Proteomics and glycoproteomics of beer and wine

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Abstract
Beer and wine are fermented beverages that contain abundant proteins released from barley or grapes, and secreted from yeast. These proteins are associated with many quality attributes including turbidity, foamability, effervescence, flavour and colour. Many grape proteins and secreted yeast proteins are glycosylated, and barley proteins can be glycated under the high temperatures in the beer making process. The emergence of high-resolution mass spectrometry has allowed proteomic and glycoproteomic analyses of these complex mixtures of proteins towards understanding their role in determining beer and wine attributes. In this review, we summarise recent studies of proteomic and glycoproteomic analyses of beer and wine including their strategies for mass spectrometry (MS)-based identification, quantification and characterisation of the glyco/proteomes of fermented beverages to control product quality.

KEYWORDS
beer, glycoproteomics, mass spectrometry, proteomics, wine

1 | INTRODUCTION

1.1 | Beer production process

Beer, an ancient and popular fermented beverage bringing enjoyment to people's daily lives the world over, is made from four main raw materials: water, malted barley, hops and yeast. The composition of each ingredient, as well as technical parameters and conditions used to combine and brew them together determine the quality of the final beer products [1]. The composition of beer changes at each stage of the brewing process due to the addition of ingredients, separation steps, changing process conditions and subsequent biochemical reactions. The protein content of beer is critical for many important functions during the beer making process and for the quality of the final beer.

The beer making process (Figure 1A) starts with harvest of barley seeds, which consist of 10%–15% protein [2] together with other components including lipids and polysaccharides [3]. These proteins are important for the biology of the barley seed, and dynamically change throughout the beer making process. Barley grain proteins are generally classified as storage proteins, structural and metabolic proteins, or protective proteins [4]. Storage proteins make up about half of the total protein content of cereal grains, and are essential for their nutritional properties by undergoing hydrolysis to free amino nitrogen (FAN) during germination and mashing [3]. Hordeins, as prolamin glycoproteins, are a very abundant class of storage protein, and can account for up to 80% of the total protein in barley seeds [2]. Late embryogenesis (LEA) proteins are also a major type of protein in barley, which function in desiccation tolerance [5], such as dehydrins which are involved in stress tolerance [6]. Harvested barley seeds are malted through steeping, germination and kilning [7]. In steeping, barley seeds are imbibed with water at an appropriate temperature to initiate germination [8]. Germination is a critical step in malting, in which starch-degrading enzymes (such as α- and β-amylase) and proteases are synthesised. These enzymes...
FIGURE 1  Diverse proteins are released and modified throughout the production process for (A) beer and (B) wine. Many proteins are post-translationally modified. Barley proteins can be glycated by reducing sugars during malting and mashing. N- and O-glycosylated proteins are secreted from yeast during beer fermentation. N- and O-glycosylated proteins are released from grapes during pressing. Highly N- and O-glycosylated proteins are secreted from yeast during wine fermentation. Created in BioRender https://biorender.com/

enzymes respectively degrade starch into small sugars, or proteins into peptides and amino acids [7, 9]. Seeds are next dried in kilns to a low final moisture content (4%–6%), which inactivates enzymes and halts nutrition usage in the barley embryo [10]. Mashing follows malting and is essentially a hot water extraction of the molecular contents of malted barley. After solubilisation, important enzymatic activities continue, including starch degradation, protein proteolysis, β-glucan degradation and fatty acid oxidation, which together produce a solution rich in fermentable sugars and FAN, as well as residual β-glucan, starch and proteins [11]. Therefore, adjusting mashing process parameters can control the profiles of these components of wort, the soluble product of mashing [11]. After separation from the spent grain, wort is boiled with hops for sterilisation, inactivation of enzymes, precipitation of proteins, evaporation of volatiles and formation of additional flavours [12]. Hops not only adds bitter flavours and diverse aromas but also contributes antimicrobial and ant-oxidant activities [13]. The hopped wort then is cooled, aerated and pitched with yeast (most commonly Saccharomyces) for fermentation. This complex biochemical process contributes numerous by-products of the yeast cellular metabolism, in which yeast assimilates fermentable sugars, amino acids, minerals and other nutrients, and meanwhile secretes a series of compounds including ethanol, carbon dioxide, higher alcohols and esters [9]. Finally, the beer production process ends with possible filtration and aging, carbonation and packaging [14].

The molecular composition of barley, malt, wort and beer changes along the beer brewing process, driven by underlying physical and biochemical processes. Barley proteins are synthesised, solubilised, digested and removed. Layered on top of these changes are further protein modifications including proteolysis and glycation [15]. Many of the proteins secreted by yeast during fermentation are also modified with abundant N- and O-glycosylation [16]. All of these proteins present in the final beer will potentially affect properties such as colour, taste, clarity and foamability. Thus, the presence, abundance and modification of proteins at each step of the beer production process is critical to the quality of the final product.

