Food Chemistry 268 (2018) 162–170

Contents lists available at ScienceDirect

Food Chemistry

journal homepage: www.elsevier.com/locate/foodchem

Wheat gluten hydrolysates separated by macroporous resins enhance the stress tolerance in brewer's yeast

Huirong Yang[a], Xuyan Zong[b], Chun Cui[a], Lixia Mu[c], Haifeng Zhao[a,*]

[a] School of Food Science and Engineering, South China University of Technology, Guangzhou 510640, PR China
[b] Liquor Making Biological Technology and Application of Key Laboratory of Sichuan Province, Sichuan University of Science and Engineering, Zigong 643000, PR China
[c] Sericultural & Agri-Food Research Institute, Guangdong Academy of Agricultural Sciences/Key Laboratory of Functional Foods, Ministry of Agriculture/Guangdong Key Laboratory of Agricultural Products Processing, Guangzhou 510610, PR China

ARTICLE INFO

Keywords:
Wheat gluten hydrolysates
Macroporous resins
Brewer's yeast
Viability
Tolerance

ABSTRACT

Wheat gluten hydrolysates (WGH) were used to examine their adsorption–desorption kinetics and thermodynamics characteristics on six macroporous resins, and their effects on the stress tolerance in brewer's yeast. Results showed that the *pseudo* second-order kinetics, Langmuir and Freundlich model could illuminate the adsorption mechanism, and the adsorption process was physical, spontaneous and exothermic. The 40% ethanol fraction separated by XAD-16 resin improved significantly the ethanol tolerance and the viability of brewer's yeast, while the 0% ethanol fraction separated by XAD-16 resin increased obviously the osmotic stress tolerance and the viability of brewer's yeast. Results from scanning electron microscopy showed that both these WGH fractions could increase budding rate and maintain normal morphology of yeast cells under various environmental stress. Thus, WGH separated by macroporous resin could be used in high gravity brewing to enhance the ethanol and osmotic stress tolerance in brewer's yeast.

1. Introduction

Very high gravity (VHG) brewing has become more and more popular in modern breweries owing to its economic, environmentally friendly and high productivity advantages (Casey, Magnus, & Ingledew, 1984; Deesuth, Laopaiboon, & Laopaiboon, 2016; Lei et al., 2013; Puligundla, Smogrovicova, Obulam, & Ko, 2011). However, yeast cells exposed to extreme environmental conditions during the VHG fermentation would lead to slow or stuck fermentation, which made the improvement on the tolerance of ethanol and osmotic stress of yeast cells a challenge for breweries. Osmotic pressure and ethanol toxicity are the two important stress-induced factors which yeast cells encounter during VHG brewing. High osmotic pressure could cause the loss of yeast viability, slow fermentations and the reduction of ethanol production efficiency during VHG brewing (Casey et al., 1984; Deesuth et al., 2016; Kawarygielska & Pietrzak, 2014; Ozmihci & Kargi, 2007). Meanwhile, the ethanol toxicity would induce DNA damage and a cell viability decrease, and even impact cellular transport systems (D'Amore, Panchal, Russell, & Stewart, 1990; Ibeas & Jimenez, 1997; Kim et al., 2016). Therefore, it is important to improve physiological activity, the tolerance to ethanol and osmotic stress and fermentation performance of yeast cells in the VHG brewing.

It has been found that amino nitrogen supplementation, such as urea, ammonium salts, amino acids, yeast extract, especially a more complex nitrogen source of peptides, could improve yeast viability, ethanol tolerance and productivity during VHG brewing (Puligundla et al., 2011; Srichuwong et al., 2009; Theerarattananoon, Lin, & Peng, 2008; Yang, Zong, Cui, Mu, & Zhao, 2017). Previous studies also indicated that supplementation with wheat gluten hydrolysates (WGH), a mixture of amino acids and peptides, in a medium could promote the growth and fermentation of yeast cells during VHG brewing (Lei et al., 2013; Mo, Zhao, Lei, & Zhao, 2013; Zhao, Wan, Zhao, Lei, & Mo, 2014). Actually, the bioactivity of WGH was mainly determined by the physiologically active peptides in WGH, which depended on their molecular characteristics (Mo et al., 2013). Therefore, isolation and enrichment of active peptides with specific molecular characteristics from WGH were prerequisites for the application of WGH in high gravity brewing. Nowadays, macroporous resins have been used for separation, purification and enrichment of bioactive compounds with high efficiency, high recovery and low cost (Lin, Zhao, Dong, Yang, & Zhao, 2012; Zhang, Zhang, & Xu, 2009), which was suitable for industrial scale application (Wang, Wang, Yuan, & Zhang, 2017; Zhuang et al., 2016). To the best of our knowledge, there was no detailed study on purifying and enriching of bioactive peptides from WGH by

