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Chemical and cytological changes during the autolysis of yeasts

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SUMMARY

Cell suspensions of *Sacharomyces cerevisiae*, *Kloeckera apiculata* and *Candida stellata* were autolyzed in phosphate buffer, pH 4.5, for up to 10 days. Cell dry weights decreased by 25–35% after 10 days. Based on initial cell dry weight, the soluble autolysate consisted of: carbohydrate (principally polysaccharide) 3–7%; organic acids 3–6%; protein 12–13%; free amino acids 8–12%; nucleic acid products 3–5%; and lipids 1–2%. The main organic acids in autolysates were propionic, succinic and acetic and the main amino acids were phenylalanine, glutamic acid, leucine, alanine and arginine. Approximately 85–90% of cellular RNA and 25–40% of cellular DNA were degraded during autolysis. Both neutral lipid and phospholipid components were degraded, with neutral lipids but not phospholipids being found in autolysates. Scanning and transmission electron micrographs showed retention of cell wall structure and shape during autolysis, but there was extensive intracellular disorganization within *S. cerevisiae* and *C. stellata*. There were differences in the autolytic behavior of *K. apiculata* compared with *S. cerevisiae* and *C. stellata*.

INTRODUCTION

Autolysis is a term that describes the breakdown of cell constituents by the action of endogenous enzymes. It occurs naturally in yeasts when they have completed their growth cycle and entered the death phase but may be induced by exposing the cells to high temperature, salts or organic solvents. Essentially, it is characterized by a loss of membranous function and cellular organization, degradation of the cell's macromolecules and the release of breakdown products into the extracellular environment. Aspects of the biology of yeast autolysis have been reviewed [4,10,26–28].

Yeast autolysis has many commercial implications. Autolysates of *Saccharomyces cerevisiae* serve as a source of additives and ingredients for food processing [15,20,30]. Yeast autolysis during beer [12,33] and wine [10] fermentations impact on the flavor profile and microbiological stability of the final product. Autolysis of compressed baker's yeast during storage decreases its leavening potential [47] and autolysis is a significant reaction during the spoilage of foods by yeasts [17]. Despite this commercial significance, there is only a general understanding of the biological changes that occur during yeast autolysis. Most studies have focused on the degradation of proteins, few have reported the breakdown of nucleic acids and less have considered the autolytic fate of carbohydrates and lipids. Moreover, nearly all studies on yeast autolysis have been conducted with only one yeast species, *S. cerevisiae*, and virtually nothing is known about the autolytic reaction of other species. A few studies have considered the autolysis of *Candida utilis* with respect to the production of single cell protein [20,39].

In this study, we report the kinetics of cell death and biomass solubilization, the degradation of proteins, nucleic acids, lipids and polysaccharides, and some cytological changes that occur during the autolysis of three yeast species, namely, *S. cerevisiae*, *Kloeckera apiculata* and *Candida stellata*.

MATERIALS AND METHODS

Yeast strains, culture and autolysis. *Saccharomyces cerevisiae* x2180a was obtained from the Department of Food Science and Technology, University of California, Davis, CA, USA. *Saccharomyces cerevisiae* EC1118 is a commercial wine yeast. *Saccharomyces cerevisiae* HB350, *Kloeckera apiculata* 202, 521 and 412, and *Candida stellata* 8008, 800MEA and 504 were isolates from wine fermentations [19,22]. Yeast cells for autolysis experiments were grown in 1% yeast extract–5% glucose broth. Inoculum cultures were grown in 10 ml of medium for 48 h at 25 °C and used to inoculate 1.5 L of medium in 2.0-L conical flasks. The large cultures were incubated for 48 h at 25 °C with orbital shaking at 100 r.p.m. Yeast cells were harvested by centrifugation at 5000 × *g* for 10 min at 4 °C and washed three times with sterile 0.9% saline. The cells were resuspended in 1400 ml of 0.1 M sodium phosphate buffer, pH 4.5 and immediately used in autolysis experiments. Autolysis was conducted by incubating cell suspensions at 45 °C for up to 10 days with orbital shaking at 100 r.p.m. During autolysis, samples of the suspension were removed for analyses of cell viability, dry weights and chemical composition. All operations were conducted under aseptic conditions.

This paper is dedicated to Professor Herman Jan Phaff in honor of his 50 years of active research which still continues.

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Cell viability. Samples of autolyzing cells were diluted in 0.1% peptone and viability determined by plate counting on Malt Extract Agar (Oxoid, Basingstoke, UK). Plates were incubated at 25 °C for 3 days. These analyses also provided a check on culture purity.

