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INFUSION OF YEAST AS COMPONENT OF MEDIUM FOR ACETOBACTERS

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Key words: acetic acid fermentation, yeast infusion, yeast autolysate.

The effectiveness of acetic infusion yeast was compared with the effectiveness of pressed baker's yeast autolysate in submerged acetic fermentation in laboratory conditions. The acetic infusion was found to be about 2.5 times more effective than the baker's yeast autolysate.

INTRODUCTION

Janke [7] divided the acetic acid bacteria with respect to their demand for nutrients into two groups: the haplotrophs and the simplotrophs. The former utilize inorganic nitrogen even when the only source of carbon is alcohol or acetic acid. The latter assimilate inorganic nitrogen only when the medium contains more complex carbon sources saccharides and amino acid and growth factors.

Simplotrophs are the bacteria usually isolated from acetic fermenters [4, 11]. It is also known that acetic acid fermentation for the production of vinegar with 10% w/v of acetic acid does not take place when the medium lacks growth factors. Thus, in order to ensure intense fermentation, the medium must contain growth factors in addition to nitrogen and carbon sources.

The growth factors most important for Acetobacters are vitamins of the B group: nicotinic acid, p-aminobenzoic acid (PAB), pantothenic acid, biotin, and β-alanine [3, 5, 8-10]. In industrial practice the growth factors sources used most often are various malt preparations [6] or yeast autolysates [2, 7].

OBJECTIVE AND SCOPE OF RESEARCH

The experimentes served to compare acetic infusion of yeast with the heretofore used pressed baker's yeast autolysate during submerged acetic acid fermentation in conditions corresponding to those during the production of 10% vinegar.

METHODS

YEAST PREPARATIONS

Pressed baker's yeast autolysate (A)

100 g of pressed baker's yeast was mixed with 100 cm^3 of water, heated in a water bath to 55°C mixing constantly, and transferred to an incubator maintaining this temperature. Autolysis in these conditions lasted 3 days, with the mixture being stirred once every 24 h. Following autolysis, the mixture was centrifuged at ca 14 000 x for 15 min, the sediment discarded, and dry mass determined in the clear fluid by drying.

Pressed baker's yeast acetic infusion (P)

Pressed baker's yeast were mixed with clear and chemically unpreserved vinegar (10% w/v of acetic acid). The suspension (100 g of yeast in 100 cm^3 of vinegar) was stored for 3 days at room temperature, and stirred once every 24 h. After this time the mixture was centrifuged and analysed like in the case of autolysate preparation.

Infusions from dried baker's and fodder yeasts

The procedure was analogous as in the case of pressed baker's yeast infusion, but with 40 g dried yeast mixed with 100 cm^3 of vinegar. The acetic infusion were prepared from dried baker's yeast (S) and from fodder yeast (Candida) dried a drum drier (W) and by an atomizer drier (R).

MICROORGANISMS

Fermentation was carried out with bacteria taken from an industrial fermenter together with fermenting medium. Taxonomic analyses performed according to the methods described in Bergey's manual of determinative bacteriology (8th edition) [1] revealed that the material represents a mixed Acetobacter culture with *A. aceti* (subspecies *orleanensis*) and *A. pasteurianus* (subspecies *pasteurianus* and *lovaniensis*). None of the isolated strains grew in media with total concentration (acid + alcohol) in excess of 7%. This may raise doubts as to their role in the technological process in which the total concentration of the medium is 11%. It may also indicate that during cultivation on plates they lose their resistance to the acetic acid and ethanol concentrations occurring in the technological process.

FERMENTATION

Fermentation was carried out in 1.8 dm^3 laboratory fermenters with self-sucking agitators revolving at 1450 r.p.m. When alcohol was fermented off to

the level of 0.4-0.6 % v/v, crude vinegar (0.9 dm^3) was removed from the fermenter which was refilled with the same amount of fresh mash with 10 % ethanol, 1 g/100 cm^3 of acetic acid, and the following medium components (all doses in mg/dm^3):

— hydrated glucose	1500
— $(NH_4)_2HPO_4$	450
— $MgSO_4 \cdot 7\ H_2O$	240
— K_2SO_4	50
— NaCl	60

The medium was supplemented with yeast preparations. Fermentation was maintained without interruptions at 29°C and an air flow of 12.5 $dm^3\ dm^{-3}\ h^{-1}$. The kind of preparation and its dose were changed in the course of fermentation. At least 10 fermentation cycles were performed after every change of preparation of preparation dose, and last three cycles, with stabilized course, were taken into consideration during results analysis.

