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Screening of autolytic yeast strains for production of L-amino acids

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Abstract

As yeast extracts are commonly used as a source of amino acids, the present work studies the potential of one *Saccharomyces cerevisiae* strain and several non-*Saccharomyces* yeasts to be used as a source of amino acids. All the strains studied were able to grow using sugar cane molasses as medium. *Pichia* strains proved to be the best biomass producers, but *Yarrowia* showed the highest rates and the best yield of hydrolysis from protein to free amino acids. *Yarrowia* strains also proved to contain the greatest quantity of essential amino acids. Finally, a phylogenetic tree was obtained from the amino acid profile which agrees with the classification of the strains.

Keywords: Yeast autolysis; Cluster analysis; Free amino acids

1. Introduction

L-Amino acids are known to play a wide range of roles in nature. They are the constituents of proteins and act as precursors of other compounds such as nucleic acids, haemo group, hormones and neurotransmitters [1,2]. From the nutritional point of view, the enrichment of food and diets with L-amino acids improves food assimilation, intensifies the metabolism of fatty acids and avoids damage to the central nervous system. The Food and Agriculture Organization together with the World Health Organization (FAO/WHO) have established a table of essential L-amino acids (EAA). Their report estimates the amino acid requirements of infants, older children and adults based on nitrogen balance [3].

In plants, exogenous amino acids play wide roles. They can modulate membrane permeability and ion uptake, for instance, and this is probably their major contribution to mitigating drought or salt stress effects [1]. Also the addition of asparagine, aspartic acid, glutamic acid, alanine, glycine and serine promotes flowering, and the accumulation of proline has been related to the adaptation to osmotic stress in tomato [4].

Yeast extracts are commonly used as a source of amino acids and vitamins, and as a food additive which is used to provide a basic flavour or enhance flavours already present. They are also used in microbiological media as growth supplements [5,6]. Several methods have been developed to release cell components from yeasts, including alkaline or acidic lysis, treatment with cell-wall-lysing enzymes, physical methods and the use of organic solvents. These methods are not applicable on a large scale because of low yields, toxicity, destruction of several amino acids or their high cost. Autolysis is a natural process caused by aging and occurs when the yeast has completed its growth cycle and entered the death phase. The autolysis of yeast is characterized by a latent period during which the yeast cells undergo active autofermentation. When the glucose formed from glycogen is exhausted, irreversible changes occur in the cytoplasm and then proteolysis starts, leading to the accumulation of polypeptides and amino acids [7]. Temperature increase is the easiest and most environment-friendly to carry out on an industrial scale, but for some yeasts it is not sufficient due to its long duration [8]. The autolytic process can be enhanced with physical, chemical and enzymatic inducers [9]. The physical methods include UV radiation [10], mechanical disruption with high pressure homogenization [11] or homogenization with glass beads [12]. These methods allow the release of the autolytic enzymes thus accelerating the process. Chemical inducers have been used to disrupt the membrane structure by dissolving lipids and proteins, and to modify pH for induction of protease promoters. These chemical inducers of the autolysis may be, detergents, organic compounds, NaOH, HCl

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or inorganic salts [13]. Finally, the addition of proteases has proven to be the most effective method for enhancing autolysis. These enzymes kill the cells but do not degrade the endogenous enzymes required for autolysis such as proteases, glucanases and nucleases [14]. Some of these methods are expensive and, therefore, not advisable on a large scale, but others, such as the change in osmotic strength in the medium, are very effective and can shorten the autolytic process. In any case the first requisite profitable for the production of yeast extract is that the strain be capable of autolysis. The strain's autolytic capacity may then be increased with one or some of the inducers described above.

Although autolysis is a commonly used process, it has been well described only in bacteria and the yeast *Saccharomyces cerevisiae* [7]. However, there is little information on the autolysis process by non-*Saccharomyces* strains. Interest in these strains, which are high biomass producers, has increased due to their natural distribution and their contribution to the wine and food industries [8,15,16]. The aim of this study was to compare the autolysis process of several yeast strains for the production of L-amino acids. We have compared two strains isolated in our laboratory and several others with interesting properties as protease secretors or high biomass producers. We have calculated the best biomass producer, the autolytic capacity and the hydrolysis velocity for obtaining of free amino acids.