Dry weight of cells and autolysates. Samples (10 ml) of the autolyzing suspension were vacuum-filtered through a pre-weighed 0.45-μm membrane filter. The residue of cells retained by the membrane was washed three times with 10.0 ml of distilled water, then dried at 60 °C for 24 h. The dry weight of the cells was determined by subtracting the weight of the filter. The filtrate and washings from this operation (termed the autolysate) were transferred to a pre-weighed crucible and evaporated to dryness in a vacuum oven (70 °C). The weight of the autolysate was obtained by subtracting the weight of the crucible, and corrections were made for the weight of the phosphate buffer in which the cells were autolyzed.

Chemical analyses. Samples of autolysate and cell residue, obtained by filtration as described already, were frozen and stored until assay.

Carbohydrates. Total carbohydrate was measured by the anthrone reagent [40] and reducing sugars were assayed by the method of Nelson and Somogyi [40]. Glucose and glycerol were determined enzymatically using the kit reagents of Boehringer-Mannheim.

Organic acids. These were determined by high performance liquid chromatography (HPLC) using an Aminex ion-exclusion cation exchange column (Bio-Rad HPX-87H, Richmond, CA, USA) and elution with 0.1% phosphoric acid at 65 °C [14]. The concentration of acetic acid was also verified by enzymatic assay using the kit reagents of Boehringer-Mannheim.

Protein and amino acids. Total protein (tyrosine material) was estimated with the Folin–Ciocalteau reagent [32]. The concentrations of individual amino acids in autolysates were determined by HPLC and detection by post-column derivatization with ninhydrin using the Amino Acid Analysis System of Waters Associates, Sydney, Australia [2]. The system was fitted with a physiological amino acid analyzer column (ANWP150) of Waters. The identities and concentrations of individual amino acids were determined by reference to the separation of a standard mixture of amino acids (Pierce Chem. Co., USA). Individual amino acids were also injected into the system to verify peak location and identity. Autolysates were filtered through an Amicon YM5 ultrafilter (MA, USA) before injection into the HPLC system.

RNA. Ribonucleic acid (RNA) in cell residues and autolysates was determined by two methods, namely, the optical density method [24,34] and the orcinol method [38]. Standards were prepared with yeast RNA obtained from Boehringer-Mannheim.

DNA. Deoxyribonucleic acid (DNA) in cell residues and autolysates was measured by the diphenylamine procedure [1,24]. Standards were prepared from salmon sperm DNA (Boehringer-Mannheim).

Lipids. Lipids were determined by a modification of the method of Watson and Rose [51]. The autolyzing suspension (250 ml) was separated into cell residue and autolysate and the fractions were frozen and freeze-dried. The dried cell residue was mixed with 15 ml of methanol containing 50 mg L^{-1} of butylated toluene (BHT) and 20 g of glass beads (0.5-mm diameter), cooled to 4 °C, and disrupted in a Braun homogenizer for 30 s. Chloroform (30 ml) containing BHT was added to the homogenate to give a chloroform to methanol ratio of 2:1. The mixture was stirred at room temperature for 2 h under nitrogen gas and the extract and residue separated by filtration. The residue was extracted a further two times with chloroform : methanol (45 ml). The extracts were pooled, washed with 0.88% sodium chloride, and evaporated at 30 °C to dryness to give the lipid material. Autolysates were extracted in the same way as the cell residue but were not disrupted in the homogenizer.

Lipid extracts, dissolved in 1.0 ml of chloroform (containing BHT), were analyzed by thin layer chromatography (TLC) on plates of silica gel 60 (Merck 5721, Germany). Phospholipids were separated with a mobile phase of chloroform : methanol : isopropanol : 0.25% potassium chloride : ethyl acetate (30 : 9 : 25 : 6 : 18) [23]. Neutral lipids were separated by a two-stage one-dimensional procedure using diethylether : benzene : ethanol : acetic acid (40 : 50 : 2 : 0.2) in the first stage followed by *n*-hexane : diethylether (96 : 4) in the second stage [37]. Lipid spots were visualized by spraying the plates with copper acetate (3%) in phosphoric acid (8%) and heating at 180 °C for 20 min [23]. Spot intensities were compared with a scanning densitometer at 590 nm [8]. Neutral and phospholipid standards (Sigma Chemical Co., USA) were chromatographed in conjunction with the samples.