CONTROL AND ASSESSMENT OF FERMENTATION COURSE

Acidity and bacteria biomass concentration in the fermenting media were analysed every several hours. Acidity was determined by the titration method, and biomass concentration nephelometrically. The content of alcohol was analysed in the final stage of the fermentation cycle in order to prevent its excessive fermentation. This was done by the modified Semichon-Flanzy method (without distillation).

The fermentation course was assessed with a coefficient calculated according to the formula

$$JPK = \frac{120}{t} (2k_k - k_p - k_b)$$

where JPK is the unit production of acetic acid expressed in g/dm^3 working capacity of the fermention per day, t is the duration of the analysed fermentation cycle (in h), and k_i is the acetic acid concentration (in g/100 cm^3); the index refer to the kind of the analysed sample: k — vinegar obtained in the analysed fermentation cycle, p — vinegar obtained in the previous fermentation cycle, b — mash used in the analysed fermentation cycle. The coefficient 120 results from

$$\frac{24 \cdot 900}{1.8 \cdot 100}$$

The effectiveness of the preparations was calculated as the ratio of JPK to dry mass of the preparation that was used (D), and as the ratio of bacteria biomass concentration (in mg dry mass/dm^3) in the obtained product (X) to the dry mass dose of preparation D.

RESULTS

The investigation of the effectiveness of a given preparation began with determinations of doses limiting fermentation rate. The limiting dose was assumed to be the preparation amount giving a JPK value lower than when an unlimiting dose of yeast autolysate was used. The X/D and JPK/D coefficients

Table 1. Unit production of acetic acid JPK and bacteria biomass concentration (X) during fermentation with pressed baker's yeast autolysate (A)

Preparation dry mass (mg dry mass/dm^3)	Preparation dose (mg dry mass/dm^3)	JPK (g/dm^3/day)		X (mg/dm^3)	
		in cycle	mean	in cycle	mean
72.6	143.2	53.0 54.2 53.3	53.5	181 185 186	184
74.0	74.0	37.1 35.2 36.6	36.3	141 146 142	143
	103.6	55.3 53.1 53.9	54.1	184 180 182	182

Table 2. Unit production of acetic acid JPK and bacteria biomass concentration (X) during fermentation with acetic infusions of pressed baker's yeast (P) and dried baker's yeast (S)

Preparation	Preparation dry mass (mg dry mass/dm^3)	Preparation dose (mg dry mass/dm^3)	JPK (g/dm^3/day)		X (mg dry mass/dm^3)	
			in cycle	mean	in cycle	mean
P	64.5	100.0	58.1 59.2 58.2	58.5	180 190 196	185.5
	52.7	40.0	47.3 45.2 45.2	45.9	184 183 182	183.0
		44.4	58.7 59.9 61.0	59.9	191 187 188	188.7
S	90.5	45.25	45.6 46.8 45.7	46.0	182 180 184	182.0
		49.77	58.7 60.9 59.8	59.8	196 191 195	194.0

Table 3. Unit production of acetic acid (JPK) and bacteria biomass concentration (X) during fermentation with acetic infusions of fodder yeasts

Preparation	Preparation dry mass (mg dry mass/dm^3)	Preparation dose (mg dry mass/dm^3)	JPK (g/dm^3/day) in cycle	JPK mean	X (mg dry mass/dm^3) in cycle	X mean
W	83.6	117	46.0 43.9 45.9	45.3	115 102 107	108.0
R	84.5	84.5	62.7 61.0 62.6	62.1	211 209 205	208.3
		42.25	50.0 51.6 49.0	50.2	173 183 184	108.0

Table 4. Effectiveness of the studied yeast preparations

Preparation	A	P	S	R	W
X/D	1.75	4.25	3.89	4.26	0.92
JPK/D	0.52	1.34	1.20	1.18	0.38

were calculated for each such dose. In the case of P and S preparations, the X/D ratio was used to estimate the preparation dose for the subsequent experiment, assuming that biomass content ought to reach 200 mg dry mass/dm^3.

The results obtained in the experiments are presented in Tables 1-3.

Several additional observations were made during the experiments. Namely, it was found that the medium foamed least when the pressed baker's yeast infusion (P) was used, while foam formation was most intense during mash fermentation of with autolysate (A).

The effectiveness of the studied preparations is given in Table 4.