2. Materials and methods

2.1. Yeast culture media and autolysis

Each strain was inoculated in 20 mL of YPD medium consisting of 2% peptone, 1% yeast extract and 2% glucose (supplied by BD, Madrid, Spain). Precultures were grown for 48 h at 30 °C under constant shaking at 200 rpm. The cultures were then prepared inoculating 10^6 cfu/mL of each preculture in 2 L of 4% (w/v) sugar cane molasses medium for 48 h at 30 °C with orbital shaking at 200 rpm, after which the cells were harvested by centrifugation at 5000 × *g* for 10 min at 4 °C and weighed. The cells were resuspended in water at a w/v ratio of 15%. Autolysis was carried out at 50 °C and with constant shaking up to 7 days. During autolysis, several aliquots of 1 mL of the suspension were removed at several time intervals for analysis. Data were obtained from three separate autolysis events and samples were taken in duplicate.

2.2. Protein and amino acid analysis

To measure the total protein content 1 mL of cell suspension was taken. This volume was then sonicated for six periods of 30 s, pulse mode 0.5 and sonic power 60%. Before measuring, the samples were diluted and quantified by the Lowry method [17].

In order to determine the concentrations of individual amino acids in autolysis media, aliquots of cell suspension were taken at several intervals up to 168 h. These were then centrifuged and the supernatant was analyzed for amino acids. For extraction and derivatization of amino acids EZ:faast GC-FID by Phenomenex (Jasco, Spain) was used. Separation and quantification were carried out in a Varian 3900 Gas Chromatographer equipped with a 10 m × 0.25 mm ZB-PAAC column. The flow-rate of the carrier gas (He) was kept constant at 1.6 mL/min. The oven temperature program was as follows: initial temperature 110 °C, a 30 °C/min ramp to 320 °C held for 1 min. The temperature of the injection was 280 °C, and that of the detector was 320 °C. 1.5 μL of sample was injected in split mode (1:15, v/v). For quantification Norvaline was used as internal standard.

Data were taken in triplicate from two autolysis experiments at each interval. The mean and the standard deviation were calculated by Microsoft Excell 2000 from these six results. For the statistical analysis, the standard deviation of the mean was calculated, establishing a Confidence Interval of 95%.

2.3. Phylogenetic analysis

Cluster analysis was used to group the yeasts according to their proportion of amino acids. A Furthest Neighbor Method was employed using the proportion of each free amino acid obtained by autolysis and the resulting tree was built by the Statgraphics Plus V5.1 (Manugistics, Inc., Rockville, MD, USA). Each amino acid concentration was normalized by dividing it by the sum of amino acids for each strain.

3. Results and discussion

We have studied 10 strains for the production of biomass and the release of free amino acids. Five of them are described as high producers of biomass: *S. cerevisiae* L2056, *Pichia anomala* (isolated in our laboratory from vinasse), *Pichia jadinii* CECT 1062 (*Candida utilis* DSMZ 70167), *Pichia pastoris* GS115, *Pichia pini* CECT 10874 (isolated in our laboratory from soil). The other five strains are described as protease secretors: *Yarrowia lipolytica* CECT 1357, 1468, 1469, 1470 and 1694. Table 1 shows the grams of wet biomass produced per litre, and it can be seen that the first group of yeasts reached the highest values. The maximum quantity produced was 23.34 g by *P. jadinii*

Table 1
Study of the parameters to establish the autolytic capacity of the strains after 96 h