Electron microscopy. Samples of autolyzing cells were fixed in glutaraldehyde (6%) in 0.2 M cacodylate buffer, pH 7.4, for 3 h at 4 °C. Scanning electron microscopy was done according to Watson and Arthur [50] using a Cambridge Stereoscan 360. Transmission electron microscopy of thin sections of samples was done according to Streiblová [42]. The grids with the sectioned sample were stained with uranyl acetate (4%) and lead citrate and observed in a transmission electron microscope (Hitachi 7000).

RESULTS

Cell viability

The viability of yeast cells decreased rapidly during autolysis (Fig. 1). Loss in viability was fastest for *K. apiculata*, followed by *C. stellata* and *S. cerevisiae*. For *K. apiculata* and *C. stellata*, more than 99% of the cells had died within 24 h and no viable cells of these species were detected after 3–5

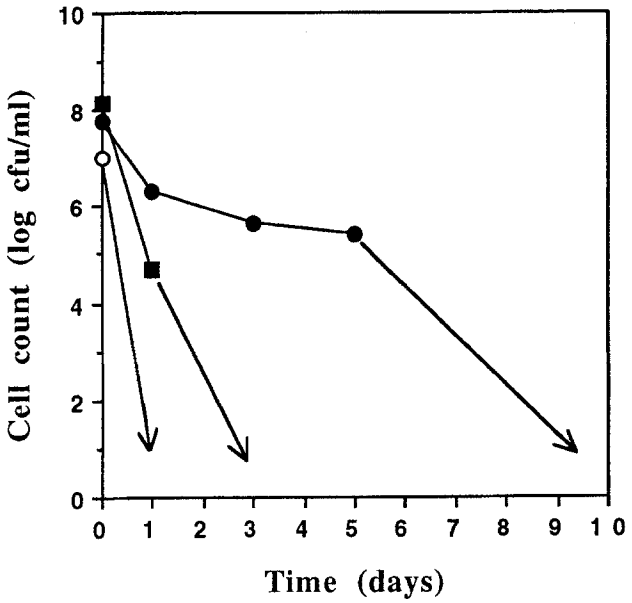

Fig. 1. Decrease in the cell viability of yeasts during autolysis. *Saccharomyces cerevisiae*, ●; *Kloeckera apiculata*, ○; *Candida stellata* ■. Cell suspensions were autolyzed in phosphate buffer pH 4.5, 45 °C. Arrows denote counts less than 5 CFU ml^{-1}.

days. Small populations of viable cells of *S. cerevisiae* were still present after 3–5 days.

Solubilization of cell biomass

Autolysis was characterized by a decrease in cell dry weight and recovery of solubilized biomass as autolysate (Table 1). For each species, the cell dry weight decreased by approximately 25–30% after 5–10 days and the soluble biomass recovered in the autolysate represented approximately 20–25% of the initial cell weight. The difference of about 5% between the cell weight lost and that recovered as autolysate was consistently observed, and probably represents loss of matter as gases (e.g. carbon dioxide and ammonia). The formation of gas bubbles in autolysates was evident. There were marginal variations between species and strains in the extent of autolysis, the most notable being the increased solubilization of *K. apiculata* strains 202 and 521 (33% loss in cell weight; 27% recovered as autolysate). Most of the autolytic solubilization occurred before day 5, with little further reaction occurring between 5–10 days.

Chemical changes during autolysis

Carbohydrate. Autolysates contained solubilized carbohydrate which, after 10 days, represented about 7.5% of the initial cell weight of *K. apiculata* 202, and about 3% for either *S. cerevisiae* 2180 of *C. stellata* 8008 (Table 2). However, as indicated by the 5-day data in parentheses, the amount of carbohydrate recovered in autolysates for *K. apiculata* and *C. stellata* varied with the strain. Only trace amounts (<0.15% dry wt of cell) of the carbohydrate in autolysates occurred as glucose or reducing sugar, indicating that the autolyzed material was mostly polysaccharide.

Protein. Protein was a main component of autolysates. It represented about 9–13% of the initial dry weight of the cells after autolysis for 5–10 days. There were no major differences between species in protein solubilization, although slightly higher amounts were consistently found in the autolysates of *K. apiculata* (Table 2).