* Corresponding author.
E-mail address: hfzhao@scut.edu.cn (H. Zhao).

https://doi.org/10.1016/j.foodchem.2018.06.082
Received 22 February 2018; Received in revised form 24 May 2018; Accepted 18 June 2018
Available online 19 June 2018
0308-8146/

macroporous resins and their potential application in enhancing the ethanol and osmotic stress tolerance in yeast cells.

Therefore, one objective of this study was to screen the optimal macroporous resin for WGH separation by comparing the static adsorption–desorption behaviours, kinetics, adsorption isotherms and thermodynamics of WGH on D101, DA-201E, AB-8, XAD-16, DM130 and HPD-826 resins. The other objective was to obtain the best active fraction by ethanol/osmotic stress tolerance tests and ethanol fermentation experiment.

2. Materials and methods

2.1. Materials and chemicals

Macroporous resins (D101, DA-201E, AB-8, XAD-16, DM130 and HPD-826) were obtained from H & E Co., Ltd. (Beijing, China). The wheat gluten was purchased from the Fengqiu Huafeng Powder Co. Ltd. (Henan, China), and Pancreatin (1.2×10^5 U/g) was obtained from Novo Co. (Novo Nordisk, Bagsvaerd, Denmark). The yeast nitrogen base (YNB) without amino acids was purchased from Jianyang Biotechnology Co. Ltd. (Guangzhou, China). The industrial yeast strain (*Saccharomyces pastorianus* CGMCC No.4466) used in this investigation was deposited in the China General Microbiological Culture Collection Center (CGMCC). All other chemicals and solvents were of the highest commercial grade and obtained from Sinopharm Chemical Reagent Co. Ltd. (Shanghai, China).

2.2. Enzymatic hydrolysis of wheat gluten

The wheat gluten hydrolysates were prepared according to the method described by Zhou et al. (2018). The wheat gluten and distilled water with a mass ratio of 1:9 was pretreated at 80 °C for 10 min, and then Pancreatin (1%, w/w) was added to the wheat gluten solution for enzymatic hydrolysis at pH 9.0, 50 °C for 24 h. The resultant slurry was centrifuged at 8000*g* and 4 °C for 10 min to get the supernatant liquid, which was then lyophilized for use.

2.3. Pretreatment of macroporous resins

The relevant characteristics of six resins are listed in Table 1. All the resins were pretreated according to the method of Zhuang et al. (2016).

2.4. Static adsorption and desorption of WGH on macroporous resins

2.4.1. Adsorption kinetics of WGH on macroporous resins

The 3.00 g of each pretreated dry resin was put into a 250 ml conical flask with 50 ml absolute alcohol, which was soaked and then washed with distilled water until alcohol-free. Subsequently, 50 ml WGH solution at a concentration of 10 mg/ml was added to different conical flasks, followed by a shaking at 160 rpm and 30 °C for 240 min in a thermostatic water bath shaker (THZ-82, Changzhou Jintan Jingda Instrument Manufacturing Co. Ltd; Jiangsu, China). The samples were collected at different time intervals from each flask for protein content analysis by Folin-Ciocalteu Colorimetric method (Zhuang et al., 2016) to establish the adsorption kinetics equation.

2.4.2. Desorption of WGH on macroporous resins

Each resin of 0.30 g was put into a series of 100 ml flasks, which was activated and washed according to the pretreatment process mentioned above. The resins were then soaked in WGH solution at the optimal concentration and temperature. When the resins reached the adsorption equilibrium in WGH solution, the WGH solutions in the flask were removed. Aliquots (25 ml) of solution at different concentrations of ethanol (0%, 20%, 40%, 60%, 80% and 100%, respectively) were mixed with the resins in the flasks. All the flasks were sealed and shaken in the water shaker at 160 rpm and 30 °C for 12 h.