Strain	WB	PC	AA	AC (%)
Saccharomyces cerevisiae L2056	18.56 ± 0.01	25.34 ± 1.89	3.64 ± 0.35	14
Pichia anomala	18.71 ± 0.01	14.93 ± 0.23	2.71 ± 0.06	18
Pichia jadinii CECT 1062	23.34 ± 0.01	21.06 ± 1.35	1.53 ± 0.17	7
Pichia pastoris GS115	19.60 ± 0.01	25.16 ± 1.32	3.45 ± 0.26	14
Pichia pini CECT 10874	18.96 ± 0.01	42.67 ± 3.77	4.34 ± 0.25	11
Yarrowia lipolytica CECT 1357	11.54 ± 0.01	9.56 ± 0.52	4.91 ± 0.33	51
Yarrowia lipolytica CECT 1468	9.74 ± 0.01	15.21 ± 5.80	7.47 ± 0.63	49
Yarrowia lipolytica CECT 1469	14.09 ± 0.01	9.88 ± 0.03	4.33 ± 0.34	44
Yarrowia lipolytica CECT 1470	9.99 ± 0.01	12.32 ± 1.80	9.18 ± 0.62	74
Yarrowia lipolytica CECT 1694	10.32 ± 0.01	7.63 ± 0.45	7.02 ± 2.31	92

WB: wet biomass production as g of wet biomass produced in 1 L of 4% sugar cane molasses at 30 °C for 48 h; PC: protein production as mg/mL of protein in 15% of cell suspension in water before autolysis; AA: Sum of free amino acids as mg/mL after autolysis; AC: autolytic capacity as % of protein transformed to free amino acids. The results data are means of six data ± S.D.

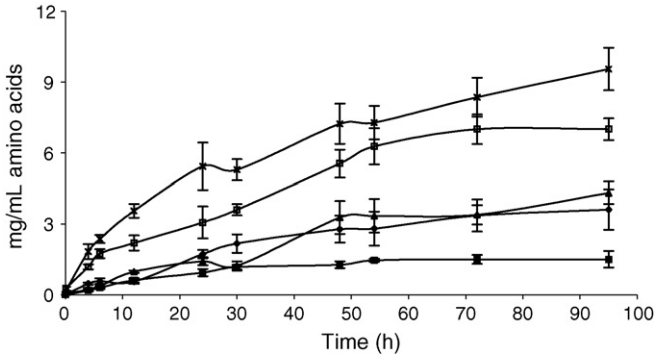

Fig. 1. Curve of release of amino acids by autolysis of *Saccharomyces cerevisiae* L2056 (♦), *Pichia jadinii* CECT 1062 (■), *Pichia pini* (▲), *Yarrowia lipolytica* 1470 (∗), *Yarrowia lipolytica* CECT 1694 (□), measured for 96 h.

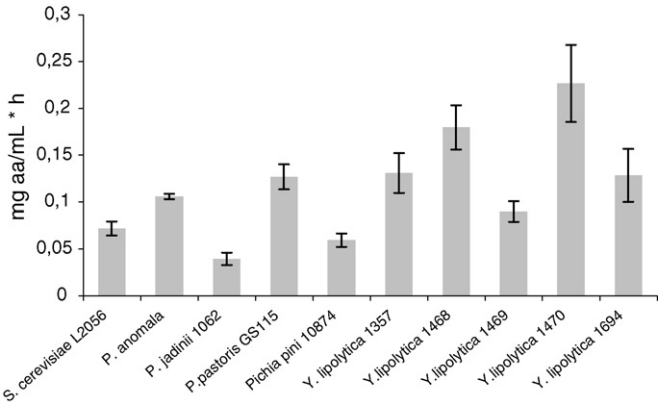

Fig. 2. Slope of the line obtained by representing mg/mL of the amino acids released vs. time in hours by each yeast strain up to 24 h of autolysis in water at 50 °C.