1.2  Wine production process

Wine is another popular fermented beverage with diverse and complex flavours, aromas and textures resulting from complex interactions between the molecular components. These stem from grapes or are generated during the winemaking process including alcohols, acids, sugars, tannins, esters, aldehydes, amino acids, minerals, vitamins, anthocyanins and other flavour compounds [17]. Various proteins from grapes and yeast, mostly between 20 and 30 kDa [18], are also present in wine in low concentrations (15–230 mg/L) but are very important because of their substantial impact on the clarity, stability and sensory properties of wine products [19]. For instance, unstable and aggregated proteins can lead to haze in white wine which is undesirable and results in wine which is considered unacceptable for sale.

The general production process for wine can be divided into pre-fermentation, fermentation and post-fermentation but with key differences for the various styles of wine (white, red, rose and sparkling) (Figure 1B). Firstly, ripe grapes are harvested and selected manually or mechanically, and then crushed and pressed to release juice. Proteins and glycoproteins from grapes are mainly released during this process together with the other components of the juice. The grape skin, pulp and seeds are removed from the juice for white wine production but are kept for red wine production to allow extraction of more colour and flavours [20]. Fermentation is initiated either through the wild yeast present on the harvested fruit, or by addition of yeast to promote efficient fermentation. As with beer fermentation, the yeast in this process generates alcohol, carbon dioxide and flavour compounds from sugars.
In addition to these small molecule components, proteins are added to the wine from yeast via both canonical secretion pathways as well as autolysis, impacting wine characteristics. The diversity and abundance of proteins in wine is affected by the fermentation conditions, as proteins from grapes or yeast can be subject to proteolysis and denaturation, triggered by proteases and changes in pH, respectively [19, 21, 22]. Secondary fermentation and aging on lees are specific processes in sparkling wine production which increase ethanol content, carbonates and matures the wine. Biochemical changes to small molecules and proteins are associated with wine maturation [23], and glycoproteins are largely accumulated during this procedure [24]. After fermentation is complete, wine is racked off from lees and stored in stainless steel vessels or oak barrels, with further optional filtration, cold stabilisation, fining and blending before packaging [20].

Wine production, like beer brewing, involves the release and modification of a many highly N- and O-glycosylated grape proteins and yeast secretory proteins, many of which have been associated with wine traits such as turbidity, effervescence, aroma and mouthfeel, critical for wine quality control.

1.3 Yeast secretory pathway and secretome

Proteins present in beer and wine do not only originate from crushed barley or grapes, but are also actively secreted from yeast during fermentation. Protein secretion is critical in all living organisms, with approximately one third of the eukaryotic proteome processed via the secretory pathway [25]. In yeast, most secreted proteins are secreted classically through the endoplasmic reticulum (ER)-Golgi pathway which is triggered by a canonical N-terminal signal peptide promoting translocation of the nascent polypeptide across the ER membrane [26]. After N-glycosylation, disulphide bond formation and correct folding, proteins are delivered to the Golgi apparatus for further modification and finally transported via small membrane-enclosed vesicles to the plasma membrane [27]. Control of flux through the secretory pathway depends on direct interactions between secretory proteins and ‘decision proteins’ which can determine the destiny of these secretory proteins [28].

Protein folding and post-translational modification involving N-glycosylation and disulphide-bond formation are both key roles of the ER [29, 30]. Most secretory proteins are N-glycosylated at select asparagine residues located in ‘glycosylation sequons’ (N-X-S/T; X≠P) [31]. Consistent with their roles in promoting productive protein folding and increasing protein solubility, N-glycosylation sequons tend to be present in solvent exposed regions and rarely in buried regions of proteins [25]. O-mannosylation catalysed by ER-localised protein O-mannosyltransferases is also a common post-translational modification of secreted yeast proteins [32]. The glycoproteome of Brewers’ yeast Saccharomyces cerevisiae is well studied, and at least one third of secreted proteins from yeast are glycosylated [16, 33, 34].

