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Proteins influencing foam formation in wine and beer: the role of yeast

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Summary. This review focuses on the role of proteins in the production and maintenance of foam in both sparkling wines and beer. The quality of the foam in beer but especially in sparkling wines depends, among other factors, on the presence of mannoproteins released from the yeast cell walls during autolysis. These proteins are hydrophobic, highly glycosylated, and their molecular masses range from 10 to 200 kDa— characteristics that allow mannoproteins to surround and thus stabilize the gas bubbles of the foam. Both the production and stabilization of foam also depend on other proteins. In wine, these include grape-derived proteins such as vacuolar invertase; in beer, barley-derived proteins, such as LTP1, protein Z, and hordein-derived polypeptides, are even more important in this respect than mannoproteins. [*Int Microbiol* 2011; 14(2):61-71]

Keywords: yeast · beer · foam · mannoprotein · sparkling wine

Introduction

Humans have produced alcoholic beverages for more than 9000 years, consuming them for medicinal, nutritional, and social purposes. Throughout history, the elaboration of alcoholic beverages has improved continuously, to the point that today winemakers can very precisely manipulate certain organoleptic properties of their products. In earlier times, fermentations relied on the spontaneous activities of a mixture of yeast and bacteria, quite often resulting in beverage spoilage. Currently, to control the outcome of fermentation, wine- and beer-makers take advantage of the use of starters, which most frequently involve strains of *Saccharomyces*

cerevisiae, although *S. pastorianus* or *S. carlsbergensis* are also employed in the production of beer [6,48].

Current biotechnological approaches to process wine and beer have been designed to respond to consumer demands. These “tailored” beverages are based on improvements in the quality of the grapes and/or barley in addition to relying on the use of particular types of yeast strains. Not only do yeast transform must sugar into ethanol, but they also greatly contribute to the aroma, taste, color and other, more complex characteristics of the final products. Traditionally, the organoleptic properties of alcoholic beverages were improved by the selection of natural yeast strains. Nowadays, however, the trend is to improve the available yeast strains by manipulating only certain specific traits. This, in turn, relies on detailed knowledge of the nature and genetic control of the trait of interest, which is not always straightforward as some traits are the result of polygenic regulation [6,21].

Foam is a major property of both sparkling wine and beer. A variety of factors including the grapes (wine), malt

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(beer), and yeast strains influence foam production and maintenance. Here we review and analyze the contribution of these factors to foaming, especially that of the yeast mannoproteins present in foam.

Foam: its importance in sparkling wine and beer

Foam is a two-phase system of gas and bubbles separated by a thin liquid layer known as the lamellar phase (Fig. 1). In the case of foods and beverages, foam is a complex system formed by gas, liquids, solids, and surfactants. Since foam development may have relevant industrial applications, it is important to understand the factors contributing to its appearance or disappearance. The number, size, and distribution of the bubbles determine a foam's texture, with uniformly distributed small bubbles resulting in a soft foam [60].

Foam accumulates at the air-liquid interface but is intrinsically unstable because surface tension tends to counteract the forces needed for its maintenance, thereby leading to collapse of the foam. Among the factors that influence foam texture are surfactants and proteins. Surfactants migrate rapidly, against a gradient, towards thinner regions of the bubble walls, whereas proteins bind to the interface and interact with it by means of electrostatic or hydrophobic forces, hydrogen bonds, or covalent linkages. These interactions lead to the formation of a viscoelastic film that is highly resistant to ten-

sion and able to withstand the film's thickness. Proteins interact with one another such that no free molecules remain. Accordingly, some proteins are good foam formers but poor stabilizers, while others are poor foam formers but good stabilizers [19,60]. Foam is derived from the process of must fermentation and has positive or negative effects depending on the timing of its formation.

During the early steps of fermentation, foam is undesirable because a thick layer implies that a void volume in the fermentation tank has to be taken into account; in addition, foaming at this stage slows down or even inhibits fermentation, resulting in a large amount of residual sugar [48]. Over-foaming during fermentation can corrupt the necessary hygiene conditions and compromise the performance of the fittings at the top of the vessel by fouling the carbon dioxide take-off pipe. It may also result in the loss of essential foaming components such as hydrophobic polypeptides, which, for example, in turn compromises the characteristic bitterness of beer or hops and reduces the amount of foam in the final product [31]. To prevent over-foaming, some breweries use authorized antifoaming agents, such as silicone and fatty acid esters. Fermentations carried out with the early generations of yeast cultures are prone to over-foaming, particularly if high aeration rates are used in the yeast propagations; this problem can be controlled by lowering the levels of dissolved oxygen.

