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The effect of yeast growth stages on the absorption of polyphenols

R.A. Harbah*, T.V. Meledina and A.A. Morozov

Saint-Petersburg State University of Information Technologies, Mechanics and Optics (ITMO University), Faculty of Food Biotechnology and Engineering, Kronversky pr., 49, RU197101 St Petersburg, Russia
*Correspondence: razan.harbah@mail.ru

Abstract. Colloidal stability of beer is one of the most critical challenges facing the brewing industry. In brewing, stabilization agents are widely used to remove colloids such as proteins and polyphenols. However, the removal efficiency of polyphenols depends on the yeast, which adsorbs these compounds on its surface. The adsorption of polyphenols on the yeast surface is associated with the zeta potential of the cell wall, which correlates with the mannan content in them. The purpose of the study was to investigate the role of yeast strains in adsorption of polyphenols (increasing colloidal stability of beer) and the correlation of this property with the mannan content in the cell wall during the fermentation. Two *S. cerevisiae* strains were used in this study, and the specific growth rate of yeast, the specific rate of change in the polyphenol content and the mannan content were determined. The results of this study showed that the highest mannan content in yeast was after 9 hours (the end of exponential growth phase). Its content was 10.97% by weight of dry matter in the yeast *S. cerevisiae Californian Lager* (M54), and 13.69% in the yeast *S. cerevisiae Belgian Wit* (M21). The desorption of polyphenols was observed during the period from 9 to 24 hours of fermentation, where an increase in the content of polyphenols in the medium was observed. Based on these results, it is necessary to remove the yeast at the end of the exponential growth phase to increase the colloidal stability of the beer.

Key words: colloidal stability, mannan, polyphenols, *S. cerevisiae* yeast.

INTRODUCTION

Beer is a complex alcoholic beverage made from malt, hops, water, and yeast (Werlen et al., 2010). Beer consists of particles: their size ranges from 0.01 to 3 μm, and because of that beer is a complex colloidal system. One of the problems facing the brewing industry is the achievement of colloidal stability of beer and reduction of its turbidity during storage. The turbidity of beer has a different nature. It can be biological turbidity, or non-biological turbidity (colloids). Beer colloids are nitrogen-containing substances, in particular proteins and other colloids such as polysaccharides and polyphenols. Polyphenols and proteins primarily form visible turbidity (haze), but interactions between polypeptides and polysaccharides, or polypeptides and minerals, can also contribute to the haziness of beer. In brewing, to increase colloidal stability, the stabilization agents are used (Mastanjević et al., 2018). The most widely used products today are silica gel for protein stabilization and Polyvinylpolypyrrolidine (PVPP) for

polyphenols stabilization. Most beer polyphenols are derived from malt (70–80%), while about 20–30% are derived from hops (Wannenmacher et al., 2018).

Yeast is an important ingredient in beer production, in addition to its role in the fermentation process, can be a stabilization agent that absorbs polyphenols on its surface. The yeast cell wall carries a negative charge due to the ionization of the carboxyl and phosphodiester groups in the cell wall proteins and phosphor-mannans, respectively; therefore, the yeast cell will attract particles that carry a positive charge, such as polyphenols. In other words, the removal efficiency of polyphenols depends on the yeast, and this phenomenon is associated with the composition of the cell wall and, in particular, with its peripheral layer (Stewart, 2018).

The cell wall of the yeast consists of three layers, and it is mainly composed of β-D-glucans, mannan proteins, and a small amount of chitin, which is mainly located at the bud scars on the surface of mother cells. The middle layer consists primarily of β-glucan, which is responsible for the stiffness and strength of the cell wall. β-D-glucans, which are the first essential polysaccharides of the cell wall in *S. cerevisiae* yeast and other yeasts are divided into two subtypes in accordance with the mode of glucose bonds. They are β-1,3-D-glucose, and β-1,6-D-glucose. These polysaccharides represented 50–60% (by weight) of the total cell wall (Liu et al., 2008). Mannan oligosaccharides (MOS) are the second essential polysaccharides in the cell wall. Most of the mannan oligosaccharides are associated with proteins that form a complex called mannoprotein.