CECT 1062, and the mean value of the other strains belonging to the biomass producer group was approximately 18 g. Protein content of the cell is even more important than biomass production because the hydrolysis of cellular proteins releases peptides and amino acids into the autolysed medium. *P. pini* showed the highest content in protein (42.67 mg/mL), which was twice that of *P. jadinii* CECT 1062. This result is even more striking if we consider that the biomass production of *P. jadinii* was 78% greater than that of *P. pini*. The yeast most widely used for biomass production on an industrial scale is *S. cerevisiae*, but *Candida*, *Kluyveromyces*, *Pichia*, *Yarrowia*, and *Schwanniomyces* yeasts have specific uses and may be grown on a similar scale using substrates unavailable to *S. cerevisiae*. For instance *C. utilis* has shown its potential as a source of single-cell protein from sulfite waste liquor and wood sugar, and *Pichia* spp. has previously been described as a biomass producer from hydrocarbons and methanol, as well as being useful in the expression of heterologous genes [18]. The results indicate that all the yeasts were able to grow using sugar cane molasses as culture medium. *P. jadinii* CECT 1062 produced the highest biomass value, but the protein content of *P. pini* CECT 10874 gives it a great advantage over the others as a source of free amino acids.

As regards the production of free amino acids, the group of protease secretors was the most active. From autolysis of *Y. lipolytica* CECT 1470 a clear solution of 9.18 ± 0.62 mg/mL of free amino acids was obtained after 96 h, followed by *Y. lipolytica* CECT 1468 with 7.47 ± 0.63 mg/mL and 1694 with 7.02 ± 2.31 mg/mL. Of these three strains the one which was able to convert most of the initial protein to free amino acids (%AC) was *Y. lipolytica* CECT 1470 (74% transformation) (Table 1).

Fig. 1 shows the evolution of autolysis for *S. cerevisiae* L2056, *P. jadinii* CECT 1062, *P. pini*, *Y. lipolytica* CECT 1470 and *Y. lipolytica* CECT 1694. The velocity of amino acid release increased linearly up to 24 h, after which the release rate entered a more or less stationary phase. These data agree with the autolysis of *Saccharomyces bayanus* in wine system, where the greatest release of amino acids occurs between 4 and 24 h [19]. Fig. 2 shows the slope of amino acid production per hour for all the strains studied. *Y. lipolytica* CECT 1470 showed the highest release rate (0.23 ± 0.04 mg/mL h), and in the group of biomass producers *P. pastoris* GS115 stood out with an autolytic velocity value similar to that of the protease secretors (0.13 ± 0.014 mg/mL h).

The content of amino acids determines the protein's nutritive value. The 19 amino acids analyzed (arginine is not detected with this method) were found in autolysates of all yeast strains examined except in *P. anomala*, *P. jadinii* CECT 1062 and *P. pastoris* GS115 where cysteine was not detected and *P. jadinii* CECT 1062 where hydroxylysine + lysine were not detected (Table 2). According to WHO guidelines, the essential amino acids are histidine, isoleucine, leucine, lysine, methionine, cysteine, phenylalanine, tyrosine, threonine, tryptophan and valine [3], and the sum of these essential amino acids in *Y. lipolytica* CECT 1357 was over 50% of the total. From the nutritional point of view *Yarrowia* strains showed better essential amino acids profiles than the strains traditionally used in the food industry.

Other authors have suggested that only part of the cell protein is degraded and released into the autolysate [8]. The protein and amino acid compositions of autolysates are not stable because the proteinases and peptidases are also released into the autolysates and continue their action. Such activity will be according to the conditions of autolysis and yeast species and will affect the amino acid composition of the autolysate. We have obtained a stable composition of amino acid release by each strain using the same time and conditions of autolysis, and we have established a phylogenetic relationship. A cluster analysis was applied to the global amino acid composition of the yeasts. The phylogenetic tree (Fig. 3) was generated using Furthest Neighbor Method, and the yeasts were separated in two well-defined groups: in one cluster *P. pastoris*, *P. pini*, *P. jadinii*, and *P. anomala* were included together with *S. cerevisiae*, and *Yarrowia* strains were grouped in the other one. In the *Yarrowia* branch 1357 appears separated from the others four strains. There was high similarity between strains 1468 and 1694 and 1469 and 1470 but not with 1357. These similarities are not evident in the behavior of the yeasts, but the grouping

Table 2
Free amino acids released to the medium by autolysis of the strains after 96 h at 50 °C