The use of pressure is another method to prevent over-foaming during fermentation [30]. On the other hand, foam

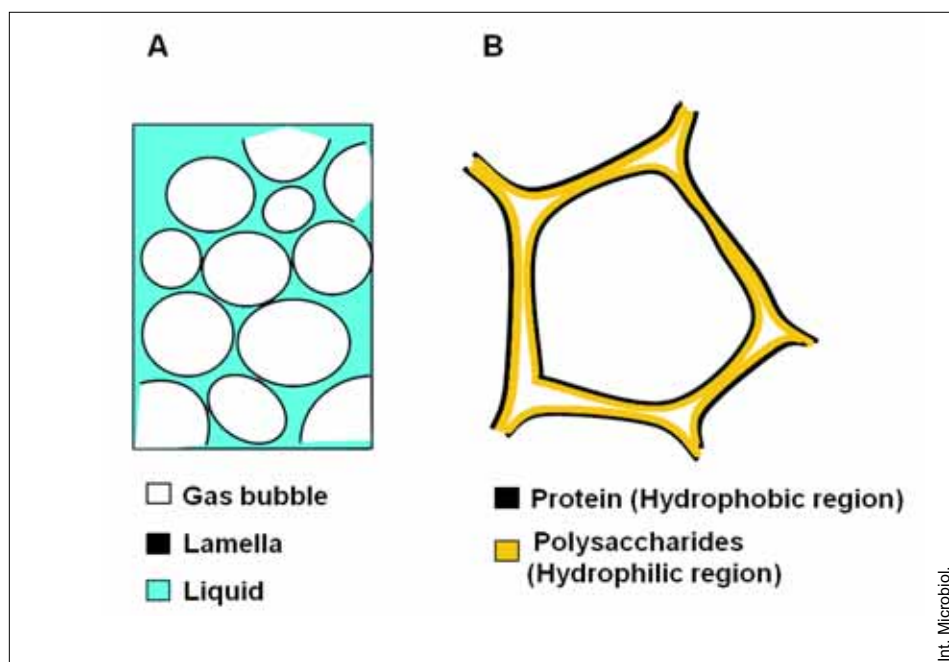


Fig. 1. (A) Foam structure. (B) Bubble glycoprotein distribution. Hydrophilic polysaccharides oriented in the liquid layer and hydrophobic proteins in the gas bubble.

formation at the end of fermentation or in the final product is a desired outcome, as clearly exemplified by its importance for sparkling wines or beers. However, the characteristic foam of sparkling wine behaves differently than that of beer. In sparkling wines there are two phases: in the initial phase, foam is abundantly produced by the carbonic gas decompression that occurs when the bottle is opened, because the gas was supersaturated inside it; in the second phase, the foam collapses a few seconds its formation, leaving a trace foam collar along the periphery of the glass, while in beers the foam must be stable and leave rings behind [18,52]. The quality of a good beer foam is defined by a combination of stability, quantity, lacing, whiteness, creaminess, and strength [18].

Yeast and foam

Foam quality is determined by several factors, such as the grape variety in the case of sparkling wines, and the barley variety and the hops in beer. In addition, the harvest conditions, the technological approach to handling the raw materials, as well as manipulation of the musts may influence foam quality [2]. The most important contributions of yeast in wine and beer production are made during fermentation, but the materials released by the yeast over the fermentation process remain in the final products and are important determinants of their organoleptic characteristics.

Yeast are involved in foaming, both positively and negatively. Sparkling wines such as cava and champagne are elaborated by the Champenoise method with two alcoholic fermentations (Fig. 2A), and sometimes an additional malolactic fermentation carried out by bacteria. The first alcoholic fermentation yields a base wine in which a secondary fermentation is conducted after the addition of yeast and further supplementation with grape syrup. This secondary fermentation takes place over a long period of time (several months) during which yeast autolysis occurs, resulting in the release of cellular components into the wine [5,38]. The released compounds include amino acids, peptides, proteins, and polysaccharides, all of which are known to be involved in foam formation and stabilization. Among the released proteins, mannoproteins, derived from the yeast cell wall, are particularly important as their hydrophobic nature causes them to preferentially adsorb to the gas/liquid interface of foam bubbles [1,39,43].

Beer brewing is a complex process (Fig. 2B) for which two types of yeast are employed: top- and bottom-fermenting

yeast. Their behavior is as distinct as the characteristics of the two main classes of beer produced by their utilization. Ales are fermented at temperatures ranging from 18 to 22 °C by top-fermenting strains, which are less flocculent and whose cells tend to be adsorbed to CO₂ bubbles. They characteristically form a yeast “head” at the top of the fermentation vessel, which is usually open and thus allows recovery of the yeast [35]. Lager beers are fermented at temperatures ranging from 7 to 15 °C by flocculent strains that precipitate at the bottom of the vessel, which is usually cylindroconical to allow recovery of the yeast for later use in repitching.

One difference between the production of sparkling wine vs. beer is that during the brewing process yeast are recovered and then employed in repitching the wort during the fermentation process. The foam level in the vessel is dependent on the generation number of the yeast. Early generations of yeast (generations 1 and 2) promote the formation of the largest amounts of foam, which reflects the presence of very high concentrations of polypeptides. As the generation number of the yeast increases, there is a decrease in both the foam level and the polypeptide content [31].