2.4.3. Adsorption isotherms and thermodynamics on macroporous resins

Three aliquots (0.30 g) of each resin were added individually to three 100 ml flasks followed by 5 ml of different concentrations (2.5, 5, 10, 20 and 40 mg/ml) of WGH, respectively. The flasks with different concentrations of WGH were shaken in a water shaker at 298 K, 308 K and 318 K (25 °C, 35 °C and 45 °C), respectively, until equilibration. The adsorption mechanism between adsorbent and adsorbate could be expounded generally by the Langmuir and Freundlich models (Wang et al., 2017; Xue, Xu, Lu, Ju, & Xing, 2016). The information of energy change and microstructural of adsorbents could be effectively reflected by adsorption thermodynamics (Bell & Tsezos, 1987; Sharma, Sinha, & Upadhyay, 2010; Xue et al., 2016).

2.4.4. The equations used in this study

In this study, the equations are expressed as follows (Xue et al., 2016; Yuanfeng et al., 2016; Zhuang et al., 2016):Adsorption capacity:

Table 1
Macroporous resins properties, adsorption kinetics parameters and equations of WGH on resins.

Resins	Polarity	Material	Specific surface area (m^2/g)	Pore dimeter (Å)	Particle size (mm)	Dynamic equations	Correlation coefficient R^2	Dynamic parameters
D101	Non-polar	Polystyrene	400	100–110	0.3–1.25	$\ln(q_e - q_t) = -k_1 t + \ln q_e$	y = −0.25677x + 4.000143	0.9493
						$t/q_t = t/q_e + 1/K_2 q_e^2$	y = 0.01791x + 0.04991	0.998 q_e = 55.8347
						$q_t = k_d t^{\wedge 1/2} + C$	y = 2.5867x + 26.892	0.462 k_2 = 0.0064
DA-201E	Polar	Polystyrene	500–550	100–120	0.3–1.26	$\ln(q_e - q_t) = -k_1 t + \ln q_e$	y = −0.23033x + 3.840849	0.9479
						$t/q_t = t/q_e + 1/K_2 q_e^2$	y = 0.02115x + 0.04635	0.9973 q_e = 47.2813
						$q_t = k_d t^{\wedge 1/2} + C$	y = 2.2778x + 22.162	0.4893 k_2 = 0.0097
AB-8	Weak polar	Polystyrene	480–520	130–140	0.3–1.25	$\ln(q_e - q_t) = -k_1 t + \ln q_e$	y = −0.30374x + 4.014409	0.9258
						$t/q_t = t/q_e + 1/K_2 q_e^2$	y = 0.01757x + 0.04969	0.9985 q_e = 56.9152
						$q_t = k_d t^{\wedge 1/2} + C$	y = 2.5313x + 28.404	0.4341 k_2 = 0.0062
XAD-16	Non-polar	Polystyrene	800	150	0.7	$\ln(q_e - q_t) = -k_1 t + \ln q_e$	y = −0.23216x + 4.048551	0.9765
						$t/q_t = t/q_e + 1/K_2 q_e^2$	y = 0.01684x + 0.03864	0.9999 q_e = 59.3824
						$q_t = k_d t^{\wedge 1/2} + C$	y = 2.7629x + 28.067	0.5576 k_2 = 0.0073
DM130	Weak polar	Methacrylate	≥100	250–300	0.3–1.25	$\ln(q_e - q_t) = -k_1 t + \ln q_e$	y = −0.10481x + 3.928252	0.9291
						$t/q_t = t/q_e + 1/K_2 q_e^2$	y = 0.01748x + 0.2248	0.9835 q_e = 57.20824
						$q_t = k_d t^{\wedge 1/2} + C$	y = 3.1683x + 16.57	0.7196 k_2 = 0.0014
HPD-826	Hydrogen-bonding	Polystyrene	350–400	80–85	0.3–1.25	$\ln(q_e - q_t) = -k_1 t + \ln q_e$	y = −0.94172x + 3.627321	0.5799
						$t/q_t = t/q_e + 1/K_2 q_e^2$	y = 0.02568x + 0.01695	0.9989 q_e = 38.9408
						$q_t = k_d t^{\wedge 1/2} + C$	y = 1.4614x + 23.047	0.4508 k_2 = 0.0389