While most secreted proteins traffic through the canonical ER-Golgi pathway, some proteins lacking signal peptides are also able to access the cell surface via unconventional secretion pathway(s) [35]. The existence of these non-classical secretion pathways is inferred from the presence of proteins outside the cell that do not traffic through the lumen of the ER–Golgi system [36]. Four types of unconventional protein secretion (UPS) pathways have been identified in eukaryotes, classified as: direct translocation of proteins via lipid pores (type I); direct translocation through ATP-binding cassette (ABC) transporters (type II); secretion through endocytic compartments (type III); and transport of integral membrane proteins from the ER to the plasma membrane bypassing the Golgi (type IV) [36]. Highly glycosylated proteins are also released slowly from the cell wall during yeast autolysis, usually occurring after fermentation with prolonged contact on lees [37]. The autolysis starts with yeast death and normally happens at the end of the stationary phase of yeast growth [38], and consists of four stages including: degradation of cell membranous systems with protease release; initial inhibition of released proteases and then reactivation owing to inhibitor degradation; hydrolysis of intracellular components with accumulation of hydrolysis products and finally, releasing of these hydrolysis products which are able to cross the porous cell wall [37, 39]. Together, this means that yeast proteins present in beer or wine can result from classical secretion of proteins and glycoproteins through the ER–Golgi, non-classical secretion of specific proteins from the cytoplasm and uncontrolled yeast cell lysis.

2 PROTEOMICS FOR BEER AND WINE

2.1 The importance of the beer and wine proteomes

The proteome is the full set of proteins present in a specific defined system [40]. As is clear from the beer and wine making processes (Figure 1), as well as consisting of water, alcohol and small molecule metabolites, these fermented beverages also contain a complex mixture of potentially post-translationally modified proteins from various biological sources: barley, hops, grapes and/or yeast. These complex biomolecules can be challenging to measure, and yet are important for many quality attributes of the beverages.

Beer contains various proteins originating from barley and yeast which can impact beer quality control. The colour, viscosity and texture of beer are largely related to barley proteins including lipid transfer protein (LTP), protein Z, peroxidase, trypsin inhibitors and α-amylase [41]. For example, protein Z and LTP from barley, and seripauperins from yeast, affect foam stability [16]; dimeric α-amylase inhibitor-1 from barley and thioredoxin from yeast positively and negatively affect foam formation, respectively; and CMe component tetrameric α-amylase inhibitor-1 from barley and thioreredoxin from yeast positively and negatively affect foam formation, respectively; and CMe component tetrameric α-amylase inhibitor, barley dimeric α-amylase inhibitor-1 and hordein are major inhibitors of beer colloidal haze [42].

Specific proteins have been clearly associated with haze in white wine, induced by protein aggregation. Pathogenesis-related (PR) proteins including β-1,3-glucanases, chitinases (CHIs), grape thiamatin-like proteins (TLPs) and LTPs, which are synthesised during grape ripening [19, 43, 44], were found to be the most abundant
proteins in unfined grape juice as determined by electrophoretic pro-
profiling. These disulphide rich, compact, globular proteins contribute to
haze formation in white wine [45], likely because of their interactions
with polysaccharides and phenolic compounds [46], together with
other minor haze-forming proteins such as β-glucanases [47]. Proteins
including yeast mannoproteins, grape invertase and grape cell wall
glycoproteins also contribute to white wine quality by stabilising wine
against heat-related protein instability, affecting the foaming prop-
erties of sparkling wines and interacting with aromatic compounds
[47].

2.2 Proteomics and analytical techniques

Proteomic analyses of yeast, beer and wine have historically used
two-dimensional gel electrophoresis [48, 49], but now standard pow-
erful bottom-up LC-MS/MS proteomics approaches are most common,
with top-down strategies also emerging [50]. Bottom-up proteomics
permits efficient analysis of highly complex samples after enzym-
atic digestion of proteins into peptides [51], separation of peptide
fragments by high-performance liquid chromatography and sensitive
detection by MS. However, there are some deficiencies of bottom-up
approaches, including potential ambiguity of the origin of redundant
peptide sequences from closely related proteins, incomplete protein
sequence coverage and loss of labile post-translational modifications
(PTMs) [50]. Data-dependent acquisition (DDA) and data-independent
acquisition (DIA), including sequential window acquisition of all theo-
retical mass spectra (SWATH-MS), are two commonly used methods
for identification and quantification of proteins within complex sam-
bles [52], which have also been applied to proteomic analysis of beer
and wine [53].