When yeast ferment the wine must or the beer wort, ethanol and CO₂ are produced, both of which interfere with foam production in different ways: the more ethanol produced, the less foam obtained; conversely, the more CO₂ dissolved, the more foam generated, because the yeast cell walls adhere to the gas bubbles, thus creating a foam layer at the surface of the fermentation tank [8]. In the case of sparkling wines, there are three carbonation sources, two of which are yeast-dependent. The first is the carbonation that develops during primary fermentation, in which some of the CO₂ produced during must fermentation remains dissolved in the base wine while the remainder is released into the atmosphere. The second source, which is forbidden in the Champenoise method, is the injection of CO₂ from either microbial or mineral sources. The third and most traditional method is the addition of CO₂ during the second fermentation, which occurs in a closed container, such as a bottle, such that the gas is maintained in the wine [24].

In beer production, after the first fermentation no CO₂ is retained; instead, carbonation of the immature beer, called “green beer,” takes place during the post-fermentation process. As in sparkling wines, carbonation in beer is attained by directly adding CO₂ after the conditioning and filtration steps; alternatively, it can be generated naturally by a secondary fermentation during the conditioning process, either in casks (ales) or in closed vessels (lagers). In some special beers, conditioning may take place in bottles [25].

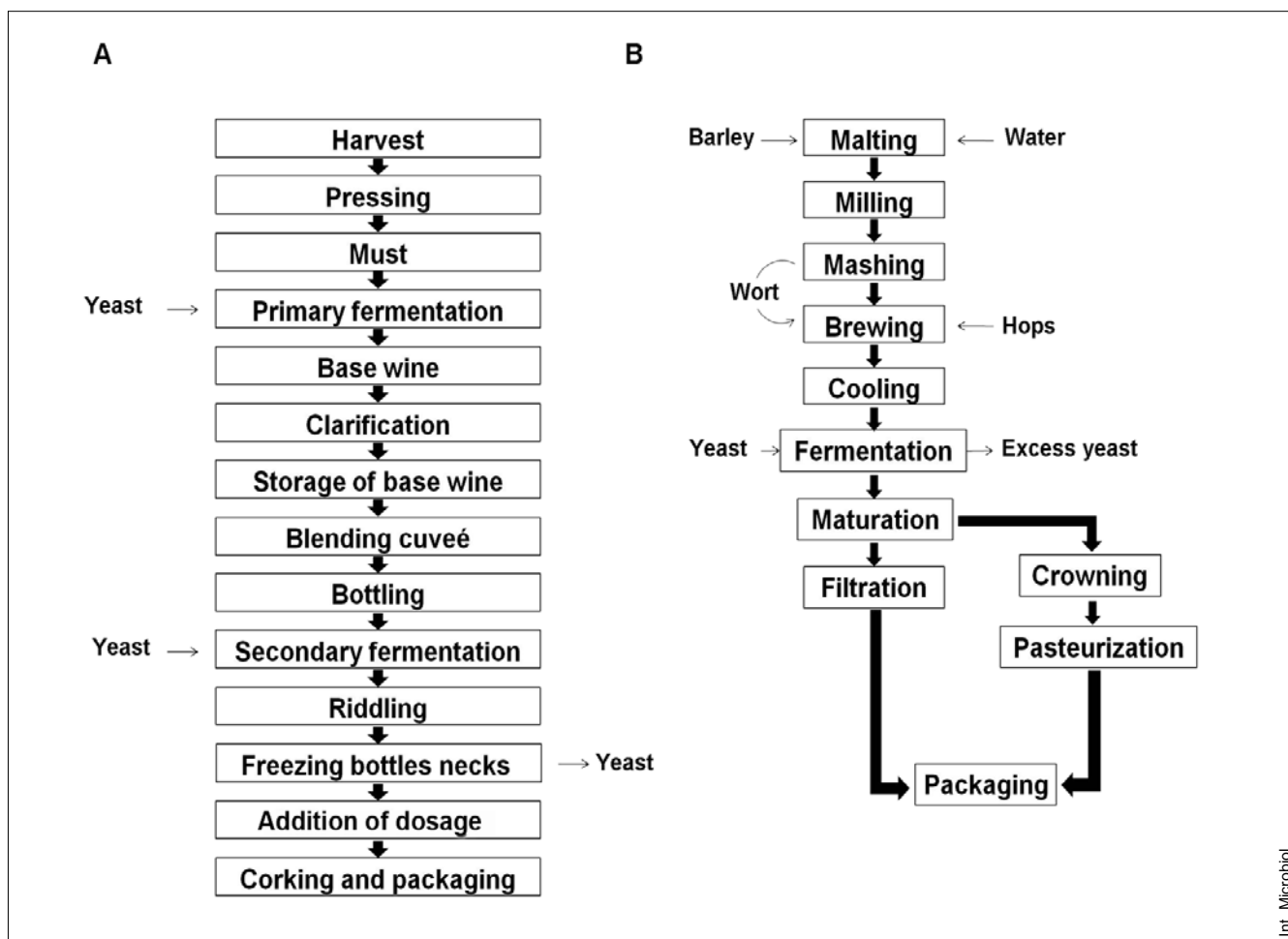


Fig. 2. Comparison of the beer- and wine-making processes. (A) Sparkling wine production. (B) Brewing.