	Saccharomyces cerevisiae L2056	*Pichia anomala*	*Pichia jadinii* CECT 1062	*Pichia pastoris* GS115	*Pichia pini* CECT 10874	*Yarrowia lipolytica* CECT 1357	*Yarrowia lipolytica* CECT 1468	*Yarrowia lipolytica* CECT 1469	*Yarrowia lipolytica* CECT 1470	*Yarrowia lipolytica* CECT 1694
Non-essential										
Ala	0.34 ± 0.07	0.290 ± 0.00	0.22 ± 0.00	0.44 ± 0.01	0.97 ± 0.05	0.41 ± 0.03	1.10 ± 0.14	0.55 ± 0.04	0.79 ± 0.06	1.13 ± 0.29
Gly	0.28 ± 0.06	0.08 ± 0.00	0.11 ± 0.01	0.15 ± 0.00	0.21 ± 0.08	0.26 ± 0.02	0.32 ± 0.05	0.22 ± 0.02	0.49 ± 0.02	0.30 ± 0.10
Ser	0.27 ± 0.04	0.25 ± 0.02	0.05 ± 0.00	0.24 ± 0.13	0.14 ± 0.06	0.55 ± 0.14	0.54 ± 0.14	0.33 ± 0.00	0.55 ± 0.15	0.41 ± 0.10
Asn + Asp	0.46 ± 0.07	0.18 ± 0.01	0.01 ± 0.00	0.16 ± 0.00	0.13 ± 0.08	0.01 ± 0.00	0.51 ± 0.04	0.15 ± 0.02	0.98 ± 0.03	0.71 ± 0.39
Gln + Glu	0.53 ± 0.02	0.70 ± 0.01	0.30 ± 0.10	0.92 ± 0.03	0.70 ± 0.05	0.08 ± 0.00	0.37 ± 0.04	0.42 ± 0.06	0.57 ± 0.15	0.76 ± 0.34
Tyr	0.08 ± 0.02	0.04 ± 0.00	0.03 ± 0.01	0.05 ± 0.01	0.09 ± 0.04	0.29 ± 0.02	0.34 ± 0.03	0.18 ± 0.01	0.36 ± 0.03	0.23 ± 0.08
Hyp + Pro	0.40 ± 0.09	0.12 ± 0.01	0.21 ± 0.01	0.54 ± 0.02	0.72 ± 0.04	0.58 ± 0.08	0.63 ± 0.10	0.38 ± 0.04	1.08 ± 0.02	0.65 ± 0.27
Total	2.36 ± 0.06	1.67 ± 0.01	0.93 ± 0.12	2.51 ± 0.20	2.95 ± 0.17	2.19 ± 0.29	3.79 ± 0.55	2.24 ± 0.19	4.82 ± 0.05	4.19 ± 1.57
Essential										
His	0.13 ± 0.03	0.32 ± 0.00	0.12 ± 0.01	0.16 ± 0.01	0.32 ± 0.13	0.19 ± 0.01	0.03 ± 0.00	0.08 ± 0.01	0.37 ± 0.01	0.13 ± 0.06
Hly + Lys	0.27 ± 0.10	0.22 ± 0.00	0.13 ± 0.04	0.19 ± 0.02	0.24 ± 0.03	0.52 ± 0.02	0.46 ± 0.09	0.39 ± 0.01	0.62 ± 0.21	0.35 ± 0.20
Ile	0.15 ± 0.02	0.09 ± 0.00	0.06 ± 0.00	0.12 ± 0.01	0.13 ± 0.00	0.29 ± 0.01	0.59 ± 0.01	0.27 ± 0.02	0.67 ± 0.03	0.43 ± 0.02
Leu	0.19 ± 0.03	0.13 ± 0.00	0.10 ± 0.00	0.13 ± 0.00	0.18 ± 0.07	0.46 ± 0.03	0.74 ± 0.09	0.37 ± 0.05	0.73 ± 0.01	0.54 ± 0.01
Met	0.03 ± 0.00	0.02 ± 0.00	0.01 ± 0.00	0.02 ± 0.00	0.03 ± 0.00	0.36 ± 0.02	0.23 ± 0.03	0.11 ± 0.01	0.21 ± 0.01	0.19 ± 0.05
Cys	0.05 ± 0.00	n.d.	n.d.	n.d.	0.04 ± 0.00	0.07 ± 0.00	0.03 ± 0.00	0.03 ± 0.00	0.07 ± 0.00	0.02 ± 0.01
Phe	0.10 ± 0.02	0.05 ± 0.00	0.05 ± 0.00	0.06 ± 0.00	0.09 ± 0.01	0.14 ± 0.00	0.49 ± 0.05	0.23 ± 0.03	0.51 ± 0.02	0.36 ± 0.10
Thr	0.14 ± 0.03	0.03 ± 0.00	0.02 ± 0.00	0.11 ± 0.00	0.19 ± 0.01	0.18 ± 0.01	0.38 ± 0.02	0.23 ± 0.02	0.39 ± 0.26	0.32 ± 0.11
Trp	0.02 ± 0.00	0.02 ± 0.00	0.01 ± 0.00	0.02 ± 0.00	0.03 ± 0.00	0.10 ± 0.00	0.12 ± 0.00	0.06 ± 0.00	0.16 ± 0.01	0.09 ± 0.03
Val	0.18 ± 0.03	0.18 ± 0.00	0.10 ± 0.00	0.12 ± 0.01	0.15 ± 0.07	0.38 ± 0.02	0.61 ± 0.12	0.32 ± 0.01	0.63 ± 0.03	0.40 ± 0.15
Total	1.28 ± 0.29	1.04 ± 0.05	0.60 ± 0.05	0.94 ± 0.06	1.39 ± 0.08	2.72 ± 0.04	3.68 ± 0.08	2.09 ± 0.15	4.36 ± 0.57	2.83 ± 0.74