2.3 Case studies of proteomics of beer and wine

2.3.1 Beer

Proteins play important roles in affecting beer properties including
colour, taste, foam stability, gushing, turbidity and texture, and MS-
based proteomic analyses have been applied to study these features
of beer. A recent study used sequential filter-aided sample prepara-
tion (FASP) combined with LC-MS/MS and identified 4692 proteins
in liquid beer and 3906 proteins in beer foam (separated based on
their high hydrophobicity), totalling 7113 beer-associated proteins.
These proteins included important proteins for beer quality such as
LTP, serpins, hordeins, gliadins and glutenins, providing an efficient
analytical workflow and offering a comprehensive map of the beer
proteome for future studies [42]. Another recent study combined pro-
tomics with an automated and robust method for assessing beer
foamability using RoboBEER and a machine learning model established
through assessment of significant correlations between the identi-
fied proteins grouped by molecular weight and physical parameters
(colour, foam and bubbles) measured by RoboBEER [54]. This identified
complex proteomic signatures of beer that correlated with 69 physico-
chemical characteristics, towards enabling the targeted production of
high-quality beer with specific quality attributes [55].

A key challenge in production of bright beer is the presence of
unfilterable haze. The causes of this unfilterable haze are not com-
pletely clear, but it is associated with high concentrations of proteins,
polyphenols and β-glucans. A proteomics based study of commercial
India Pale Ale diagnosed that overall protein and β-glucan content
were associated with this haze, and postulated that cell wall manno-
proteins secreted by yeast were the primary cause [56]. A follow-up
study used protein fractionation, SDS-PAGE and LC-MS to reveal that
both yeast cell wall mannoproteins and flocculins Flo1 and Flo9 were
the most abundant proteins associated with unfilterable haze, provid-
ing avenues for future research to avoid problematic haze formation
[57].

The proteome of beer changes throughout the production process,
and so effective proteomic profiling at each stage can provide insights
into the biochemistry at key steps towards controlling the final product
quality. A recent study revealed the complex and dynamic proteome
at various stages throughout the beer brewing process, using SWATH-
MS analysis of sweet wort, hopped wort and bright beer produced at
a nanobrewery scale [41]. As well as quantifying protein abundances,
this study revealed that the beer proteome was extensively diversified
with PTMs including glycation, oxidation and abundant proteolysis.
Another study also applied SWATH-MS to quantify proteins and their
site-specific proteolysis throughout the mashing process. Proteolysis of
proteins to generate FAN is a critical biochemical process that takes
place during malting and mash. This study found that sequence-specific
proteolytic cleavage determined protein stability and consequently
controlled the dynamic beer brewing proteome based on the ther-
mal stability of proteolytically clipped and unclipped proteoforms [14].
Using proteomics, this study also confirmed some common concerns
of the brewing industry concerning biochemistry during the mash: that
α-amylase and β-amylase were stable and active below 65°C, that pro-
teolysis was extensive at 53°C and that both processes were inhibited
by protein aggregation at higher temperatures. Finally, a recent study
proposed a novel high-throughput and low-cost mashing strategy at a
micro scale, using a common laboratory benchtop incubator to allow
robust statistical analysis. This small-scale micro-mashing approach
could be used to efficiently test the effect of altered mash conditions or
barley varieties [58]. The validity of this method was confirmed by pro-
tecomic comparison at both peptide and protein levels via DDA and DIA
LC-ESI-MS/MS between the 1 ml micro-mash and a 23 L mash, which
found high similarity and consistency. Quantification of fermentable
sugars (glucose, maltose and maltotriose) and free amino acids through
MRM LC-MS/MS also showed equivalent abundances [58].

In addition to analysis of the global beer proteome, more targeted
analysis of the gluten content of beer is of particular importance. The
allergenicity of gluten proteins is a critical contributor to celiac disease
[59], for which current treatment relies on maintaining a gluten-free
diet [60]. Gluten proteins make up a large proportion of the beer pro-
teome, including hordeins from barley [61] and in some beer styles
also gliadins from wheat [62, 63]. However, analysis of gluten proteins
in beer is quite challenging because of the nature of these proteins; they can have low solubility, they lack tryptic cleavage sites and they are modified during the beer making process by glycation and partial proteolysis [16]. Therefore, while measuring gluten in beer is of high importance, it has technical challenges. While ELISA is a standard quantitative method for measuring protein antigens, including glutens in beer, targeted MRM-MS is more reliable, particularly in avoiding false negative results, due to the high specificity of ELISA for protein epitopes that may not always be able to be detected after proteolysis during beer production [64, 65]. Approaches to make gluten-free beer include the use of an enzyme called proline endopeptidase (PEP), which digests gluten by cleaving at proline residues. Treatment with PEP removes gluten proteins, but LC-MS/MS approaches have shown that immune-reactive gluten peptides can still be present at high levels in beer [60, 66, 67]. This demonstrates the utility of LC-MS/MS for measuring gluten in beers in both regulatory settings and in quality control during commercial production. The complexity of gluten proteins remains a challenge, especially for traditional bottom-up proteomics, and advances in middle-down and top-down analyses [68] or degradomics [69] may open opportunities for more robust detection or complete characterisation of these complex proteins.