Characteristically, the mannans of *Saccharomyces cerevisiae* consist of long D-mannose chains, (where mannose molecules of 50–200 units bind to each other via α1-6 bonds) with short side chains in α-1-2 and α-1-3 bond. These macromolecules bind to asparagine in the protein. Also, mannan has short oligomannose units with α-1-2 and α-1-3 bonds that are associated with serine and threonine in the protein. Thus, the mannose chains bind to the polypeptide part by two methods. The first method consists of using an O-glycosidic bond between the hydroxyl group of amino acids (serine and threonine) and mannose residues. The second method is through the di-N-acetylchitobiose bridges that are formed by the N-glycosidic bond between the N-acetylglucosamine residue and the amide nitrogen of the asparagine polypeptide chain (N-linked mannoprotein) (Maru et al., 2015).

Mannoprotein, which represents 30–40% (by weight) of the total cell wall (Moreno et al., 2008), is mainly localized in the outer layer of the yeast cell wall and plays an essential role in determining the charge on the surface of the cell wall, in other words, it directly affects the zeta potential of yeast (Lipke & Ovalle, 1998).

For each yeast strain, the adsorption properties depend on the zeta potential of the cell wall, which in turn, correlates mainly with the mannan content in yeast (Lavaisse et al., 2019).

The amount of mannan polysaccharides in *Saccharomyces cerevi*siae varies depending on the characteristics of the yeast strain, their physiological state, as well as the physicochemical conditions of cultivation (Bzducha-Wróbel et al., 2013). In the same context, Lavaisse and others showed that the zeta potential of yeast varies depending on the stages of yeast growth and cultivation conditions (Lavaisse et al., 2019). It is well known that the surface properties of yeast cells determine processes such as adsorption, flocculation, and adhesion to various surfaces. The surface charge, which value can be determined indirectly through the measurement of the zeta potential, is one of these properties.

Afonin and colleagues showed that yeast adsorbs the colloidal particles, which have size ranged from 0.31 to 0.39 μm on their surface, while the phenolic compounds in beer are significantly smaller (from 1 nm to 10 μm). The yeast cell in good physiological condition adsorbs these low molecular weight compounds due to negative surface charge (Afonin et al., 2012). In context, the purpose of this study was to investigate the role of yeast strains in the absorption of polyphenols on its cell wall during wort fermentation and establish the correlation this characteristic with the mannan content in yeast cells at the various stages of growth yeast.

MATERIALS AND METHODS

Strain and growth conditions

Research objects were dry yeast *S. cerevisiae Californian Lager (*M54) (Mangrove Jacks, New Zealand) and *S. cerevisiae Belgian Wit* (M21) (Mangrove Jacks, New Zealand). The inoculum was 3×10^7 cfu mL^{-3}. The flasks with capacity 300 mL were used, and the usable volume was 264 mL.

Spraymalt light barley wort extract (Muntons, England) was used to prepare the medium. 200 g of extract was mixed with 1,500 mL of water in conical flask. Then, the mixture was heated for 20 minutes.

The concentration of dry matter of nutrient medium was determined using refractometer PTR-46 (Shimadzu, Tokyo, Japan). The dry matter content after adding the inoculum was 8.6%. The fermentation process was carried out in incubator (TC-1/80 CPU, Russia) on the principle of a batch culture without forced aeration at temperature 28 °C for 29 hours.

The specific growth rate of yeast, the specific rate of change in the polyphenol and mannan content were determined in this study.

Extraction and estimation of crude mannan:

The yeast biomass was separated from nutrient medium using centrifuge (ULAB, Beijing, China) at 6,000 rpm for 15 min. The supernatant was decanted and the sedimented yeast was washed with distilled water twice and dried according to Huang et al. (2010). Weight of dry cell mass estimated in grams. The mannan were obtained from 2 g of dry yeast extraction with 1% NaOH (50 mL) at 100 °C for 2 hours, cooling and neutralizing to pH 7 with dilute HCl solution.

Deprotienization by the TCA (trichloroacetic acid) method: The concentrated solution of crude mannan oligosaccharides were adjusted to pH 3 with 10% TCA solution overnight. The centrifugation at 5,000 rpm 10 min^{-1} was used to obtain the mannan in the supernatant (Huang et al., 2010). The quantitative estimation of mannan was determined by the phenol-sulfuric acid method using glucose as standard (Maru et al., 2015).

Determination of polyphenols:

The polyphenols were determined according to the EBC method 9.11 using a 'UV 1240' spectrophotometer from 'Shimadzu' at the wavelength of 600 nm (Dvořáková et al., 2007).

The specific rate of change in the polyphenol content in the culture fluid (adsorption, desorption) was calculated using the equation:

$$\rho = \ln(y - y_0)/(t - t_0) \quad (1)$$

where ρ – the specific rate of polyphenols adsorption, h^{-1}; y – concentration of polyphenols at the beginning of measurement (t_0), mg L^{-1}; y – concentration of polyphenols at the end of measurements (t), mg L^{-1}; t-t_0 – the time interval between measurements, h.

