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Study on spent brewer's yeast hydrolysis by acid

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Summary

In this study, acid induced autolysis of spent brewer's yeast was carried out with sulfuric (*SA*) and lactic acid (*LA*). The aim of this study was to estimate the success of autolysis induced with inorganic acid compared to autoysis induced with organic acid. The reaction was performed at pH and temperature range which enable the optimum activity of the yeast endoenzymes, so that the process can be considered on acid induced autolysis of yeast biopolymers. Process of hydrolysis was monitored by measuring the increase in the free amino nitrogen (*FAN*, α-amino *N*) concentration. Hydrolysis with sulfuric acid was conducted at the temperature range T = 45 - 60 °C, pH 5.0 - 5.4 and in the period of 12 - 32 h. Hydrolyses with lactic acid was carried out at the temperature range T = 48 - 62 °C, pH 4.8 - 6.0 and in the period of 12 - 44 h. The best results (γ_{FAN} = 4917.45 mg/L) obtained with *SA* were at the following process conditions: T = 52 °C, pH = 5.2 and t = 32 h. On the other hand, the best results (γ_{FAN} = 5789.36 mg/L) obtained with *LA* were at the T = 55 °C, pH = 5.5 and t = 44 h. In both performed acid hydrolysis, α-amino *N* content was not detected at temperatures higher than 60 °C, suggesting the possible inactivation of yeast proteases.

Keywords: yeast autolysis induced with acid, free amino nitrogen (*FAN*, α-amino *N*)

Introduction

Brewer's yeast extract production is the usual way of processing spent industrial yeast whose hydrolyzate has a broad application in food industry, microbiology and pharmaceuticals (Baras et al., 1996; Ferreiraa et al., 2010; Chae et al., 2001). Industrial procedures which are used in brewer's yeast extract production are based on transformation of insoluble protein yeast cell components into soluble form that is easier to use. Procedures for brewer's yeast extract include disruption of cell wall using mechanical, chemical or enzyme methods, followed by hydrolysis of intracellular biopolimers (proteins) (Peppler, 1982). Hydrolysis can be carried out by activating endoenzymes of yeast itself or by adding egzoenzymes (protease) or acid. Depending on type of catalysts hydrolysis procedures can be divided into: autolysis (yeast endoenzymes), enzyme hydrolysis (egzoenzymes) and acid hydrolysis

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(increase of $[H^+]$ using anorganic acids- HCl and H_2SO_4). Compared to the other two procedures, acid hydrolysis is the oldest procedure and it has substantial number of flaws (increased share of carbohydrates and nucleic acids in hydrolyzate). Obtained hydrolyzate has to be neutralized afterwards. Furthermore, the unfavorable effect of acid hydrolysis is reflected as destructive activity on chemically unstable components of yeast (vitamins and thioamino acids) (Reed and Nagodwithana, 1990). To avoid these disadvantages when using mineral acids, it is recommended to apply pH values that are not destructive to cellular compounds but activate yeast endoenzymes (acid-assisted yeast autolysis). If pH values are set so they ensure optimal conditions for yeast protease activity, this procedure can be called acid-assisted proteolysis. Yeast proteases have different pH and temperature optimum, and it is necessary to adjust these parameters so they provide maximal activity. To adjust pH values for yeast suspensions, mineral, but also organic acids that do not have destructive oxidative activity, can be used. Considering legislative regulations, acetic, citric, formic, gluconic and lactic acid can be used. Lactic acid has a few advantages because it positively affects nutritive and sensory properties of obtained hydrolyzate. Also, after hydrolysis it is not necessary to neutralize and filtrate the hydrolyzate.

Materials and Methods

Brewer's yeast hydrolyzate was obtained from industrial yeast used in "Osječka pivovara d.d.". Washing and debittering of yeast was carried out according to Shotipruk et al. (2005). Yeast was suspended in water and then pH value was adjusted to values shown in Table 1 (autolysis initiated with sulphuric acid) and Table 2 (autolysis initiated with lactic acid). pH range that covers area for yeast endoproteases was chosen according to Baras et al. (1996). Hydrolysis kinetics was monitored by increas of α-amino N (FAN). α-amino N was determined by EBC ninhydrin method that gives values that meet the values of free α-amino N from aminoacids. Ninhydrin is an oxidative chemical that sets off oxidative decarboxilation of aminoacids with separation of CO_2 and NH_3 and aldehide development which has one C atom less regarding the original aminoacid. Reduced ninhydrine reacts with nonreduced ninhydrin and free NH_3 causing blue coloration (prolyne causes yellow coloration). In this reaction fructose also takes part as a reducer. Sample was heated with ninhydrin at pH 6.7 and the intensity of developed color was measured spectrophotometrically at 570 nm (European Brewery Convention, 1998).

Results and Discussion

Results in Table 1 show α-amino N (FAN) concentrations during hydrolysis with sulphuric acid at different temperatures and pH values. Furthermore, from Table 1 it is visible that the content of FAN is increasing over time of hydrolysis, and reaches saturation limit at higher pH values during the same process time.