Values are expressed in mg/mL as mean of six determinations. Alanine (Ala), glycine (Gly), serine (Ser), asparagine (Asn), aspartic acid (Asp), glutamine (Gln), glutamic acid (Glu), tyrosine (Tyr), 4-hydroxiproline (Hyp), proline (Pro), histidine (His), lysine (Lys), hydroxylysine (Hly), isoleucine (Ile), leucine (Leu), methionine (Met), cystine (Cys), phenylalanine (Phe), threonine (Thr), triptophan (Trp), valine (Val). The upper table shows the non-essential amino acids and the lower table the essential ones, as described by the World Health Organization. n.d.: non detected. The results data are means of six data ± S.D.

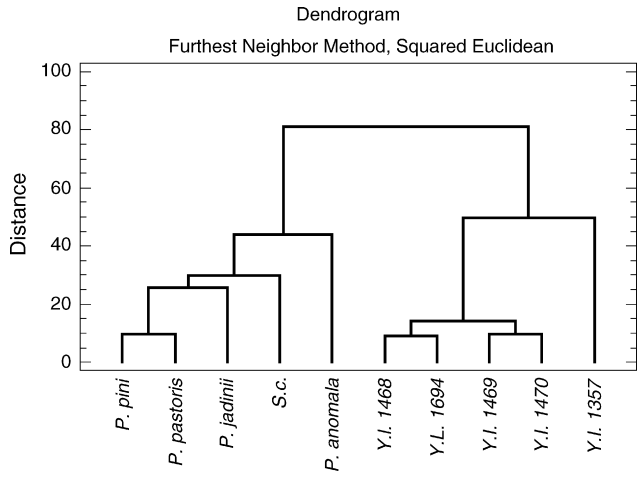

Fig. 3. Dendrogram generated using a cluster analysis.

based in the L-amino acids profile is clear. In the other branch *P. pastoris* and *P. pini* showed the highest similarity; these strains are the only methylotrophic yeasts included in this study. *P. jadinii*, *S. cerevisiae* and *P. anomala* do not have such a specific relationship as the two *Pichia* strains above mentioned. This differentiation in two clear clusters and the inter-cluster grouping, based on the L-amino acids profile, agrees with the physiological characteristics of the yeasts and their biochemical classification.