2.3.2 Wine

Haze is a common defect in white wine and is a challenging problem to eliminate during winemaking. Haze is mainly associated with the aggregation of thermolabile proteins such as TLPs and CHIs. A recent study used trypsin surface accessibility and molecular dynamic simulations to detect regions on TLPs and CHIs that became accessible to enzymes when protein conformation changed due to variation in pH and temperature, revealing some of the mechanisms that controlled haze formation [70]. Structural rigidity was found to be the main reason proteins escaped from proteolytic cleavage during winemaking, suggesting that proteolysis of unstable proteins could be controlled by appropriately adjusting temperature and pH control to prevent wine haze. Another recent study of wine made from botrytised grapes used SDS-PAGE, in-gel digestion and LC-MS/MS identification of proteins extracted from wine. This study found that proline rich proteins were also associated with haze formation [71]. The use of diverse defined yeast in addition to S. cerevisiae can increase the diversity and complexity of wine sensory features in a controlled manner, and also leads to interesting and complex biochemical interactions between the various yeast species. The roles of extracellular vesicles (EVs) appear to be critical in these interactions, but are still not well understood. A recent study characterised EVs produced by six wine yeast species grown in synthetic grape must through a process including: isolation of EV-enriched fractions, visualisation via transmission electron microscopy (TEM), separation by SDS-PAGE, quantification by fluorometry and protein identification via trypsin digestion and LC-ESI-MS/MS [72]. Glycolytic enzymes and cell-wall-related proteins were enriched in EVs from S. cerevisiae and Torulaspora delbrueckii, with the cell-wall-degrading enzyme exo-1,3-β-glucanase being the most abundant protein identified. Further biochemical and functional analyses of EVs and the impact of different culture conditions on the content of EVs are required to fully understand their role in inter-species interactions in wine making.

Grapes and yeast are not necessarily the only ingredients in wine; fining agents are commonly used to clarify wine, and may remain present at low levels in the final product. Their presence is potentially problematic if they are allergenic. Fining is a common step in commercial wine production to increase clarity, stability and sensory attributes of final wine products, and relies on various fining agents: animal-based (gelatin, casein or ovalbumin proteins); plant-based (gluten proteins) or chemical-based (bentonite) [73]. Animal- and plant-based fining agents are commonly used for high-quality wine, leading to potential risks for consumers who are allergic to proteins from those sources [74]. Although ELISA is the current standard method used for monitoring fining agent residues in wine, LC-MS/MS offers several advantages including the ability to measure many proteins in a single assay, robustness to the folding status of the protein and even higher sensitivity [75–77]. Methods developed for monitoring residual fining proteins tend to use targeted analyses with parallel reaction monitoring (PRM) or selected reaction monitoring [75, 76, 78], and quantification using stable isotope dilution analysis has also been reported [79]. The protease digestion step is a bottleneck in standard proteomic workflows, normally taking several hours. This has been overcome for analysis of wine samples, using ultrasound-assisted digestion, which allows efficient protease digestion in only 3 min [80]. Current mass spectrometry instruments already allow detection of residual fining proteins at concentrations in wine below the legally required limits, and further improvements in sample preparation, LC-MS/MS and data analysis pipelines promise to make these technologies useful for industrial application.

3 GLYCOPROTEOMICS FOR BEER AND WINE

3.1 The importance of the beer and wine glycoproteomes

Glycosylation refers to the process in which carbohydrates (including monosaccharides, oligosaccharides, polysaccharides or their derivatives) are covalently attached to proteins. It is one of the most important PTMs in eukaryotes, with more than half of all proteins modified with glycosylation [81]. The structural diversity of protein glycosylation is central to a series of biological processes including cellular development [82], lectin-mediated cell-cell communication [83], adhesion-based host microbial interactions [84, 85] and immunity [86, 87]. Glycans also assist with protein folding, affect the solubility and stability of glycoproteins and modulate the susceptibility of glycoproteins to proteolysis [88–90].