DISCUSSION AND CONCLUSIONS

The experiments demonstrated that the most effective of the studied preparations was the acetic infusion of pressed backer's yeast (P) which gave the highest JPK/D ratio. Compared with the autolysate of these same yeasts (A) that was used heretofore in fermentation, this ratio was over 2.5 times higher. The disproportion between the X/D ratios of both these preparations is analogous.

A slightly lower effectiveness, but still almost twice higher than that of autolysate (A), was demonstrated by preparations S (acetic infusion of dried baker's yeast) and R (acetic infusion of fodder yeast dried by an atomizer dried).

The only preparation that was less effective than the autolysate was the infusion of fodder yeast dried by a drum drier (W).

Valuable information was supplied by studies of differences in the chemical composition of the considered preparations. Preliminary research [12] revelated that the acetic extract of pressed backer's yeast (P) contains over 40% more thiamine than the autolysate of these same yeasts: 4.34 mg/100 g dry mass of the preparation as opposed to 3.05 mg/100 g dry mass of the autolysate.

The observed course of fermentations as well as the greater thiamine content in the acetic extract than in the autolysate of baker's yeast justify the surmise that the former is a better source of growth factors than the latter. The process used to produce the acetic extract probably ensures better eluation and preservation of yeast cell components required for the growth and activity of Acetobacters than the process used in autolysate preparation. The low pH and temperature of processing inhibit the enzymatic and nonenzymatic reactions decomposing the compounds playing the role of growth factors for the bacteria responsible for fermentation.

The distinctly lower free amino acids content in the acetic extract (2.89 mg/100 g dry mass) than in the autolysate (7.33 mg/100 g dry mass) [12] and the about twice lower dose of the extract (44.4 m dry mass/dm^3 of mash) than the autolysate dose (103.6 mg dry mass/dm^3 of mash) that is required in the fermentation are probably the reason for the lower foam production in the medium with extract P than in the medium with autolysate A. Given the fact that air bubbles transport part of the bacteria biomass to the foam, the greater amounts of foam produced during fermentation with autolysate may also the reason for the inferior course of this fermentation.

The drastic heat treatment during the drying of *Candida* yeasts by a drum drier may be responsible for the lower effectiveness of this preparation, compared with the effectiveness of the other investigated preparations.

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WYCIĄG Z DROŻDŻY JAKO SKŁADNIK POŻYWKI DLA BAKTERII KWASU OCTOWEGO

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S t r e s z c z e n i e

Źródłem czynników wzrostowych podczas fermentacji octowej są najczęściej preparaty ze słodu lub autolizaty drożdżowe. Badano efektywność wyciągów octowych z drożdży piekarskich prasowanych (P) i suszonych (S), drożdży paszowych (*Candida*) suszonych na suszarni walcowej (W) i suszarni rozpyłowej (R) w porównaniu z efektywnością autolizatu z prasowanych drożdży piekarskich (A). Miarą efektywności badanego preparatu były stosunki wskaźnika szybkości fermentacji (JPK) oraz stężenia biomasy bakterii (X) do suchej masy wprowadzonego preparatu (D).

Fermentację prowadzono w fermentorach laboratoryjnych z zachowaniem warunków występujących podczas produkcji octu spirytusowego o zawartości kwasu octowego ok. 10 g/100 cm^3. W odróżnieniu od warunków przemysłowych, podczas tych doświadczeń, stosowano natężenie przepływu powietrza oraz dawki składników pożywki (poza dawką preparatów drożdżowych) nieograniczające wzrostu biomasy bakterii.

W tabeli 1 zestawiono wskaźniki fermentacji (JPK) i uzyskiwane stężenie biomasy (X) podczas fermentacji z użyciem autolizatu z prasowanych drożdży piekarskich (A). Tabela 2 zawiera wyniki fermentacji (JPK i X) podczas doświadczeń z użyciem wyciągów octowych z drożdży piekarskich (P i S), a tab. 3 zawiera wyniki uzyskiwane przy użyciu preparatów z drożdży *Candida* (W i R). W tabeli 4 zebrano efektywności badanych preparatów.

Przedstawione wyniki fermentacji świadczą o tym, że wyciąg octowy z prasowanych drożdży piekarskich (P) znacznie (ok. 2,5-krotnie) lepiej spełnia rolę składnika pożywki dla bakterii kwasu octowego niż stosowany dotychczas autolizat (A). Również i inne badane preparaty, poza wyciągiem z drożdży paszowych suszonych na suszarni walcowej (W), wykazały znacznie (ok. 2-krotnie) większą efektywność niż autolizat z prasowanych drożdży piekarskich.