Amino acids. Free amino acids were found in the autolysates and for some strains constituted 9–12% of the initial cell weight after autolysis for 5–10 days. However, some strains of *K. apiculata* and *C. stellata* gave autolysates with much lower concentrations of amino acids (Table 2). The same complement of 17 amino acids was found in autolysates of all nine yeast strains examined, except that lysine was not

TABLE 1

Solubilization of biomass of yeast species during autolysis

Yeast species	Autolysis time (days)	Cell dry weight[a] (% decrease)	Autolysate (% initial cell dry wt)
Saccharomyces cerevisiae 2180	1	10.9	6.3
	5	25 (26,26)[b]	17.2 (22,23)[b]
	10	26.6	21.9
Kloeckera apiculata 202	1	16.7	11.1
	5	27.8 (33,26)[b]	22.2 (27,22)[b]
	10	33.3	27.8
Candida stellata 8008	1	11.4	6.3
	5	27.8 (25,30)[b]	17.7 (20,25)[b]
	10	30.4	20.3

[a] Initial cell dry weights of the three yeasts were: *S. cerevisiae* 2180, 6.40 mg ml^{-1}; *K. apiculata* 202, 3.6 mg ml^{-1}; *C. stellata* 8008, 7.9 mg ml^{-1}.

[b] Data in parentheses for day 5 are the values for two other strains of the same species: *S. cerevisiae* HB350 and EC1118; *K. apiculata* 521 and 412; and *C. stellata* 800MEA and 504.

TABLE 2

Recovery of carbohydrate, protein, free amino acids and glycerol in yeast autolysates

Yeast species	Autolysis time (days)	Total carbohydrate[a]	Protein[a]	Amino acids[a]	Glycerol[a]
Saccharomyces cerevisiae 2180	1	0.9	3.3	2.1	0.5
	5	2.5 (1.6,1.8)[b]	9.3 (10.9,11.7)[b]	9.1 (8.2,7.9)[b]	0.6
	10	3.1	11.6	11.7	0.6
Kloeckera apiculata 202	1	1.5	7.8	6.4	0.1
	5	5.9 (4.2,2.1)[b]	11.5(13.2,12.6)[b]	7.2 (4.4,1.8)[b]	0.2
	10	7.5	13.2	8.4	0.2
Candida stellata 8008	1	0.7	4.3	2.8	0.3
	5	1.6 (1.6,5.6)[b]	10.9 (8.7,10.8)[b]	8.7 (5.1,4.9)[b]	0.3
	10	2.7	12.3	10.6	0.3

[a] Data expressed as % initial cell dry weight.
[b] Data in parentheses for day 5 are the values for two other strains of the same species: *S. cerevisiae* HB350 and EC1118; *K. apiculata* 521 and 412; and *C. stellata* 800MEA and 504.

detected in autolysates of *K. apiculata* 202 and 412, tyrosine was absent from autolysates of *K. apiculata* 202, methionine was not found in autolysates of *K. apiculata* 412, and cystine was absent from autolysates of *C. stellata* 800MEA and 504. The five most prevalent amino acids in autolysates were phenylalanine, glutamic acid, leucine, alanine, and arginine, but their order of significance in terms of concentration varied with the species and strain (Table 3).

Organic acids. Organic acids were recovered in the autolysates of all yeast species (Table 4). They were detected after the first day of autolysis, with increased concentrations being found after 5 and 10 days. Propionic, succinic and, to a lesser extent, acetic were the main acids found for all three species. Lactic acid was not detected in autolysates of any strain of *S. cerevisiae* although it occurred in the autolysates of *K. apiculata* and *C. stellata*. For *S. cerevisiae* and *C. stellata*, the total concentration of released acids represented about 5% of the initial cell weight, although there was some variation between strains. The autolysates of *K. apiculata* contained lower concentrations (about half) of the acids compared with the other species.

Nucleic acids. The initial RNA content of the cells ranged between 4–6% depending on the species. The content of cellular RNA decreased throughout autolysis (Table 5) and by 10 days had decreased by 85–90% for each of the species. Ribonucleic acid material was recovered in the autolysates, although after 5 days this was 10–15% less than that expected from the amount lost by the cells. Thus, while 86% of the cellular RNA of *S. cerevisiae* was lost after autolysis for 10 days, only 74% of RNA or RNA material was recovered in

TABLE 3

Concentrations[a] of the five most prevalent amino acids in autolysates of yeasts after autolysis for 10 days

Amino acid	*Saccharomyces cerevisiae* 2180	*Kloeckera apiculata* 202	*Candida stellata* 8008
Glutamic acid	14.7	14.9	12.5
Phenylalanine	14.0	6.2	14.8
Leucine	12.1	9.9	8.0
Alanine	9.1	13.9	11.5
Arginine	8.5	7.5	13.2

[a] Expressed as μg of acid mg^{-1} of dry weight of cells before autolysis.
Lysine at 3–6 μg mg^{-1} was the next most prevalent amino acid in autolysates of *S. cerevisiae* HB350, and EC1118, *K. apiculata* 521 and *C. stellata* 800MEA. It was the fourth most significant acid in *C. stellata* 504.

