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STUDIES ON YEAST EXTRACTS ENRICHED IN 5'NUCLEOTIDES FLAVOUR ENHANCERS OBTAINED FROM SPENT BREWERY YEAST

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ABSTRACT

The aim of the research was to develop a method of obtaining yeast extracts to the needs of food industry. The yeast extracts were enriched in nucleotide flavour enhancers (5'-GMP and 5'-IMP) obtained from brewery spent yeast. The influence of the directed brewery yeast cell autolysis, preceded by ultrasonic yeast cells' disintegration and a cytolytical enzymes complex (Lyticase), 5'- phosphodiesterase (5'PD) and 5'-adenylic deaminase (5'DA), on the 5'-nucleotides content in the extracts, was evaluated. It was proved that in the spent brewery yeast there was a lysis of nucleic acids into 5'-nucleotides and deamination of the majority of 5'-AMPs into 5'-IMPs. By ultrasonic disintegration, the production of 5'-nucleotides could be increased with a parallel decrease in the level of hypoxanthine. A complex of lytical enzymes favours the production of 5'-GMP and 5'-IMP during a directed autolysis of spent brewery yeast. As a result of the use of Lyticase preparation, a 2.5 times increase in the content of 5'-nucleotides in the extract was observed, which was accompanied by an insignificant increase in the content of hypoxanthine. The use of 5'PD in the same experimental conditions caused an increase in the content of 5'-nucleotides in the extracts with a parallel considerable increase in the content of hypoxanthine. The use of 5'DA insignificantly influenced the change of 5'-AMP into 5'-IMP in the autolysates being the subject of the study. After attempts to intensify the salty taste in the model sample, it was observed that an addition of obtained extract, containing 1.4% 5'-GMP + 5'-IMP makes it possible to decrease the concentration of salt.

Key words: spent brewery yeast, flavour enhancers, yeast extract, 5'-nucleotides, waste utilization.

INTRODUCTION

A group of chemical compounds acting as "improvers" of taste features are commonly called flavour enhancers. These compounds having no significant taste, or having no taste at all, increase the taste sensation of other substances, by modifying or diminishing its intensity or by mystifying any redundant tastes.

The notion of flavour enhancers is reserved to a group of over a dozen of compounds: glutamic acid, guanylic acid and inozylic acid and their salts [2,10,11,18]. Flavour enhancers have been drawn on the GRAS list and are approved by FDA to be the safe food additives not requiring a daily ADI dose. At present 5'-nucleotides together with yeast extracts are basic additives in ready-made food, which is easy to prepare [12,14,16,17]. The two intensifiers mostly used in food production, i.e. disodium 5'-inozynate and disodium 5'-guanylate in combination with monosodium glutamate (MSG), develop the "umami" taste – acknowledged especially in Asia to be the fifth basic taste [3]. Yeast, featuring high nucleic acids'content, attaining even over 10% RNA in their dry mass substance, could be the source of obtaining 5'-nucleotides: 5'-GMP and 5'-IMP [10]. After the process of autolysis and a partial hydrolysis of nucleic acids with the use of different methods, among the most frequently used enzymatic method with 5'-phosphodiesterase (5'FD), 5'-nucleotides are extracted from the yeast biomass or left in the extract, which results in obtaining an enriched extract. A hypothesis implying that monosodium glutamate (MSG) in a joint form (e.g. with protein) does not cause allergies, influenced an increase in popularity of these extracts used as taste and scent enhancers in food technology. The demand of this kind triggered the production of yeast extracts enriched in 5'-IMP and 5'-GMP [4].

One of the elements of complex use of spent yeast in the brewery, would be the way of them being the source of obtaining extracts enriched in flavour enhancers [8].

This would make the possibility of minimalizing the contamination by sewage, which regards the environment protection requirements, feasible [5,7,13]. Another argument speaking in favour of taking action in this domain of life is the consumer fearfulness linked to the allergic reaction caused by MSG being added to a number of meals also in Poland [9,10,19]. The direct aim of the research was to obtain the maximum level of nucleic acids' degradation into 5'-nucleotides in the spent brewery yeast extracts.

MATERIALS AND METHODS

Materials

The research material was postproductive, waste brewer's yeasts, the production strain, belonged to Weihen Stephan Collection, no 4/79 (Browary Królewskie SA, Warszawa). The gist part of spent brewery yeasts was debittered with the use of a method developed exclusively for the sake of the research [15].

Quantitative determination of 5'nucleotides

The 5'-nucleotides content and the hypoxanthine content was determined by using of HPLC (Waters, column mi-Bondpak C18, detection UV 254 nm) with the use of external standards of 5'-nucleotides and hypoxanthine (Sigma).

Autolysis of brewery yeasts

Optimal conditions of releasing the cytoplasmatic gist from yeast cells during the process of autolysis were chosen on the basis of a former research [6]. after preceding ultrasonic disintegration (by Sonics VC-601, Sonics and Materials Inc., Vibra Cells TM) with the application of a 13 mm tip in chosen conditions.