Over-foaming during fermentation can be detrimental for the foaminess capacity of the final product, as foaming is responsible for the loss of a large amount of foam-active compounds. This potentially detrimental effect is particularly seen in high-gravity wort fermenters when yeast secrete proteolytic enzymes into the fermenting wort, degrading the hydrophobic polypeptides [11]. The loss of these polypeptides also may be due to their adhesion onto the sides of the fermentation vessel during transfer from the fermenter to the storage vessel. Furthermore, cold break precipitation, which is carried out to cool the wort to the temperature required for pitching after wort boiling, may be another source of hydrophobic polypeptide losses. In this case, the proteins and polyphenols derived from malt and hops extensively precipitate in the relatively cold media [8,11].

During beer fermentation, yeast subjected to stress conditions can negatively influence the formation of a beer head, due to the secretion of lipids that promote the coalescence of

bubbles, which results in foam collapse, and of proteinase A, which hydrolyzes malt proteins involved in foam stabilization [18]. The release of proteinase A increases under stress conditions that reduce yeast viability, such as high-gravity brewing, nitrogen starvation, high levels of alcohol, CO₂ and pressure. Proteinase A levels are higher at the end of the fermentation in high-gravity brewed beer than in low-gravity brewed beer. Foam is also destabilized by the ethanol produced by yeast during the fermentation process [11,18].

Foam-forming proteins in wine and beer

Quantitatively, proteins are minor components in wine but they are of technological and oenological interest as some are responsible for foam stabilization in sparkling wines, the reduction of haze in white wines, interactions between aroma-

producing compounds, and the protection of wine against tartaric salt precipitation, etc. Other grape proteins, however, may have negative effects such as haze formation [1,12,59].

Wine. The proteins found in wines come either from the grapes (*Vitis vinifera*) or from yeast, which release mainly mannoproteins and proteases [47]. The protein content in must does not correlate with that in wine because proteolytic activities and changes in pH during fermentation cause protein denaturation [12]. Overall, however, wine proteins (usually present at concentrations of 4–20 mg/l) are highly resistant to both proteolysis and low pH. They are heterogeneous in size, ranging from 10 to 100 kDa. Grape-derived proteins have sizes of 14–60 kDa, and those having a pI close to 3.9 are essential proteins [37]. Yeast-derived proteins present in wine are mainly glycoproteins involved in the assembly and disassembly of the cell wall and, specifically, in lipid catabolism [47]. Several studies have been carried out on the isolation and characterization of wine mannoproteins derived from yeast. Waters et al. [59] isolated a 420-kDa mannoprotein with a 30 % protein content, while the remaining 70 % corresponded to sugar residues, 98 % of which were mannose and 2 % were glucose. In white wine, Gonçalves et al. [22] reported three groups of mannoproteins, with molecular masses of 53.4, 252, and 560 kDa. Size-exclusion chromatography of these mannoproteins yielded two fractions, one containing the highest-molecular mass mannoproteins (containing 10 % protein and 90 % mannose) and the other containing mannoproteins of lower molecular mass (87.5%

mannose and 2.5% protein) [22]. Dambrouck et al. [12] used yeast antibodies and were thus able to detect a protein fraction in wine that corresponded to mannoproteins with sizes between 20 and 100 kDa (Table 1).

Foam due to grape proteins is influenced also by the grape variety, as reported in several studies showing that the Chardonnay variety has the best foaming ability, both in juices and base wines [2]. In the case of sparkling wines that are aged with yeast, the release of mannoproteins and other macromolecules from the yeast cells prior to autolysis contributes to foam stability [2,30,44,45]. Proteins therefore play a major role in foam stabilization, serving as macromolecular surfactants [9,49].

Some studies have shown that glycoproteins are the dominant macromolecules in the foam of sparkling wines [43,52]. The hydrophobic nature of these proteins explains why they are better foam stabilizers and foam producers than non-glycosylated proteins [23]. Certain strains of *S. cerevisiae* can rise to the surface of a liquid medium—a trait that is involved in foam formation and stabilization. The hydrophobicity of foam-forming strains reflects that of cell-wall proteins, including mannoproteins, which are able to adhere to the CO₂ bubbles [10]. This is so because the hydrophilic glycans are located at the liquid layer, among the bubbles, corresponding to the oxidic zone of the protein. Hence, when the layer surrounding the bubbles becomes thinner, the viscosity increases and drainage of the liquid is delayed. Hydrophobic polypeptides increase the surface tension of the bubbles, resulting in a stabilized foam [13,37,45,52].