TABLE 4

Concentrations[a] of organic acids in yeast autolysates after autolysis for 10 days

Acid	*Saccharomyces cerevisiae* 2180	*Kloeckera apiculata* 202	*Candida stellata* 8008
Propionic	1.7	0.5	1.4
Succinic	1.2	0.6	1.2
Malic	0.4	0.2	0.4
Formic	0.6	0.1	0.2
Acetic	0.9	0.4	0.4[b]
Oxalic	0.5	0.3	0.8
Citric	0.2	0.1	0.2
Tartaric	0.1	0.1	0.2
Lactic	–	0.1	0.2
Total	5.6	2.5	5.0
	(3.3,7.5,4.0)[c]	(1.8,2.3,1.9)[c]	(3.9,5.5,4.8)[c]

[a] Data expressed as % initial cell dry weight.
[b] *Candida stellata* 504 gave 0.7% after 5 days.
[c] Data in parentheses represent the total acids (% initial dry weight of cells) found in 5-day autolysates for strains *S. cerevisiae* 2180, HB350, EC1118, *K. apiculata* 202, 521, 412 and *C. stellata* 8008, 800MEA, 504, respectively.

TABLE 5

Changes (%) in the concentrations of RNA and DNA in cells and autolysates of yeasts during autolysis

Yeast species	Autolysis time (days)	RNA[a] In cells	RNA[a] In autolysates	DNA[a] In cells	DNA[a] In autolysates
		Decrease (%)	Increase (%)	Decrease (%)	Increase (%)
Saccharomyces cerevisiae 2180a	1	28	26	11	7
	5	82	73	32	28
	10	86	74	41	37
Kloeckera apiculata 202	1	23	26	7	7
	5	78	68	21	18
	10	85	70	24	21
Candida stellata 8008	1	29	25	15	13
	5	84	75	34	32
	10	90	76	42	40

[a] The percentage change was calculated with reference to the concentration of RNA and DNA in the cells before autolysis.

the autolysate. The data shown in Table 5 were obtained by measuring the concentration of RNA by the orcinol method. Similar data were obtained by measuring RNA by the optical density method. Similar trends for RNA behavior were also found for the strains of the species.

DNA represented 0.1–0.2% of the initial cell dry weight, depending on species. Its content decreased throughout autolysis and, by 10 days, had decreased by approximately 40% for both *S. cerevisiae* and *C. stellata* and 24% for *K. apiculata.* DNA material was recovered in autolysates at concentrations reflecting the decrease found in the cells. However, in one series of experiments where all nine strains were examined, DNA material recovered in the autolysates was substantially less than that expected from the decrease within the cells. The reason for this discrepancy is not clear at this stage.

Lipids. The dry weights of lipid material recovered in autolysates and extracts of cell residues were too low to be measured accurately. An approximate estimation of lipid content was obtained by comparing the intensities of the spots of the different lipid fractions obtained on TLC plates, with that of known standards. On this basis, lipid material represented 4–5% of the initial cell dry weight. It decreased during autolysis, the reduction after 10 days being approximately 80%, 15% and

45%, respectively, for *S. cerevisiae* x2180, *K. apiculata* 202 and *C. stellata* 8008. Approximately half of the lipid lost from the cells was recovered in the autolysates. The degradation of cell lipid is supported by the recovery of glycerol in the autolysates (Table 2).

By comparing the intensities of the spots on TLC plates, we observed progressive decreases in the concentrations of the different neutral and phospholipid components of the cells during autolysis. Table 6 shows the decrease (%) in contents of particular lipid components after 10 days. For *S. cerevisiae* and *C. stellata*, most components had decreased by more than 40–50%. By comparison, the lipid components of *K. apiculata* were less degraded. All neutral lipid fractions were found in autolysates of each of the three yeast species, but phospholipids were not detected in autolysates of any of the species.