The process of autolysis of debittered yeast suspension (10% dry mass) was conducted in the presence of adipic acid (0.5% w/w by Sigma) acting as the inductor of autolysis in the laboratory shaker (New Bruswick Scientific Co, USA) (conditions: 50°C; 24 h) An optimal character of the conditions of autolysis was chosen.

Enzymatic hydrolysis

Both the yeast cell walls (enzyme complex: Lyticase *Rhizoctonia solani,* ICN Biomedical Inc.) and the yeasts RNA were exposed to the influence of hydrolytic enzymes, with the application of 5'-phosphodiesterase (*Arthrobacter luteus,* INC Biomedical Inc.) (5'FD), and in the aim of bioconversion of 5' -AMP into 5'-IMP by 5'-adenylic deaminase (5'DA) (Sigma).The hydrolysis was conducted during periods of the different time; subsequently the yeast suspension was autolysed (pH 4.5) in the previously stated conditions.

A dose of 5'-phosphodiesterase was selected for obtaining the maximum 5'-nucleotides content in the extracts that were previously exposed to ultrasonic disintegration. 5'PD was added to suspension of debittered brewery yeast, then incubated for 4 hours in 37°C, pH 7.5. In order to increase the amount of brewery yeast cells cytoplasmatic compounds released, a complex of cytolytical enzymes (Lyticase) containing beta-1,3-glucanase, protease, hemicelulase, pectinase and amylase was used. 8, 16 and 32 mg/g (4h, 25°C) doses of the enzyme were used, followed by an autolysis conducted in previously established conditions. Two series of experiments were carried out, each made in triplicate.

Preparation the yeast extract enriched in 5'nucleotides

To conduct both a preliminary evaluation of the applicability of obtained extracts enriched in 5'nucleotides as taste enhancers and a general evaluation of the efficiency of the process, a majority part of the suspension being the subject- matter was obtained in the previously stated conditions. An inductor was added to a 10% suspension of debittered brewery yeast in the volume of 3 kg and pH adjusted to 4.5, which was followed by a 24-hour autolysis in 50°C.

The autolysis was preceded by a 1-minute ultrasonic disintegration. Subsequently the suspension was exposed to the Lyticase enzyme in the amount of 150 mg/kg for 4 hours. After aforesaid treatment, with the use of a centrifuging machine (9000 spins/minute), the extract obtained by extracting if from the cell walls subsequently dried (90°C/120°C).

Sensory evaluation

Sensory evaluation was conducted in accordance with a PN ISO 3972 norm [1] for an 8-member group. The threshold of detectability and recognizability of salty taste and of the extract was evaluated. After determining the concentration of extract below the threshold of detectability, an attempt to intensify the salty taste was conducted. The basic solution applied to sensory evaluation was a 0.2% solution of brewery spent yeast extract containing 1.4% of 5'-GMP and 5'-IMP.

The reaction of the release of nucleotides 5'-GMP and 5'-IMP as a result of a controlled autolysis of yeast cells, assisted by the activity of egzogenic hydrolytic enzymes and ultrasonic disintegration was being studied.

Subsequently adipic acid was added, acting as an inductor of autolysis, and pH adjusted to 4.5, the suspension being subsequently exposed to autolysis (50°C, 24h).

RESULTS AND DISCUSSION

The aim of the research was to obtain the highest degradation of nucleic acids to 5'-nucleotides in obtained extracts of spent brewery yeasts. Disintegration of yeast cells was conducted with the use of ultrasounds, autolysis in chosen conditions and enzymatic hydrolysis, with the use of a complex of enzymes lysing the yeast cell wall. Spent brewery yeast originating from the Browar Warszawski brewery was chosen to the needs of the research. The influence of the time of ultrasonic disintegration of debittered brewery yeast on the 5'-nucleotides content in the obtained extracts was evaluated. Optimal ultrasonic disintegration conditions were established: the duration of impulses and the percentage of power of the apparatus used in the research. A 3-second impulse and a 90% power of the apparatus were established as the best conditions. 1 minute was established to be the optimal time of disintegration.

The level of release of 5'-nucleotides and hypoxanthine was then measured, where pH parameter of the autolysing suspension of 10%dry mass brewery yeast was being established (table 1).

pH 4.5 was determined to the most beneficial condition.

The extend to which alkaline hydrolysis, with the use of 0.3M KOH influences the increase of 5'nucleotides content in the extract was evaluated. Hydrolysis was conducted after the process of autolysis had finished. Alkaline hydrolysis of brewery yeast extract didn't increase the 5'-nucleotides content in the extract.

Table 1. The influence of pH of the autolysis on the process of 5'-nucleotides release from brewery yeast

pH of autolysis	Content of (mg/g dry mass)				
	AMP	GMP+IMP	UMP	CMP	hypoxanthine
3.5	0.76	4.55	0.55	0.00	0.22
4.0	5.02	11.93	0.58	1.02	2.87
4.5	6.37	14.10	0.46	1.48	3.50
5.0	7.79	11.63	3.02	2.36	3.73
5.5	13.94	13.28	0.43	1.65	3.77

Optimal conditions of 5'nucleotides release with the use of 5'-phoshodiesterase (5'FD) was evaluated (table 2). Preparations was made from another part of the material - spent brewery yeast.