Table 1. Molecules involved in foam formation in fermented beverages

Name	Molecule type	Molecular mass (kDa)/pI	Foam effect	Origin	Ref.
LTP1	Protein	9.7/9	Beer foam generation	Barley	[56]
Protein Z	Protein	40/5–6	Beer foam stabilization	Barley	[18]
Hordeins	Protein	17–33/6–8	Beer foam stabilization	Barley	[18]
Invertase	Protein	60/3.9	Wine foam stabilization	Grape	[13]
Proteinase A	Protein	44/4.54	Beer foam degradation by lowering the hydrophobicity of LTP1	Yeast	[18,33]
Awa1p	Protein	166.8/4.18	Foam generation in sake mash	Yeast	[54]
Fpg1p	Protein	72.5/4.23	Wine foam generation	Yeast	[7]
<i>FRO1</i>	Gene	–	Wine foam generation	Yeast	[57,58]
<i>FRO2</i>	Gene	–	Wine foam generation	Yeast	[57,58]

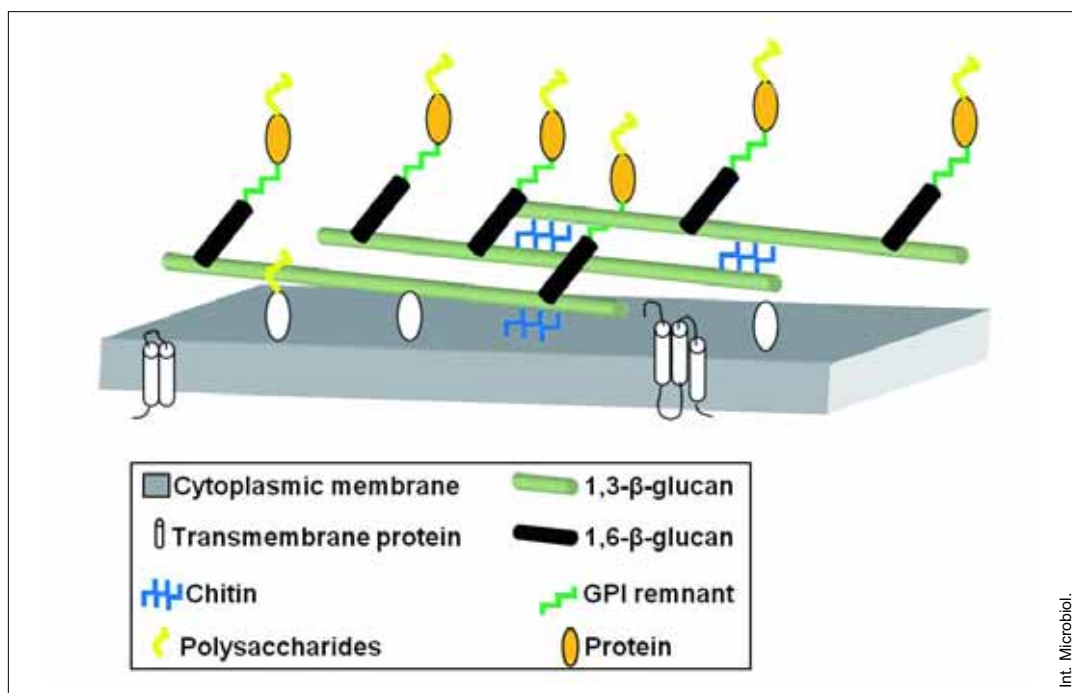


Fig. 3. Yeast cell wall structure.

Several studies have correlated the decrease in macromolecules and proteins in wine with a decrease in its foaming ability. The same trend was observed following the use of protein-lowering treatments of wines [50]. The relationship between proteins and foam quality was also obvious when hydrolytic enzymes were added to wine and musts. In all cases, there was a decrease in the foaming properties [32]. Other studies have related foaming ability to ethanol and to the SO_2 content, or to the total acidity of the wines, given the effects of ethanol and acidity on the surface properties of the hydrophobic proteins and the denaturing action of SO_2 [9,52].

The glycoproteins present in wine also originate from grapes and yeast. Although a large proportion of wine glycoproteins derived from grapes are involved in foaming, only one of them—vacuolar invertase—has so far been identified. Invertase is one of the most abundant proteins present in wine (representing 9–14 % of the total protein content in Chardonnay wines) [13,27,49]. Grape invertase is a 60–65-kDa *N*-glycoprotein with a pI of 3.9. This enzyme maintains its activity in wine and is of high hydrophobicity; significant decreases in the invertase content in wines have been shown to correlate with decreases in foam quality [13].