Electron microscopy of autolyzing yeasts

Scanning electron micrographs of cells of the species before and after autolysis for 5 and 10 days revealed no obvious changes in cell shape or surface appearance. The walls were not disrupted and bud scars were evident. Electron micrographs of thin sections of cells of *S. cerevisiae* and *C. stellata* showed shrinking of the cell membrane and cytoplasmic contents away from the cell wall as autolysis occurred. Decreases in the density of the cytoplasmic material were also evident (Figs 2 and 4). Such changes were not apparent during autolysis of *K. apiculata* (Fig. 3). These micrographs also confirmed that the cell walls of the yeasts remained entire and unbroken throughout autolysis. The layered composition of the walls remained unchanged but wall thickness decreased by approximately 20% for each of the species after autolysis for 10 days. (Wall thickness data were averaged from measurements on five different cells at five different wall locations $cell^{-1}$, for each species).

DISCUSSION

Yeast autolysis is characterized by the degradation of cellular proteins, nucleic acids, lipids and polysaccharides, leading to a loss in cell viability and solubilization of cell biomass. We have confirmed this general behavior for *S. cerevisiae* and extended this conclusion to include *K. apiculata* and *C. stellata*, two other yeast species that are significant in wine fermentations [18] and food spoilage [17].

According to Joslyn and Vosti [28] and Arnold [4], cell death is essential to initiate yeast autolysis, but the conditions causing death should not inactivate hydrolytic enzymes, such as proteases, lipases and nucleases that are considered necessary for the autolytic process. For the three species tested, incubation in buffer at 45 °C was sufficient to cause rapid loss in viability (Fig. 1) and initiate autolysis. The faster death of *K. apiculata* at 45 °C compared with the other species probably reflects the increased susceptibility of this species to heat inactivation [7].

Proteins represent 45–50% of the dry weight of yeast cells [20,35]. 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 are responsible for these reactions, but details of the specific enzymes involved and the factors that regulate their action remain poorly understood [4,10,26]. Our values for the recovery of proteins (10–13%) and amino acids (8–12%) in autolysates approximate those reported by Hough and Maddox [26] for brewer's yeast, and suggest that only part of the cell protein is degraded and released into the autolysate. This conclusion is evident from the data of others [25,41,44–46]. However, the protein and amino acid compositions of autolysates are not stable because proteinases and peptidases are also released into the autolysates and continue their action. Such activity will vary with the conditions of

TABLE 6

Decrease (%) in the concentration of neutral lipids and phospholipids of yeasts after autolysis for 10 days

Lipid component	*Saccharomyces cerevisiae* 2180	*Kloeckera apiculata* 202	*Candida stellata* 8008
Triacylglycerol	54	17	69
Diacylglycerol	68	48	39
Monoacylglycerol	57	6	53
Sterol esters	65	23	25
Sterols	43	33	40
Free fatty acids	70	25	43
Phosphatidyl choline	44	66	70
Phosphatidyl ethanolamine	–[a]	–[a]	–[a]
Phosphatidyl inositol	76	37	43
Phosphatidyl serine	79	42	90
Phosphatidyl glycerol	33	25	33

[a] Not detected.