The accumulation of yeast biomass was determined by the weight method after drying the suspension of washed yeast to constant weight at 105 °C for 24 h in cabinet dryer ES-4610 (Reaktivsnab, Shymkent, Kazakhstan).

The specific growth rate of yeast was calculated by the equation:

$$\mu = \ln(x - x_0)/(t - t_0) \quad (2)$$

where μ – the specific growth rate, h^{-1}; x_0 – biomass at the moment of the inoculation (t_0), g; x – biomass at the time τ, g; $t - t_0$ – the time interval between measurements, h.

Data Analysis

Data generated were subjected to analysis of variance (ANOVA) using Origin statistical software (version 6.1) at 95% significance. All measurements were made in at least triplicate. Results were reported as means ± standard deviations.

RESULTS AND DISCUSSION

The growth and multiplication of yeast in a batch culture is described by the growth curve (Figs 1, 2). The growth curve of the yeast *Saccharomyces cerevisiae Californian Lager* (M54) (Fig. 1) showed that the lag phase lasted 5 hours, then, after a short phase of increasing growth, a log phase was started and lasted 3 hours. Then from 9 to 25 hours, there was a slow decrease in biomass growth and the cell death phase was observed for the next 4 hours, as evidenced by the decreasing value of the specific growth rate of yeast (Table 1). Fig. 2 shows the growth curve of *Saccharomyces cerevisiae Belgian Wit (*M21) yeast. The lag phase lasted 2 hours, and then the growth acceleration began and lasted 1 hour. The decrease in biomass was started after the log phase, which lasted 5 hours, and the cells entered the stationary phase after 9 hours of cultivation. The stage of the cell death began in the last 6 hours of cultivation as shown at the growth curve and this evidenced by the decreasing value of the specific growth rate of yeast at the last hours of cultivation (Table 1).

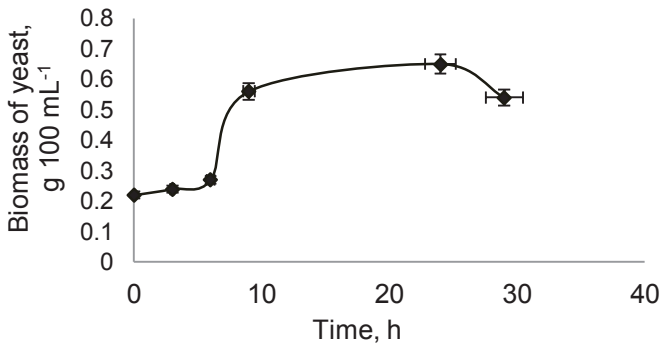

Figure 1. The growth curve of the *Saccharomyces cerevisiae Californian Lager M54* in a batch culture.

Therefore, the log stage of the *Saccharomyces cerevisiae Belgian Wit (*M21), which lasted 5 hours was 1.6 times more compared to the *Saccharomyces cerevisiae Californian Lager* (M54), and in both yeasts, biomass began to decrease after 9 hours of cultivation, while the yeast cells of *Saccharomyces cerevisiae Belgian Wit (*M21) entered the phase of death before other yeasts.

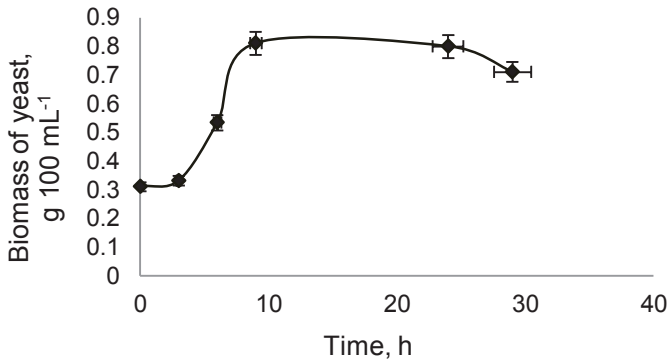

Figure 2. The growth curve of the *Saccharomyces cerevisiae Belgian Wit M21* in a batch culture.