Yeast are also a source of foaming glycoproteins in wines, especially in sparkling wines [43,52]. These glycoproteins are mainly mannoproteins present in the yeast cell walls and they are released by the lytic action of β -1,3 glucanase dur-

ing fermentation. In the case of sparkling wines, they are released during the second fermentation and aging processes, as a consequence of yeast autolysis [22]. The yeast cell wall is composed of polysaccharides (85 %) and proteins (15 %). The polysaccharides include 1,3- and 1,6- β -D-glucans, a small amount of chitin, located mainly at the bud scars, and mannoproteins. Thus, the overall glycan fraction of the yeast cell wall is formed by glucose and *N*-acetyl-D-glucosamine (85 %), with the remaining 15 % corresponding to mannoproteins, in which the glycan moiety is bound to the protein moiety through *N*-glycosidic and *O*-glycosidic bonds. Mannoproteins are anchored to the cell wall through glycerolphosphatidyl inositol (GPI). Consequently, the inner face of the cell wall has a fairly rigid network of 1,3- β -D-glucan interlinked with 1,6- β -D-glucan and chitin and with the mannoprotein layer (Fig. 3), which is mainly located on the outer face of the supramolecular structure [34]. Mannoproteins contain *N*- or *O*-linked carbohydrates bound, respectively, to asparagine (through a bridge of di-*N*-acetyl-chitobiose) or to serine or threonine residues. The *O*-glycosylated mannoproteins have short mannose chains linked to the hydroxyl end of serine or threonine residues through α -glycosidic linkages with the hydroxyl group of the mannose anomeric carbon. These short chains are normally formed by five mannose residues, in which the inner first two are 1,2- α -linked while the others contain 1,3- α -D linkages [34] (Fig. 4A). The final

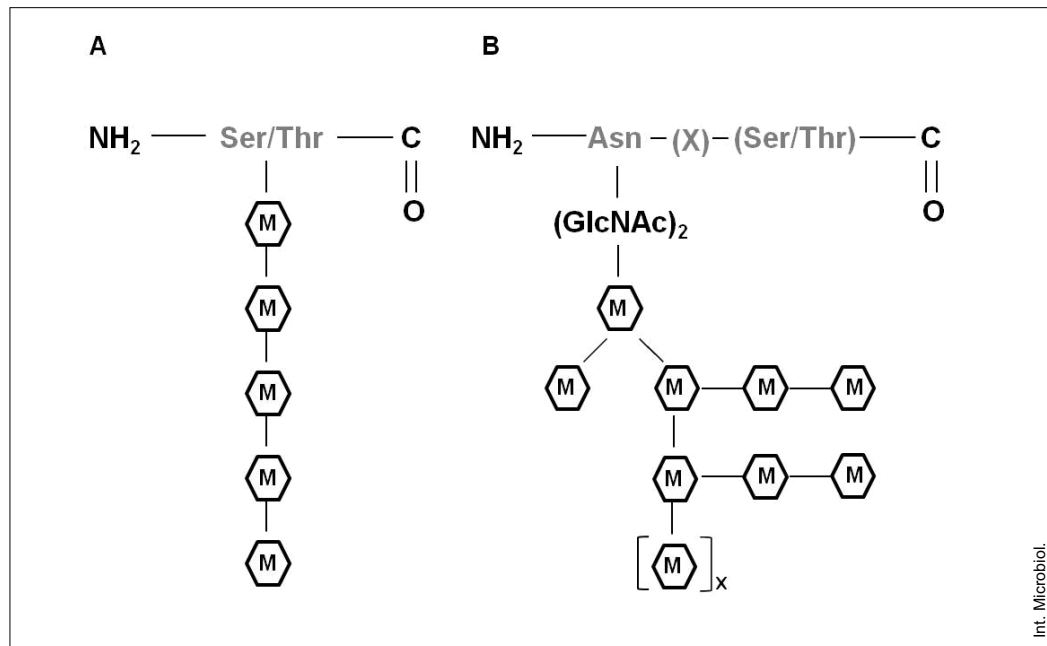


Fig. 4. Mannoprotein glycosylation pattern. (A) *O*-glycosylation. (B) *N*-glycosylation. Asn: asparagine; GlcNAc: *N*-acetylglucosamine; Ser: serine; Thr: threonine; X: any amino acid; M: mannose.

result is a mannoprotein containing up to 200 mannose residues, *N*-glycosidically linked in chains of 1,2- α -linked mannose that end in 1,3- α -linked mannose residues (Fig. 4B).