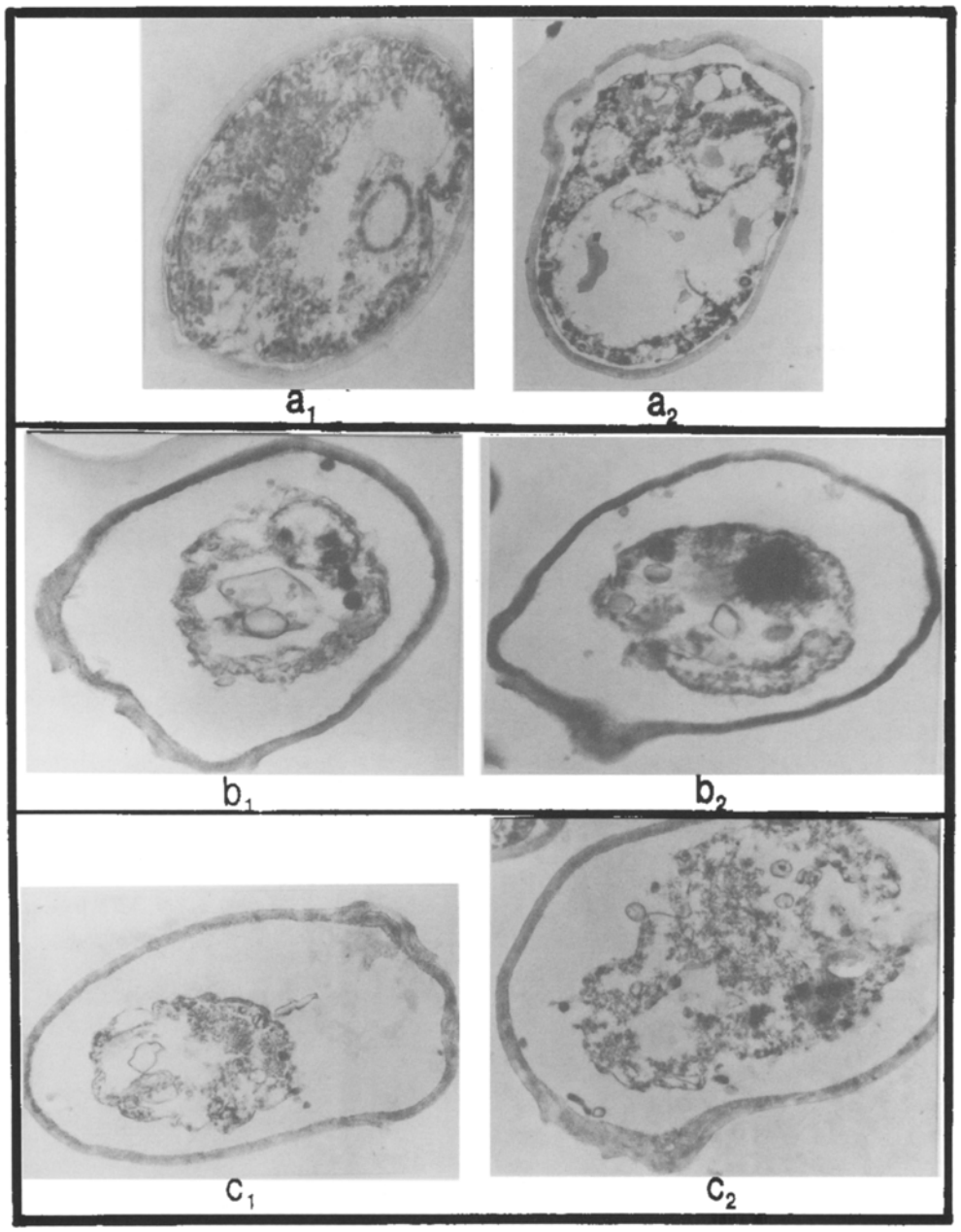

Fig. 2. Transmission electron micrographs of sections of *Saccharomyces cerevisiae* 2180 after autolysis for: (a) 0 days; (b) 5 days; and (c) 10 days. Magnification 9000–10 800.

autolysis and yeast species and will affect the amino acid composition of the autolysate (Table 3). The amino acids found in autolysates of baker's and brewer's yeasts have been reported [27,30,31,33] and reflect considerable variation but, nevertheless, include those shown in Table 3.

The concentrations of RNA found in the yeasts are within the range of 6–8% reported for *S. cerevisiae* [20,29,45]. The RNA contents of *K. apiculata* and *C. stellata* have not been reported previously. Rapid and extensive (80–90%) degradation of RNA is another characteristic reaction of yeast autolysis [26,44,45]. The products of RNA degradation, presumably nucleotides, nucleosides and purine and pyrimidine bases, are recovered in the autolysate. However, we recovered about 15% less RNA material in the autolysate than expected from the amount degraded within the cell (Table 5). A similar finding was reported by Hough and Maddox [26]. These data suggest that some of the RNA degradation products become entrapped or associated with the cell residue that is removed from the autolysate before analysis.

DNA represents only a small proportion (0.2–1.5%) of the dry weight of yeast cells (20,46). For the three species examined it was only partially degraded during autolysis (Table 5). Trevelyan [46] also noted this phenomenon during the autolysis of baker's yeast. The tendency for DNA to complex with protein may protect it from the action of DNA-ases that, presumably, are involved in its hydrolysis. While a range of

