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Evaluation of *Kluyveromyces marxianus* as a source of yeast autolysates

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Abstract Cells of *Kluyveromyces marxianus* FII 510700 and *Saccharomyces cerevisiae* CBS 1907 were autolysed in phosphate buffer, pH 4.5, for a maximum of 10 days to compare chemical changes that occur in the carbohydrate, protein, amino acid and nucleic acid content. Approximately 2.2–3% carbohydrate, 9.5–12% protein, 0.6–1.0% DNA and 6–7% RNA were recovered in the autolysates. The main amino acids were β-alanine, phenylalanine, cysteine, methionine, glutamic acid and isoleucine. No significant differences in the yeast autolysates of *K. marxianus* and *S. cerevisiae* were observed. Consequently, *K. marxianus* produced from lactose-based media has potential as a source of yeast autolysates used in the food industry.

Keywords Autolysate · Yeast · *Kluyveromyces marxianus* · *Saccharomyces cerevisiae*

Introduction

Autolysates are defined as concentrates of soluble components of yeast cells produced by autolysis. During this process, yeast constituents are degraded by their own endogenous enzymes. Further, the process is characterized by the loss of membranous function and cellular organization, alteration of porosity of the cell wall, and subsequent leakage of the degradation products along with some cell components into the extracellular environment [3, 14]. At the completion of autolysis, cell components are separated from insoluble cell walls and the resulting autolysate concentrated in agitating evaporators or falling film evaporators [31].

Yeast autolysates are used mainly in the fermentation industry as substrates and in the food industry as flavor improvers [31]. In Europe, the major raw material for producing yeast extract is primary-grown high protein strains of *Saccharomyces cerevisiae* cultivated on molasses-based media [21, 31, 38]. In the United Kingdom and in the United States, yeast extracts are also manufactured from debittered brewers' yeast, consisting of strains of *S. cerevisiae*. Other raw materials used for the production of yeast biomass include waste products of the timber and agriculture industries, and ethanol [21, 38].

Autolysis of *S. cerevisiae* has been the subject of numerous articles, and its mechanism is well understood [5, 6, 19, 27]. However, for the food grade lactose-utilizing yeast *Kluyveromyces marxianus* there is very little data available on yeast extract production [2, 8, 19].

In this paper we aim to contribute to the understanding of extracts from *K. marxianus* FII510700 grown on lactose-based media compared to extracts from the traditional *S. cerevisiae* (CBS 1907) grown on glucose-based media, focusing on DNA and RNA hydrolysis, carbohydrate, protein and amino acid contents.

Materials and methods

Yeast strains, culture conditions and autolysis methods

K. marxianus (FII 510700) was obtained from the Culture Collection of the University of New South Wales, UNSW 248 (World Data Center for Microorganisms). *S. cerevisiae* (CBS 1907) was obtained from the Department of Food Science and Technology, UNSW, Sydney, Australia.

For autolysis experiments, *K. marxianus* cells were grown in a lactose medium containing: lactose, 20 g l^{-1}; $(NH_4)_2$ SO_4, 5 g l^{-1}; $MgSO_4 \cdot 7H_2O$, 2 g l^{-1}; KH_2SO_4, 4 g l^{-1} and yeast extract 2 g l^{-1}. *S. cerevisiae* was grown in a glucose medium (GM) containing: glucose, 20 g l^{-1}; $(NH_4)_2$ SO_4, 5 g l^{-1}; $MgSO_4 \cdot 7H_2O$, 2 g l^{-1}; KH_2SO_4, 4 g l^{-1} and yeast extract, 2 g l^{-1}. The yeast were cultured in 500-ml conical flasks containing 100 ml medium and incubated for 24 h during which time the cultures reached stationary phase.

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The temperature was controlled at 30°C in an orbital shaker (New Brunswick, Edison, N.J.) at 180 rpm. The yeast cells were harvested by centrifugation at 5,000 *g* for 10 min at 4°C and washed three times with 0.9% (w/v) saline. One gram of cells was suspended in 30 ml sodium phosphate-citric acid buffer (0.2 M), pH 7.0. Autolysis was initiated by incubating the suspension at 40°C with orbital shaking at 180 rpm for 10 days [39]. During autolysis, samples of the suspension were removed daily under aseptic conditions to check by microscopy for possible contamination, and for analyses of chemical composition. Cell residue and supernatant (autolysate) fractions were recovered from 5-ml aliquots of autolysing cell suspensions at days 5 and 10. These were separated by centrifugation at 5,000 *g* for 5 min at 4°C. The autolysate fraction was filtered through a 0.45 μm membrane and both fractions were stored at 4°C until analyzed.