Mannoproteins are involved in a number of functions in yeast cell physiology, and many of these functions are determined by the glycosylation status. Thus, *O*-linked mannose chains, which are short and rigid and intercrossed with 1,3- β -D-glucan, confer cell-wall strength, which is further increased by *N*-glycosylated mannoproteins. Other functions of mannoproteins include cell wall porosity, water retention, cell-cell adhesion, virulence, biofilm formation, astringency, iron uptake, hydrophobicity, and enzymatic activity [14]. As glycoproteins, the yeast mannoproteins present in wine are involved in foam formation based on their hydrophobic proteinaceous moieties and hydrophilic domains, which are exposed to the air bubble, as well as a sugar moiety (containing mannose residues), which is usually hydrophilic and exposed to the aqueous medium [37,52]. The relationship between yeast mannoproteins and foam was first noted by Núñez et al. [45], who were able to improve the foaming of sparkling wine by adding yeast cell-wall mannoproteins. During alcoholic fermentation, mannoproteins are released by actively growing yeast in a hydrolytic process controlled by the mother cell wall to allow emergence of the daughter's bud [20]. In sparkling wine, mannoproteins and other intracellular compounds are released also as part of the wine-

aging process, during yeast autolysis. In this step, glycoproteins and polysaccharides in the cell wall are hydrolyzed by β -glucanases, thus releasing mannoproteins inserted in or covalently linked to glucans [40].

A positive correlation was shown between proteins, polysaccharides, and the quality of foam in sparkling wines. Foam quality has an optimum aging time, after which it is dramatically diminished [1,43]. Núñez et al. [44], employing an autolysis-defective *S. cerevisiae* strain, showed that with accelerated autolysis a quality sparkling wine with improved foam properties could be obtained in a shorter time. These authors also showed that mannose was the main sugar component of the polysaccharides released by this particular yeast strain, and their presence correlated with better foam quality. The authors [45] then enriched a model wine with a thermal extract of yeast cell wall. The preparation was mainly composed of mannoproteins with molecular masses between 10 and 21.5 kDa; this produced a substantial improvement of the foam quality.

Although several studies have identified a variety of yeast proteins released in wine, and the role of mannoproteins as foam enhancers has been reported, only two yeast mannoproteins have so far been identified as foaming proteins. Awa1p, from the cell wall of *S. cerevisiae* sake strain K7, was the first mannoprotein to be identified as a foaming protein [54]. It is highly hydrophobic and encodes a putative GPI-anchoring

site. Fermentations carried out with the *Awa1p* null mutant failed to produce foam in the sake mash, thus confirming this protein's role in foaming [41,54]. Recently, based on sequence analogy with the *AWA1* gene sequence, a new foaming gene, *FPG1*, was identified in *S. cerevisiae* wine strain 145A211 [7]. This gene codes for a cell-wall manno-protein, *Fpg1p*, that is highly homologous to *Awa1p*, hydrophobic, and involved in foaming during wine fermentation. The role of *Fpg1p* was demonstrated by its over-expression in a non-foaming *S. cerevisiae* strain, which resulted in the strain's acquisition of foaming ability.

The first report of foaming genes in *S. cerevisiae* sake strains was that of Kasahara [29]. Based on the results of hybridization experiments between non-foaming and foaming strains, two genes were determined to be responsible for the foaming phenotype. Thornton [57,58] used tetrad analysis to study foaming in a *S. cerevisiae* oenological strain and identified two genes involved in foam formation: *FRO1* and *FRO2*, located on chromosome VII and 21 centimorgans apart. These genes were found to be dominant, non-additive, and allelic to the genes determined in the sake strains; but studies of the respective proteins products have yet to be published.

Beer. Foaming in beer is mainly due to the interactions between proteins and hop acids [3]. Unlike in wine, the main proteins involved in beer foaming derive from barley, while yeast proteins play a secondary role in enhancing the foam, although they are important in attenuating the effects of proteinase A [33]. Beer contains approximately 500 mg protein/ml, mostly in the form of polypeptides with molecular masses of 5–100 kDa. The small size of the polypeptides found in beer is due to the hydrolytic effects of malting, mashing, boiling, and fermentation. The most important proteins derived from barley and involved in foam formation are the lipid transfer protein *LTP1p*, a 9.7-kDa polypeptide; protein Z, a 40-kDa polypeptide; and various hordein-derived polypeptides ranging in size from 10 to 30 kDa [18,56]. Additionally, there are small quantities of polypeptides directly derived from yeast [42].

Brewing is a complex process, in which malt and other cereals are mashed and boiled together with hop to obtain the wort, which is yeast-fermented; the yeast are used several times, such as for wort repitching. The foaming proteins from barley and wheat have been studied [28]. Modification of the barley during germination causes protein hydrolysis, with the release of amino acids and polypeptides. Malt modification negatively correlates with foam stability, due to the degrada-

tion of foam-positive protein effectors and to the presence of large polypeptides. In addition, the degradation of non-starch polysaccharides reduces the viscosity and thus the drainage of liquid from the foam [46].