As a crucial PTM, glycosylation can affect diverse biophysical properties of proteins, including folding, trafficking, solubility, stability, protein–protein interactions and susceptibility to proteases, as well
TABLE 1  Key functional glycoproteins in beer or wine

| Origin | Glycoprotein | Modification type | Potential functions |
|--------|--------------|-------------------|---------------------|
| Grape  | Chitinases    | N-glycosylation   | Primary role in haze formation due to increased viscosity and elasticity [113]; contributes to haze formation due to inhibition of wort filterability [114]. |
|        | Thaumatin-like proteins (TLP) | N-glycosylation | Positive role in disease resistance and stress defence in grape [110]; involved in haze formation due to interactions with polysaccharides and phenolics [46]. |
| Barley | Hordeins     | N-glycosylation   | Contributes to beer foam stabilisation [111]. |
|        | Lipid transfer proteins (LTP) | Glycation | Contributes to beer foam formation [112]. |
|        | Protein Z/serpins | Glycation and N-glycosylation | Contributes to beer foam stabilisation due to increased viscosity and elasticity [113]; contributes to haze formation due to inhibition of wort filterability [114]. |
| Yeast  | Seripauperins | O-glycosylation(O-mannose) | Negative association with beer foam lifetime and number of bubbles [16]; negative association with gushing caused by excessive foaming [103]. |

as the biological activity of proteins [91]. Importantly, glycosylation protects proteins against proteolysis and increases proteins thermal stability [92]. Glycosylation is abundant on proteins secreted from yeast during fermentation, including high-mannose N-glycosylation and oligomannose O-glycosylation [93]. A study focusing on glycoproteomic analysis of white wine identified 28 glycoproteins with 44 N-glycosylation sites both from grape (including PR proteins, class IV CHI, class IV endochitinase, putative TLP and vacuolar invertase GIN1) and yeast (15 glycoproteins with 28 N-glycosylated sites) [94]. When present in high concentrations, glycosylated PR proteins from grapes are a major contributor to haze formation in white wine, but glycosylated mannoproteins from yeast inhibit haze formation by preventing protein aggregation and precipitation [95–97]. Additionally, high concentrations of reducing sugars in wort during the high temperature stages of beer production can lead to glycation, which impacts the properties of final beer products [98]. For instance, beer foam stability and flavour can be affected by glycation of protein Z, LTP1 and LTP2 [99]. The main classes of functional glycoproteins found in beer and wine are summarised in the Table 1, with their origins, modification types and potential functions.

3.2 Glycoproteomics and analytical techniques

Glycoproteomics systematically analyses glycans linked to proteins or peptides to identify and quantify glycoprotein abundance, site occupancy (macro-heterogeneity) and glycosylation structural diversity at individual sites (micro-heterogeneity) [100]. Approaches applied in proteomics can theoretically also be employed in glycoproteomics. However, the inherent heterogeneity and dynamic nature of glycosylation adds layers of complexity to identification and quantification approaches. Specialised software is required identify the glycan component attached to peptides or proteins, and MS2 spectra from these species are more complex owing to glycan fragmentation. In addition, glycosylated species are typically lower in abundance than peptides due to glycan heterogeneity and reduced ionisation efficacy during MS. For MS-based quantification of glycosylation events, label and label-free approaches including DDA, DIA and multiple reaction monitoring (MRM) are commonly used [101].

3.3 Case studies of glycoproteomics of beer and wine

3.3.1 For beer

Compared to the many studies of the beer proteome, the beer glycoproteome remains largely unexplored. A study recently used DIA/SWATH-MS and revealed a very high diversity of PTMs in 23 commercial beers with a focus on proteolysis, glycation and glycosylation [16]. Consistent with glycation during malting or mashing, 111 unique glycation events were detected on 18 barley proteins, with oligohexose modification of lysine residues. Extensive glycosylation was identified on yeast proteins with 60 unique glycosylation sites on 21 proteins. O-mannosylation was the most abundant form of glycosylation, but an unusually highly truncated N-glycan, a single HexHAc, was identified on yeast cell wall mannoprotein Pst1. Linear-regression correlation between the glyco/proteome and foamability was also studied using the RoboBEER workflow, detecting a significant negative correlation between the abundance of O-glycosylated yeast seripauperins and foam lifetime and bubble numbers [16]. Moreover, this study also found that the brewery was the main factor differentiating the beer glyco/proteome, with clear molecular differences in the glycoproteome distinguishing beer made by international breweries from craft beer.

3.3.2 For wine

The surprising complexity of the glycoproteome of sparkling wine was described and investigated in a recent study based on quantitative DIA glycoproteomics [34]. This study presented a sensitive analytical strategy requiring only small volumes (250 μl) of sparkling wine to generate robust MS data for identification and quantification of glycoproteins and their site-specific modifications. A SWATH/DIA glycopeptide
ion library was built in this study, based on glycopeptide identification with the commonly used Byonic software (Protein Metrics), and was then used to compare the glycoproteomes of three sparkling wines. Yeast glycoproteins dominated the glycoproteome, with 93 glycopeptides identified from yeast compared to 11 glycopeptides from grape. Seripauperins were the most abundant yeast glycoproteins present in sparkling wine, including the Pau5 glycoprotein, which has been reported to inhibit gushing in sparkling wines [102, 103]. The high abundance of secreted yeast glycoproteins highlights the importance of secondary fermentation in the production of sparkling wine, and shows that the glycoproteome reflects the production process.

