concentration.

Peonidin derivatives exhibited the weakest binding capacity: however, they showed an increased tendency to form complexes with salivary proteins.

Overall, this study reveals specific interactions between grape-derived anthocyanins and salivary proteins, potentially influencing in-mouth sensory perceptions. Further experimental models are required to validate these preliminary findings.

4. Conclusion

The results of this study demonstrate that the chemical affinity and binding capacity between anthocyanins derived from pre-purified Cabernet Sauvignon/Merlot and salivary proteins are highly dependent on the specific structure of the anthocyanins. Using UHPLC Q-ToF MS analysis, anthocyanin-saliva interactions were quantitatively monitored, revealing that malvidin derivatives had the highest binding affinity, while peonidin derivatives showed relatively weaker interactions. These findings suggest that anthocyanin composition and structural modifications play a critical role in the formation of anthocyanin-saliva complexes, which could have implications for the sensory attributes of wine. The study highlights the importance of understanding anthocyanin-

protein interactions and their potential impact on oral perceptions, though further research is necessary to validate these results in broader sensory models.

5. References

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