The addition of 5'-phosphodiesterase to all applied doses was not considerably influencing the 5'-GMP and 5'-IMP levels in extracts obtained for one pH value, nor it was changing the hypoxanthine content.

Table 2. The influence of hydrolysis conducted with the use of 5'-phosphodiesterase and the autolysis on the release of 5'nucleotides from brewery yeasts (37°C)

pH	Enzyme concentration [j /g dry mass]	Content of (mg/g dry mass)				
		AMP	GMP+IMP	UMP	CMP	hypoxanthine
4.5	0	0.00	8.40	2.02	0.00	4.03
	0.3	0.04	8.87	1.43	2.35	5.75
	0.6	0.00	8.70	0.72	1.05	5.92
	1.5	0.56	9.08	1.84	0.00	0.72

The influence of the time of hydrolysis and the dose of 5'-adenylic deaminase on the level of 5'- nucleotides was conducted. With a dose of 0.45 units of enzyme, after a 50 minutes of hydrolysis, an increase in the content of 5'-GMP + 5'-IMP in the extract was observed with a parallel increase in the content of hypoxanthine. At a dose of a 1.35 units of enzyme after a 60-minutes-long hydrolysis a double increase in 5'-GMP and 5'-IMP content was observed with a parallel almost quadruple increase in the content of hypoxanthine.

A hydrolysis of brewery yeast dry mass with a use of cytolytical enzymes complex (Lyticase) was conducted (table 3).

Table 3. The influence of cell wall hydrolysis with the use of a enzyme Lyticase and a subsequent autolysis on the 5'-nucleotides content in a spent brewery yeast extract duration of enzyme treatment

The amount of enzyme/g dry mass of yeast [mg]	Content of (mg/g dry mass)		
	AMP	GMP+IMP	hypoxanthine
0	0.70	1.30	0.99
8	1.65	2.18	1.31
16	1.92	3.35	1.15
32	0.98	1.22	1.18

The addition of a lytic enzyme in the concentration of 16 mg/g increased the content of 5'-nucleotides in the post-productive brewery yeast extract more than two times, with a parallel slight increase in the content of hypoxanthine.

The sensory analysis was conducted in a team of 8 people. For 6 members of the team the threshold of salt concentration detectability was established at 0.48 g/l (dissolution D5), for the remaining - 2, at 0.69 g/l (D4). After the evaluation of the salty taste detectability threshold, it was observed that it appears at 0.69 g/l salt concentration (4 assessors) and at 0.98 g/l (4 assessors), (which corresponds with a statistic threshold of salty taste detectability). Subsequently the "umami" taste threshold detectability was evaluated, for the solutions of a spent brewery yeast's extract in the concentration from 0.08 to 1g/l. 5 members of the sensory team found the 0.12 g/l (D6) concentration to be the threshold of detectability, 1 member the 0.17 g/l concentration and 1 member the 0.08 concentration.

It was established that for the evaluation of taste intensification (i.e. lowering of the salty taste detectability threshold), a concentration of 0.08 g/l shall be optimal, being lower than the threshold of detectability at the level of which a different taste is not introduced. Series of salt solutions were prepared, with the first experiment, where the preparation concentration levels were 0.08 g/l. After the experiment it was stated that 5 of 8 members of the sensory team determined the detectability threshold at the concentration of 0.24 g/l (dissolution D7), while the salty taste detectability threshold was established for the dissolutions D5 and D4.

Application of obtained preparations to the evaluation of their usefulness in a particular food product requires further research.

CONCLUSION

Spent brewery yeast may constitute a material for obtaining yeast extracts enriched with 5'-nucleotides. As a result of induced autolysis (24 h at 50°C) in adjusted conditions pH (4.5) with the participation of autolysis inductor of (0.5% adypic acid), there was a considerable disintegration of nucleic acids towards 5'-nucleotides and a deamination of the majority of 5'-AMP to 5'-IMP. By the hydrolysis with the use of cell wall lytic enzymes complex (Lyticase), and directed autolysis of spent brewery yeast, there was even a 2.5 times increase in the 5'-nucleotides content in the extract observed, with a parallel slight increase of hypoxanthine content. In the same experimental conditions, application of 5'-phosphodiesterase and 5'-adenylic deaminase caused a slight increase in the process of 5'-nucleotides release with a parallel increase in the content of hypoxanthine. Similarly incorporating the enzymes of living yeast into the process of hydrolysis of nucleic acids into autolysis of spent brewery yeast did not cause an increase of 5'-GMP+5'-IMP content in the autolysate.

Using optimal ultrasonic disintegration parameters it is possible to increase the concentration of the 5'-nucleotides release, parallel to a decrease in the level of hypoxanthine.

After the essays to intensity the salty taste with the use of obtained extract (containing 1.4% 5'-GMP+5'-IMP) it was observed that an additive of this kind makes a double diminution of salt arrangement feasible.

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