Table 1. The variation of the specific growth rate of yeast in the culture fluid and the content of mannan in yeast during the time of cultivation

Period of the cultivation (h)	*S. cerevisiae Californian Lager* (M54)		*S. cerevisiae Belgian Wit (*M21)	
	Mannan (%*)	Specific growth rate (h^{-1})	Mannan (%*)	Specific growth rate (h^{-1})
0	6.96 ± 0.007	-	6.88 ± 0.006	-
3	7.63 ± 0.006	0.029	9.35 ± 0.008	0.081
6	8.85 ± 0.007	0.034	11.21 ± 0.002	0.090
9	10.97 ± 0.008	0.10	13.69 ± 0.003	0.106
24	10.45 ± 0.007	0.045	13.16 ± 0.002	0.040
29	9.77 ± 0.005	-0.04	12.75 ± 0.004	-0.028

* mg mannan mg^{-1} dry cell weight.

The content of polyphenols in the culture fluid during the time of cultivation was identified (Table 3). The results showed the decrease of their content when the cells were in good physiological activity, and this confirms the ability of yeast to adsorb these colloids on its surface, as Afonin and colleagues showed through his study (Afonin et al., 2012).

To clarify the relationship between the growth phases and polyphenol adsorption, the change in polyphenol content at different time intervals corresponding to a specific yeast growth phase was determined. Also, the mannan content in yeast was determined at the various phases of growth in order to study its effect on the adsorption properties of yeast.

When the growth of yeast slow down, and the cells entered the stationary phase as reported in Table 1, the desorption of polyphenols from the surface of yeast was observed. It can be seen from the increasing of the content of the polyphenols in the medium (the positive value of the Specific Adsorption Rate of Polyphenols) (Table 2, 3).

Table 2. The specific rate of change in the polyphenol content in the culture fluid during the time of cultivation

Time intervals (h)	The specific rate of change in the polyphenol content (h^{-1})	
	S. cerevisiae Californian Lager M54	*S. cerevisiae BelgianWit M21*
0–3	- 0,155	-0.046
3–6	- 0,102	-0.073
6–9	-0,021	-0.045
9–24	0,016	0.023
24–29	0,026	0.030

Determination of mannan in yeast showed that it accumulated in the cells when the cells were in good physiological activity.The highest mannan content in yeast was at the end of the exponential phase of growth (log phase), i.e. after 9 hours of the cultivation. Its content was 10,97 % in the yeast *S. cerevisiae Californian Lager* (M54) and 13,69 % in the yeast *S.cerevisae Belgian Wit* (M21) by the weight of dry matter of yeast. A decrease in the amount of mannan was observed when the yeast entered the stationary phase (after 9 hours). According to these results, the mannan content in yeast cells varies depending on the phases of yeast growth (Moreno et al., 2008).

In both yeast strains, the highest amount of mannan was when the cells were in the log phase. The mannan content in *S. cerevisiae Belgian Wit* (M21) was more than its content in *S. cerevisiae Californian Lager* (M54). At the same time, the adsorption of polyphenols on the surface of this strain was more than the other during this phase (Table 3). When the physiological activity of yeast cells decreased, the content of mannan decreased, and at the same time, the desorption of polyphenols from the surface of yeast started. In other words, the decrease of the physiological activity of cells leads to colloid adsorption reduction and an increase of beer turbidity and this corresponds to the results obtained by Meledina and colleagues (Meledina et al., 2015).

Table 3. The polyphenol content in the culture fluid during the time of cultivation

Period of the cultivation (h)	Content of the polyphenols (mg L^{-3})	
	S. cerevisiae Californian Lager M54	*S. cerevisiae BelgianWit M21*
0	171.60 ± 2.333	168.91 ± 1.003
3	109.33 ± 1.320	156.66 ± 0.333
6	79.83 ± 3.083	117.33 ± 3.333
9	72.86 ± 1.653	102.66 ± 2.583
24	94.93 ± 4.763	109.73 ± 1,063
29	110.06 ± 1.013	119.03 ± 2.403

CONCLUSIONS

Based on the results obtained from the study conducted on two strains, we conclude that the concentration of mannan varies depending on the yeast growth stages and the yeast strains. At the same time, the adsorption of polyphenols depends on the mannan content in the yeast, which was the highest when the yeast was on the exponential phase (log phase). Therefore, in order to increase the colloidal stability of beer, and reduce the consumption of stabilizers in particular PVPP; it is necessary to remove the yeast from

the fermentation tanks at the end of the exponential phase of growth, that is, before the desorption of polyphenols from the surface of the yeast. This conclusion is valid for the yeast *S. cerevisiae Californian Lager* (M54) and *S. cerevisiae Belgian Wit* (M21).

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