Table 1. Content of free amino nitrogen in brewers yeast extract during hydrolisys catalysed by sulphoric acid at the different pH and temperature

FREE AMINO NITROGEN (mgL^{-1})							
Time (h)	pH	temperature (°C)					
		45	47	50	52	55	60
1	5.0	98.74	122.74	178.99	457.64	569.13	778.10
6		1248.33	1344.57	1024.89	1884.65	1104.00	1168.42
12		1989.44	2117.98	2445.51	3753.63	1877.07	1632.01
24		2246.12	2478.88	3124.65	4008.35	1964.56	1897.45
28		2657.66	3398.65	3872.40	4122.06	2963.65	1873.03
32		3078.45	3862.33	4403.11	4536.22	3442.36	2114.31
1	5.2	180.01	167.48	156.40	116.78	137.85	701.53
6		1767.55	2004.31	1827.08	1984.22	3102.04	1263.47
12		2201.36	2941.03	3157.56	2103.65	3433.11	1386.67
24		2869.67	3104.44	3804.74	3642.86	3441.50	1773.21
28		3144.63	3561.97	3894.21	4098.78	3646.55	2047.04
32		3955.67	4126.58	4891.45	4917.45	3714.01	3001.47
1	5.4	100.01	132.66	105.00	131.05	423.28	504.69
6		1869.23	1879.01	2971.58	1463.87	1065.49	699.10
12		2144.50	2604.71	3056.44	2763.46	2498.36	1103.66
24		2687.12	3564.77	3665.01	4131.04	2897.48	1699.58
28		3241.62	3876.58	4201.44	4331.04	3564.74	2130.65
32		3892.06	3964.22	4123.04	4766.34	3688.63	3004.47

Table 2. Content of free amino nitrogen in brewers yeast extract during hydrolisys catalysed by lactic acid at the different pH and temperature

FREE AMINO NITROGEN (mgL^{-1})							
Time (h)	pH	temperature (°C)					
		48	52	55	58	60	62
12	4.8	1145.50	1548.88	1695.44	1006.58	1036.58	964.85
24		1324.58	1669.87	1746.36	1348.22	1102.58	950.69
36		1864.66	2006.58	1936.99	1489.69	1130.25	987.58
40		2214.58	2265.58	2201.63	1569.47	1003.69	992.67
44		2314.25	2445.78	2632.41	1894.22	1233.01	1102.58
12	5.0	2004.65	2678.25	2961.22	2311.58	1142.69	1033.77
24		2164.25	2794.47	3006.84	2744.69	1641.33	1124.69
36		2248.56	3195.47	3101.56	2945.57	1744.25	1421.02
40		2846.95	3045.47	3226.78	3140.01	1747.22	1178.25
44		3301.47	3497.25	3687.56	3457.41	1875.69	1096.14
12	5.3	2897.54	3356.10	3778.55	3210.02	1240.63	1116.44
24		3310.55	3755.24	3938.36	3741.65	1410.23	1332.47
36		3754.69	3849.36	4011.74	4101.34	191810	1632.33
40		3887.26	4221.36	4536.30	4471.63	1663.48	1741.68
44		4132.25	4132.24	4497.33	4689.55	2036.66	2105.64
12	5.5	3134.26	3448.64	4778.03	3174.62	1778.65	1479.36
24		3874.21	4062.41	4897.33	3487.65	2003.33	1648.25
36		4331.10	4513.55	5214.33	3689.14	2213.03	1596.22
40		4013.45	4732.68	5301.56	4665.15	3102.44	1659.47
44		4115.66	4885.36	5789.36	5475.56	3641.54	1874.11
12	6.0	4463.58	4965.47	5264.74	4834.20	3065.47	1687.44
24		4201.39	4623.56	5003.01	4713.54	2697.41	1574.69
36		4174.11	4012.56	5104.56	4544.22	2471.69	1569.23
40		3946.25	4102.36	4633.56	4013.58	2513.03	1466.47
44		3846.22	4236.47	4547.63	4014.62	3847.11	2241.56

Maximum FAN concentration is reached at pH 5.2 and temperature 52 °C over 32 h. This agrees with results obtained by Baras et al. (1996) obtained. However, it should be mentioned that process parameters in this experiment are adjusted according to the latter author, and no research considering pH and temperature range were conducted that would significantly deviate from the values that are represented in Baras's paper. Results in Table 2 represent concentrations of α-amino N (FAN) during hydrolysis with lactic acid at different pH values and temperatures. It is determined that optimal parameters for this process are: pH range 5.3 – 6.0, temperature range 52 - 58 °C, and over time of 40 - 44 h. The best results are obtained at pH 5.5, temperature 55 °C and during 44 h.

Conclusions

Results of brewer's yeast hydrolysis with sulphuric and lactic acid have shown that concentrations of developed FAN is a function of time, temperature and pH values.The best results (γ_{FAN} = 4917.45 mg/L) obtained with sulphuric acid were at the following process conditions: T = 52 °C, pH = 5.2 and t = 32 h. On the other hand, the best results (γ_{FAN} = 5789.36 mg/L) obtained with lactic acid were at the T = 55 °C, pH = 5.5 and t = 44 h. In both performed acid hydrolysis, α- amino N content was not detected at temperatures higher than 60 °C, suggesting the possible inactivation of yeast proteases. Furthermore, it was determined that during hydrolysis with lactic acid similar FAN concentrations were observed as when hydrolysis with sulphuric acid was performed.

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