Amino acids

The concentrations of amino acids in the autolysates were determined using a Beckman 6300/7300 amino acid analyzer (Model 6300; Beckman, Palo Alto, Calif.) fitted with an ion exchange column (Beckman, 100 mm × 4 mm). The samples and standards were treated in accordance with the Beckman System 6300/7300 Training Guide [7]. Samples (1 ml) of autolysate were collected in hyparnized tubes and transferred to 5 ml conical centrifuge tubes. The samples were centrifuged at 400–800 *g* for 15 min. Supernatant (200 μl) was withdrawn and quantitatively transferred to a 400 μl microfuge tube to which 20 μl 30% sulfosalicylic acid (Sigma, Sydney, Australia) in water was added and mixed thoroughly. The mixture was centrifuged for 5 min in a microfuge (Sigma, Munich, Germany) at 11,000 *g*. Clear supernatant (100 μl) was withdrawn and mixed in a 1:1 ratio with a mixture of Li-A and Li-S (lithium citrate; Beckman, Palo Alto, Calif.) buffer, pH 2.2. Each sample was then loaded on a sample storage coil (Beckman) and eluted with Li-A buffer at 20 ml h^{-1}.

Carbohydrates

Total carbohydrate was measured using the anthrone reagent method [4, 32].

Protein

Protein was estimated with the Folin-Ciocalteau reagent as outlined by Lowry et al. [23]. Optical density was read at 500 nm. A standard curve (0–200 mg l^{-1}) was constructed with bovine serum albumin as the standard.

Ribonucleic acid

RNA was estimated by the orcinol method [17]; for this analysis the optical density of the reaction is proportional to the concentration of RNA in the range 20–250 μg ml^{-1}.

The RNA from 200 mg (dry weight) yeast cells was extracted after the cells were washed twice with 5 ml 0.9% saline solution and then with 0.2 M $HClO_4$ to remove sugars and acid-soluble materials [29]. Cells were hydrolyzed in 5 ml 0.5 M $HClO_4$ by incubating the suspension in a water bath at 70°C for 15 min with occasional stirring. The mixture was then centrifuged at 5,000 *g* for 10 min, and the supernatant was transferred to a volumetric flask. The cells were extracted twice in the same manner and the combined extracts made up to 20 ml with cold 0.5 M $HClO_4$. RNA was estimated by reaction with the orcinol reagent according to the method of Ogur and Rosen [26] as modified by Schneider [29]. Autolysates were assayed directly, whereas cell residues were extracted in 0.5 M $HClO_4$ as described above, prior to RNA estimation by the orcinol procedure. RNA standards (200, 300, 400, 500 and 600 μg ml^{-1}) in 0.5 M $HClO_4$ prepared from standard yeast RNA (Boehringer Mannheim 109223, Mannheim, Germany) were similarly treated with orcinol.

Deoxyribonucleic acid

DNA in yeast cells, residues and autolysates was measured by the diphenylamine procedure [1, 10, 13]. Sample preparation was similar to that for RNA analysis. The DNA was estimated by reaction with diphenylamine [13]. A standard curve of DNA concentration against optical density was prepared from a stock solution of calf thymus DNA (400 μg ml^{-1}, Boehringer Mannheim).

Results

Cell composition

The gross chemical compositions of *K. marxianus* FII510700 and *S. cerevisiae* CBS 1907 grown in batch culture on lactose-based medium and glucose-based medium, respectively, are shown in Table 1. *S. cerevisiae* had a slightly lower content of RNA and DNA compared to *K. marxianus*, and a slightly higher carbohydrate content.

Changes during autolysis

Carbohydrate

Autolysates of both *K. marxianus* and *S. cerevisiae* contained solubilized carbohydrate (Table 2), which, after 10 days, represented about 2% of the initial weight of *K. marxianus* and about 3% of the initial weight of *S. cerevisiae*. These low values suggest that the cell walls were poorly degraded.