The *LTP1* protein originates from barley aleurone and is expressed at the final stages of grain development. This protein, which is concentrated in beer foam, constitutes up to 1 % of beer proteins [16]. Its foaming potential increases during the kettle boil, when it is irreversibly denatured [36]. *LTP1* has different modes of action in relation to beer foam quality. When isolated from beer, it is a good foam-forming factor but a poor stabilizer. Its stabilizing properties, however, are substantially enhanced when it is combined with low-molecular-weight hordeins or with high-molecular-weight foam proteins, including the protein Z [56]. However, the role of *LTP1* in foam stabilization is not clear. On the one hand, an increase in *LTP1* content seems to imply an improvement in foam stability, as confirmed by Lusk et al. [36]. However, Evans et al. [17], employing the Rudin apparatus, obtained ambiguous results, in which either a positive or negative relationship or none at all between *LTP1* and foam stability was determined. These discrepancies arise from the fact that *LTP1* is a lipid binding protein, such that foam stability varies according to the amount of *LTP1* denaturation achieved by boiling [18].

The first specific protein suggested to promote foam stability was protein Z. This protein originates from barley albumin and represents 2 % of the total protein in beer. It has two isoforms, Z4 and Z7. Z4 is predominant in beer and it is the beer protein with the highest elasticity and surface viscosity, but it is not as abundant in foam as *LTP1* [16,56]. Evans et al. [17] showed that the level of protein Z did not always correlate well with the foam stability values measured using the Rudin apparatus. On the other hand, by employing an immuno-affinity column, the authors observed that foam stability decreased as protein Z was increasingly removed from the tested beer. These contradictory results no doubt reflect the malt modification level. Thus, when malt is under-modified, the contribution of protein Z to foam stability is low; when malt modification is higher, the contribution of protein Z to foam stabilization increases [17]. Bamforth [4] explained the influence of malt modification on protein Z action as a consequence of the hydrolysis of hordeins, since hydrolyzed hordeins exclude albumins, such as protein Z, from beer.

Hordeins are the major storage proteins in barley; they are insoluble in aqueous solution and become soluble upon hydrolysis [16]. There are several groups of hordeins, classi-

fied as B, C, D, and γ on the basis of their molecular sizes. The diversity of these groups and their interactions with proteases to enable solubilization are such that they give rise to desirable foam-promoting but also to undesirable haze-promoting hordein species [18]. Sheehan and Skerritt [53] used monoclonal antibodies to identify a 23-kDa polypeptide present at relatively high concentrations in foam; this protein is thought to be active on the bubble surface, where it promotes foam stability. A later study demonstrated that in moderately carbonated beer the half-life of foam correlates with the combined presence of LTP1 and a 17-kDa protein, but this is not true for highly carbonated beer. Although denatured LTP1 enhances foam stability to a greater extent than denatured hordeins, when proteolysis is reduced, the stabilizing properties of the latter are better than those of albumins [28].

Proteins from yeasts also influence the foam in beer, but in two different ways: (i) they are involved in foam formation during fermentation and during the final steps of beer brewing; (ii) they release proteinase A, an enzyme that actively degrades LTP1, thus decreasing the foam level. The presence of yeast proteins in beer has been described in a number of studies, many of which were aimed at improving the technological processes and the final quality of beer. These reports include data from studies on yeast flocculation and on the involvement of mannoproteins in haze elimination. However, only a few investigated the relevance of yeast in foam formation in beer [15,26,51,55]. Immunological assays performed on beer foam conclusively demonstrated the presence of yeast antigens in foam. Although most of these antigens are in the 70- to 120-kDa range, some studies described antigens as large as 200 kDa [42]. The relationship of yeasts and foam was established in assays in which synthetic must was fermented; the results showed that the artificially produced foam was more stable than that obtained from a non-fermented synthetic must. These studies also established differences in foam stability as a function of the yeast strain employed, thus confirming yeast involvement in foam formation. While this involvement is limited, yeast are, however, important to foam stabilization [40].

There are also several reports describing factors that negatively affect foam formation. For example, yeast stress is responsible for a decrease in foam stability; in high-gravity brewing, only low levels of hydrophobic polypeptides are present in beer and foaming is concomitantly diminished [11]. Both events can be related to the presence of proteinase A, which degrades hydrophobic foam-promoting proteins and is released from living yeast cells under stress conditions, such as high-gravity worting, or improper post-fermentation stor-

age. It has been shown that yeast vacuolar proteinase A, encoded by *PEP4*, lowers the hydrophobicity of LTP1, thus affecting foam stability. In barley, LTP1 in its native form is resistant to the protease, but after processing during malting it becomes sensitive to protease digestion [18,33,56].

Conclusions

Proteins play a major role in the development and maintenance of foam during the production of beer and sparkling wines. Among the malt proteins found in beer, LTP1 and protein Z are the main contributors to foaming. Although yeast proteins are present in beer foam, their function is more closely associated with foam stabilization. Indeed, yeast play a rather negative role in foaming, due to their release of proteolytic enzymes, such as proteinase A, which degrade the LTP1 protein. By contrast, in sparkling wines, yeast mannoproteins are the major foam promoters. This is due to their structure, which favors adhesion to the foam bubbles' gas/liquid interface. This review also highlighted the need for further studies to identify and characterize the foaming proteins that originate from the yeast cell wall, information that will surely be of interest to brewers and wine-makers.

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