4 | FURTHER APPLICATIONS OF PROTEOMICS TO BEER AND WINE

Proteomics and glycoproteomics are clearly powerful analytical techniques for describing the molecular complexity of beers and wines. Beyond characterisation of the final beverages, proteomics and glycoproteomics have also been used for diverse other applications associated with beer and wine production. For example, recent studies have used proteomics to profile the main ingredients in beer and wine production, barley and grapes, providing the possibility for more precise ingredient quality control. Proteomic analysis of barley has been used to identify changes in the barley seed after infection with fungal pathogens, proteomic signatures associated with malt-grade barley [104], and to follow changes in the proteome of barley during germination or malting [6, 105]. Proteomics of grapes has identified the grape proteins responsible for production of volatile flavour compounds and ethylene during ripening [106]. Proteomics of yeast has characterised the importance of cytosolic thioredoxins under winemaking conditions in carbohydrate and lipid metabolism as well as protein biosynthesis [107], and identified the yeast proteins associated with sugar utilisation and production of flavour compounds produced during secondary fermentation of sparkling wines [108].

5 | CONCLUSION

Although proteins are present at relatively low concentrations in beer and wine, they are associated with or directly responsible for many important quality traits and production efficiencies. Moreover, the proteins present in fermented beverages are highly modified with PTMs including proteolysis, glycation and glycosylation. The application of proteomic and glycoproteomic workflows to understanding the molecular complexity of beer and wine has been shown to provide insights into the dynamic changes to the biochemistry of these beverages, which in turn can advise more accurate quality control during the production process and for the final beverage products. Advances in sample preparation, mass spectrometry and data analysis support the identification, quantification and characterisation of proteins and their modifications. All of these aspects of improvements in proteomic and glycoproteomic workflows have benefitted analysis of beer and wine, in particular ingredient-efficient micro-scale sample preparation and data analysis for characterisation and quantification of PTMs. Looking forward, opportunities are emerging in the use of proteomics and glycoproteomics to further understand the complexity of beer and wine, and to understand how this molecular complexity can be used in ingredient quality control, process optimisation, process monitoring, product provenance and identifying and controlling diverse aspects of the sensory quality of these popular fermented beverages.

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CONFLICT OF INTEREST

The authors declare no conflict of interest.

DATA AVAILABILITY STATEMENT

Data sharing is not applicable to this article as no new data were created or analysed in this study.

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REFERENCES

1. Parker, D. (2012). Beer: Production, sensory characteristics and sensory analysis. In Alcoholic beverages. Woodhead. https://doi.org/10.1533/9780857095176.2.133
2. Finnie, C., & Svensson, B. (2009). Barley seed proteomics from spots to structures. Journal of Proteomics, 72(3), 315–324. https://doi.org/10.1016/j.jprot.2008.12.001
3. Olsen, O.-A. (2001). Endosperm development: Cellularization and cell fate specification. Annual Review of Plant Biology, 52, 233–267. https://doi.org/10.1146/annurev.arplant.52.1.233
4. Shewry, P. R., & Halford, N. G. (2002). Cereal seed storage proteins: Structures, properties and role in grain utilization. Journal of Experimental Botany, 53(370), 947–958. https://doi.org/10.1093/jexbot.53.370.947
5. Bartels, D., Schneider, K., Terstappen, G., Piatkowski, D., & Salamini, F. (1990). Molecular cloning of abscisic acid-modulated genes which are induced during desiccation of the resurrection plant Craterostigma plantagineum. Planta, 181(1), 27–34. https://doi.org/10.1007/BF00202321
6. Osama, S. K., Kerr, E. D., Yousif, A. M., Phung, T. K., Kelly, A. M., Fox, G. P., & Schulz, B. L. (2021). Proteomics reveals commitment to germination in barley seeds is marked by loss of stress response proteins and mobilisation of nutrient reservoirs. Journal of Proteomics, 242, 104221. https://doi.org/10.1016/j.jprot.2021.104221
7. Stewart, G. G. (2013). Biochemistry of brewing. In Biochemistry of foods. Elsevier (pp. 291–318).
8. Kerr, E. D., Fox, G. P., & Schulz, B. L. (2021). Grass to glass: Better beer through proteomics. Comprehensive Foodomics, 407–416. https://doi.org/10.1016/b978-0-08-100596-5.22869-2
85. Vickers, N. J. (2017). Animal communication: When I'm calling you.