Protein

Protein was the main component in the autolysates. It represented about 8–12% of the initial weight after 5–10 days of autolysis (Table 2). Differences in protein content between the two species of yeast or the method of determination were not significant.

Amino acids

The six most prevalent amino acids in the extracts – β-alanine, phenylalanine, cysteine, methionine, glutamic acid and isoleucine – were present in insignificant amounts [1–4 μg (mg dry weight of cells)$^{-1}$ before autolysis].

Table 1 Gross chemical composition of *Kluyveromyces marxianus* FII510700 and *Saccharomyces cerevisiae* 1907 grown in batch culture

Material	*K. marxianus* (% dry weight)	*S. cerevisiae* (% dry weight)
Protein	56	57
Carbohydrate	26	30
RNA	10	8.0
DNA	2.7	1.2

Table 2 Recovery of carbohydrate and protein in cell residue and autolysate of *K. marxianus* FII 510700 and *S. cerevisiae* CBS 1907

Yeast	Autolysis time (days)	Total carbohydrate ($\mu g\ mg^{-1}$)		Protein ($\mu g\ mg^{-1}$)	
		Remaining in cells	In extract	Remaining in cells	In extract
S. cerevisiae	5	280	20	470	93
	10	270	30	450	120
K. marxianus	5	240	18	475	82
	10	235	22	463	95

Changes in DNA

DNA represented 1.2 and 2.7% of the initial cell dry weight in *S. cerevisiae* and *K. marxianus*, respectively (Table 1). DNA content decreased during autolysis (Table 3) and by 10 days had decreased by approximately 42% in *K. marxianus* and 55% in *S. cerevisiae*. DNA was recovered in the autolysates at concentrations slightly less than the decrease found in the cells. The reason for this discrepancy could be due to degradation by DNAse activity, or to DNA binding.

Changes in RNA

The initial RNA content was about 8% in *S. cerevisiae* and about 10% in *K. marxianus* (Table 1). Cellular RNA decreased throughout autolysis (4), and by 10 days had decreased by 85% in *K. marxianus* and 90% in *S. cerevisiae*. Only 70% RNA material was recovered in the autolysate of *K. marxianus*, while 76% was recovered in the autolysate of *S. cerevisiae*. These amounts were less than the loss from the cells.

Discussion

The gross cellular composition of yeasts is expected to vary according to species, method of cultivation and the phase at which cells are harvested. Nonetheless, from the results of the gross composition of *K. marxianus* and *S. cerevisiae*, it can be concluded that no significant differences were found in the carbohydrate and protein content of the two species. *K. marxianus*, however, contained more DNA and RNA than *S. cerevisiae*. The amount of RNA estimated in *S. cerevisiae* (6–8%) was within the range reported [18, 35]. No value has been reported previously for *K. marxianus*. The difference in the RNA content can be attributed to species variation and/or culture conditions.

DNA represents a small proportion (0.2–1.5%) of the dry weight of *S. cerevisiae* [38]. For both species examined, DNA was only partially degraded during autolysis. This may be due to the tendency of DNA to complex with protein, which may protect it from the action of DNAses that presumably are involved in its hydrolysis [37]. While a range of DNAses and RNAses have been reported from yeasts [9, 12, 30], their involvement in the autolytic reaction has not been studied systematically [14].

Rapid and extensive degradation of RNA is a well-studied characteristic reaction of yeast autolysis [16, 34, 35]. The products of RNA degradation, presumably nucleotides, nucleosides, and purine and pyrimidine bases, are recovered in the autolysates. In this study, however, about 10% less RNA material was recovered in the autolysate than would be expected from the sum of degradation products assayed (Table 5). A similar finding was reported by Hough and Maddox [16] and by Hernawan and Fleet [14]. These data suggest that some

Table 3 DNA in yeast cells and autolysate of *K. marxianus* and *S. cerevisiae* during autolysis

Yeast	Autolysis time (days)	% DNA in cells	% DNA in autolysate
S. cerevisiae	0	1.2	–
	5	0.77	0.38
	10	0.54	0.60
K. marxianus	0	2.7	–
	5	2.0	0.73
	10	1.0	1.0

Table 4 RNA in yeast cells and autolysate of *K. marxianus* and *S. cerevisiae* during autolysis