The hydrolysis of cellular proteins and release of degraded proteins, peptides and amino acids into the autolysates are the principal reactions in yeast autolysis [9]. Nevertheless, the combination of high biomass production and efficiency of hydrolysis is the key to a good strain. Based on the protein content and the capacity and velocity of autolysis, the most profitable combination for the industrial production of L-amino acids is a mixed cell suspension of *P. pini* CECT 10874 and *Y. lipolytica* CECT 1470. However, for human and animal diets *Yarrowia* strains have proved to have a balanced content in essential amino acids that has not been described previously.

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References

[1] Rai VK. Role of amino acids in plant responses to stresses. Biol Plant 2002;45:481–7.
[2] Voet D, Voet JG. Biochemistry. 2nd ed. John Wiley and Sons; 1995, ISBN 0-471-58651-X.
[3] FAO/WHO/UNU (Expert Consultation) Energy and Protein Requirements. FAO/WHO Nutrition Meeting, Report Series 724, Geneva, 1985.
[4] Handa S, Handa AK, Hasegawa P, Bressan RA. Proline accumulation and the adaptation of cultured plant cells to water stress. Plant Physiol 1986;80:938–45.
[5] Verduyn C, Suksomcheep A, Suphantharika M. Effect of high pressure homogenisation and papain on the preparation of autolysed yeast extract. World J Microbiol Biotechnol 1999;15:57–63.
[6] Barrete J, Champagne CP, Goulet J. Growth-promoting properties of yeast extracts produced at different pH values, with different autolysis promoters and bacterial populations. J Chem Technol Biotechnol 2001;76:203–9.
[7] Vosti DC, Joslyn MA. Autolysis of baker's yeast. Appl Microbiol 1953:70–8.
[8] Hernawan T, Fleet G. Chemical and cytological changes during the autolysis of yeasts. J Ind Microbiol Biotechnol 1995;14:440–50.
[9] Babayan TL, Bezrukov MG, Latov VK, Belipkov VM, Belavtseva EM, Titova EF. Induced autolysis of *Saccharomyces cerevisiae*: morphological effects, rheological effects, and dynamics of accumulation of extracellular hydrolysis products. Curr Microbiol 1981;5:163–8.
[10] Yokoi K-J, Kawasaki K-I, Taketo A, Kodaira K-I. Characterization of lytic enzyme activities of *Lactobacillus gasseri* with special reference to autolysis. Int J Food Microbiol 2004;3:273–9.
[11] Verduyn C, Suksomcheep A, Suphantharika M. Effect of high pressure homogenisation and papain on the preparation of autolysed yeast extract. World J Microbiol Biotechnol 1999;15:57–63.
[12] Inouhe M, Sugo E, Tohoyama H, Joho M, Nevins DJ. Cell wall metabolism and autolytic activities of the yeast *Saccharomyces exiguus*. Int J Biol Macromol 1997;21:11–4.
[13] Breddam K, Beenfeldt T. Acceleration of yeast autolysis by chemical methods for production of intracellular enzymes. Appl Microbiol Biotechnol 1991;35:323–9.
[14] Conway J, Gaudreau H, Champagne P. The effect of the addition of proteases and glucanases during yeast autolysis on the production and properties of yeast extracts. Can J Microbiol 2001;47:18–24.
[15] Strauss MLA, Jolly NP, Lambrechts MG, van Rensburg P. Screening for the production of extracellular hydrolytic enzymes by non-*Saccharomyces* wine yeasts. J Appl Microbiol 2001;91:182–90.
[16] Vosti DC, Joslyn MA. Autolysis of several pure culture of yeasts. Appl Microbiol 1953:79–84.
[17] Lowry OH, Rosebrough NJ, Farr AL, Randall RJ. Protein measurement with Folin-phenol reagent. J Biol Chem 1951;193:265–75.
[18] Walker GM. Yeast physiology and biotechnology. John Wiley and Sons; 1998, ISBN 0-471-964476.
[19] Martínez-Rodríguez AJ, Polo MC. Characterization of the nitrogen compounds released during yeast autolysis in a model wine system. J Agric Food Chem 2000;48:1081–5.