87. Ugonotti, J., Chatterjee, S., & Thaysen-Andersen, M. (2020). Structural and functional diversity of neutrophil glycosylation in innate immunity and related disorders. Molecular Aspects of Medicine, 79, 100882. https://doi.org/10.1016/j.mam.2020.100882

86. Pereira, M. S., Alves, I., Vicente, M., Campar, A., Silva, M. C., Padrão, N. A., Pinto, V., Fernandes, Â., Dias, A. M., & Pinho, S. S. (2018). Glycans as key checkpoints of T cell activation and function. Frontiers in Immunology, 9, 2754. https://doi.org/10.3389/fimmu.2018.02754
103. Kupfer, V. M., Vogt, E. I., Ziegler, T., Vogel, R. F., & Niessen, L. (2017). Comparative protein profile analysis of wines made from Botrytis cinerea infected and healthy grapes reveals a novel biomarker for gushing in sparkling wine. *Food Research International*, 99, 501–509. https://doi.org/10.1016/j.foodres.2017.06.004

104. Kerr, E. D., Phung, T. K., Caboche, C. H., Fox, G. P., Platz, G. J., & Schulz, B. L. (2019). The intrinsic and regulated proteomes of barley seeds in response to fungal infection. *Analytical Biochemistry*, 580, 30–35. https://doi.org/10.1016/j.ab.2019.06.004

105. Mahalingam, R. (2018). Temporal analyses of barley malting stages using shotgun proteomics. *Proteomics*, 18(15), 1800025. https://doi.org/10.1002/pmic.201800025

106. Kambiranda, D., Basha, S. M., Singh, R., Snowden, J., & Mercer, R. (2018). Proteome profile of American hybrid grape cv. Blanc du Bois during ripening reveals proteins associated with flavor volatiles and ethylene production. *Proteomics*, 18(8), 1700305. https://doi.org/10.1002/pmic.201700305

107. Picazo, C., Mcdonagh, B., Peinado, J., Bárcena, J. A., Matallana, E., & Aranda, A. (2019). *Saccharomyces cerevisiae* cytosolic thioredoxins control glycolysis, lipid metabolism, and protein biosynthesis under wine-making conditions. *Applied and Environmental Microbiology*, 85(7), e02953–e02918. https://doi.org/10.1128/AEM.02953-18

108. González-Jiménez, M. D. C., Garcia-Martínez, T., Mauricio, J. C., Sánchez-León, I., Puig-Pujol, A., Moreno, J., & Moreno-Garcia, J. (2020). Comparative study of the proteins involved in the fermentation-derived compounds in two strains of *Saccharomyces cerevisiae* during sparkling wine second fermentation. *Microorganisms*, 8(8), 1209. https://doi.org/10.3390/microorganisms8081209

109. Marangon, M., Van Slyter, S. C., Neilson, K. A., Chan, C., Haynes, P. A., Waters, E. J., & Falconer, R. J. (2011). Roles of grape thaumatin-like protein and chitinase in white wine haze formation. *Journal of Agricultural and Food Chemistry*, 59(2), 733–740. https://doi.org/10.1021/jf1038234

110. Vigers, A. J., Wiedemann, S., Roberts, W. K., Legrand, M., Selitrennikoff, C. P., & Fritig, B. (1992). Thaumatin-like pathogenesis-related proteins are antifungal. *Plant Science*, 83(2), 155–161. https://doi.org/10.1016/0168-9452(92)90074-V

111. Blasco, L., Viñas, M., & Villa, T. G. (2011). Proteins influencing foam formation in wine and beer: The role of yeast. *International Microbiology*, 14(2), 61–71.

112. Van Nierop, S. N. E., Evans, D. E., Axcell, B. C., Cantrell, I. C., & Rautenbach, M. (2004). Impact of different wort boiling temperatures on the beer foam stabilizing properties of lipid transfer protein 1. *Journal of Agricultural and Food Chemistry*, 52(10), 3120–3129. https://doi.org/10.1021/jf035125c

113. Maeda, K., Yokoi, S., Kamada, K., & Kamimura, M. (1991). Foam stability and physicochemical properties of beer. *Journal of the American Society of Brewing Chemists*, 49(1), 14–18. https://doi.org/10.1094/ASBCJ-49-0014

114. Jin, Z., Li, X.-M., Gao, F., Sun, J.-Y., Mu, Y.-W., & Lu, J. (2013). Proteomic analysis of differences in barley (Hordeum vulgare) malts with distinct filterability by DIGE. *Journal of Proteomics*, 93, 93–106. https://doi.org/10.1016/j.jprot.2013.05.038

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