Yeast	Autolysis time (days)	% RNA in cells	% RNA in autolysate
S. cerevisiae	0	8.0	–[b]
	0.5	1.6	5.6
	10	0.8	6.0
K. marxianus	0	10	–
	5	3.5	6.0
	10	1.5	7.0

Table 5 Recovery of carbohydrate, protein, DNA and RNA in the autolysate of *Kluyveromyces marxianus* compared to other yeast species (expressed as % of initial dry weight of cells)

Yeast species	Autolysis time (days)	Carbohydrate	Protein	DNA	RNA
Kluyveromyces marxianus	5	1.8	9.3	27	60
	10	2.2	12	40	70
S. cerevisiae 2180	5	2.5	9.3	28	73
	10	3.1	11.6	37	74
Kloekera apiculata 202	5	5.9	11.5	18	68
	10	7.5	13.2	21	70
Candida stellata 8008	5	1.6	10.9	32	75
	10	2.7	12.3	40	76

of the RNA degradation products become entrapped or associated with the cell residue that is removed from the autolysate before analysis.

Proteins represented 55–57% of the dry weight of both *S. cerevisiae* and *K. marxianus*, and approximate the values reported by Peppler [28]. The hydrolysis of cellular proteins and release of degraded proteins, peptides and amino acids into the autolysates are principal reactions of yeast autolysis. A complex of proteinases and peptidases is responsible for these reactions; however, details of the specific enzymes involved and factors that regulate their action remain poorly understood [3, 11, 16]. The values for the recovery of protein in this study (8.2–12%) in the extracts (Table 6) approximate those reported by Hough and Maddox [16] and by Hernawan and Fleet [14], and suggest that only part of the cell protein is released undegraded into the autolysate. This conclusion is also evident from the data of others [14, 15, 33, 34, 35, 36]. However, when yeast autolysis was induced by addition of accelerators such as commercial lytic enzymes, the amount of protein released was significantly higher (25% of dry cell weight) in comparison to yeast suspensions without inductors [19]. These experiments were performed at elevated temperatures and for shorter periods of time.

The presence of amino acids in the autolysates supports the suggestion that cell protein is degraded and released into the autolysate [14, 15, 33, 34, 35, 36]. In this study, insignificant amounts of amino acids were detected in the extracts after autolysis for 10 days. The amino acid composition, however, was time-dependant. The small quantities of amino acids detected could be attributed to proteinases and peptidases that may also have been released into the autolysates and continued their action during the autolysis period. Furthermore, degradation of the amino acids released could have been exacerbated by the conditions of autolysis and the yeast species used [15]. The amino acid composition of autolysates of baker's and brewer's yeasts has been reported previously [17, 20, 21, 25] and reflects considerable variation. The presence of glutamic acid, although in insignificant amounts, would be important for the production of flavor profiles, as the threshold value for flavor enhancement is only 0.01–0.03% [31].

Table 6 Changes (%) in the concentrations of carbohydrate and protein in cells of *K. marxianus* FII 510700 and of *S. cerevisiae* CBS 1907

Yeast	Autolysis time (days)	Total carbohydrate		Protein	
		Cells	Extract	Cells	Extract
S. cerevisiae	0	30	–	57	–
	5	28	2	47	9.3
	10	27	3	45	12
K. marxianus	0	26	–	56	–
	5	24	1.8	47.5	8.2
	10	23.5	2.2	46.3	9.5

From the results presented, it is concluded that differences between the yeast autolysates of *K. marxianus* and *S. cerevisiae* are relatively minor. Consequently, we further conclude that *K. marxianus* has potential as a source of yeast autolysates for use in the food industry. The autolysis process could be accelerated by addition of autolysis promoters such as NaCl or ethyl acetate to keep the autolysis time under 24 h. A short autolysis time would have significant economic benefits, prevent the development of contaminants and curb the actions of proteinases and peptidases to yield desired components [24].

The greater metabolic flexibility of *K. marxianus* enables it to be grown on a wider range of waste products. Hence in this study, cells of *K. marxianus* were grown in a lactose-based medium to evaluate production of yeast autolysates, a value added product, and to propose a treatment method for whey, a lactose-containing waste stream of the dairy industry [22].

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