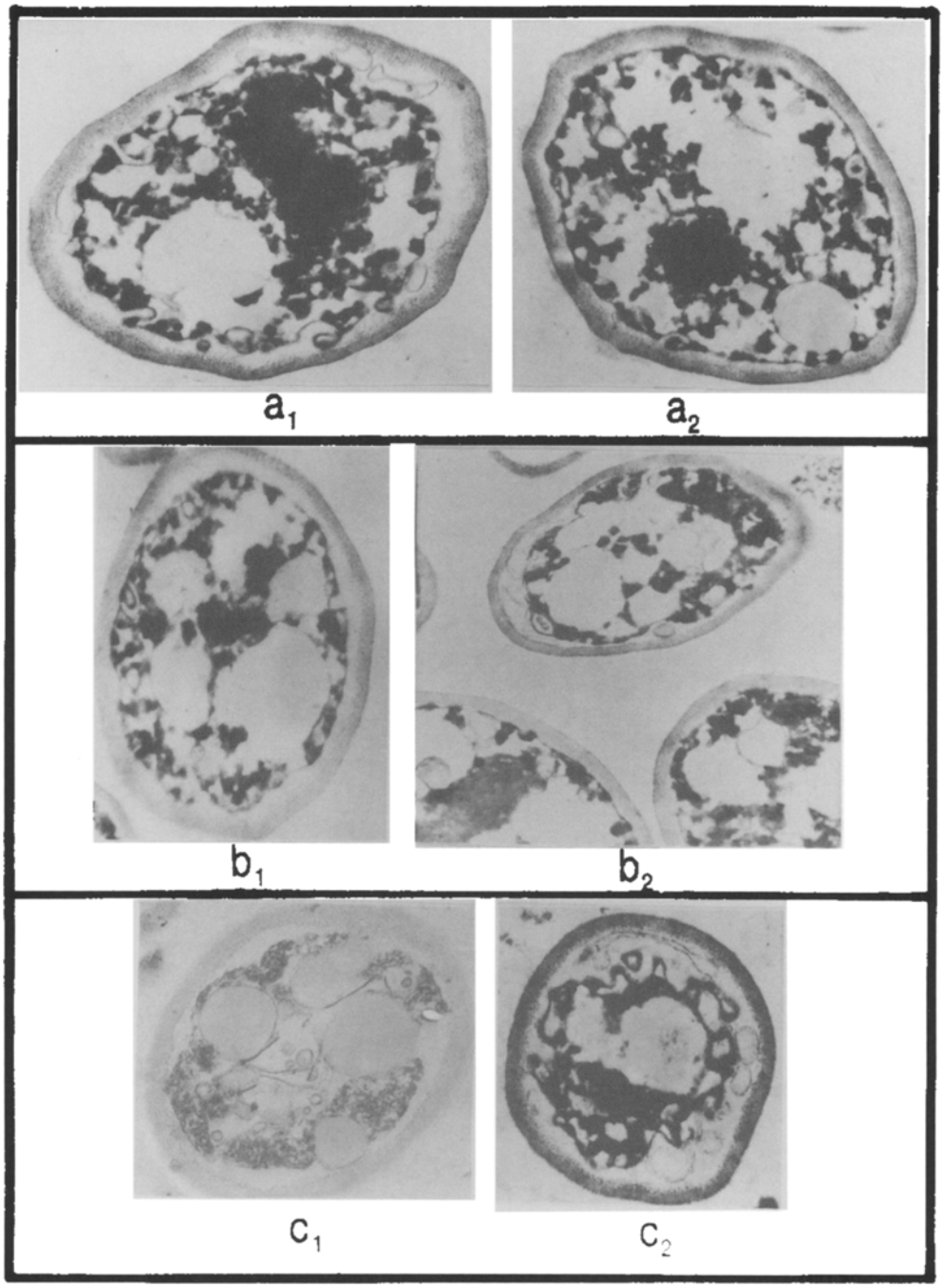

Fig. 3. Transmission electron micrographs of sections of *Kloeckera apiculata* 202 after autolysis for: (a) 0 days; (b) 5 days; and (c) 10 days. Magnification 9000–13 500.

DNA-ases and RNA-ases have been found in yeasts [9,13,39], their involvement in the autolytic reaction has not been systematically studied.

Loss of membrane function and breakdown in cellular organization are believed to be primary events that trigger yeast autolysis [3]. In accepting the principal role of lipids in membrane composition and structure [3,36], lipid degradation should be an important reaction in autolysis. However, this issue is quite complex since lipids embrace components of acylglycerols, sterol esters, sterols, phospholipids and free fatty acids and, moreover, different membranes within the cell have different proportions of these components [3,36]. It is not surprising, therefore, that only a few studies have examined the fate of lipids during autolysis. According to some reports [21,43,49], phospholipid degradation is the most significant change to lipids during the autolysis of baker's yeast. The recovery of a range of free fatty acids and acylglycerols in beer and wine have been attributed to lipid degradation during the autolysis of brewer's and wine yeasts [12,33,48]. For *S. cerevisiae* and *C. stellata*, we observed substantial losses

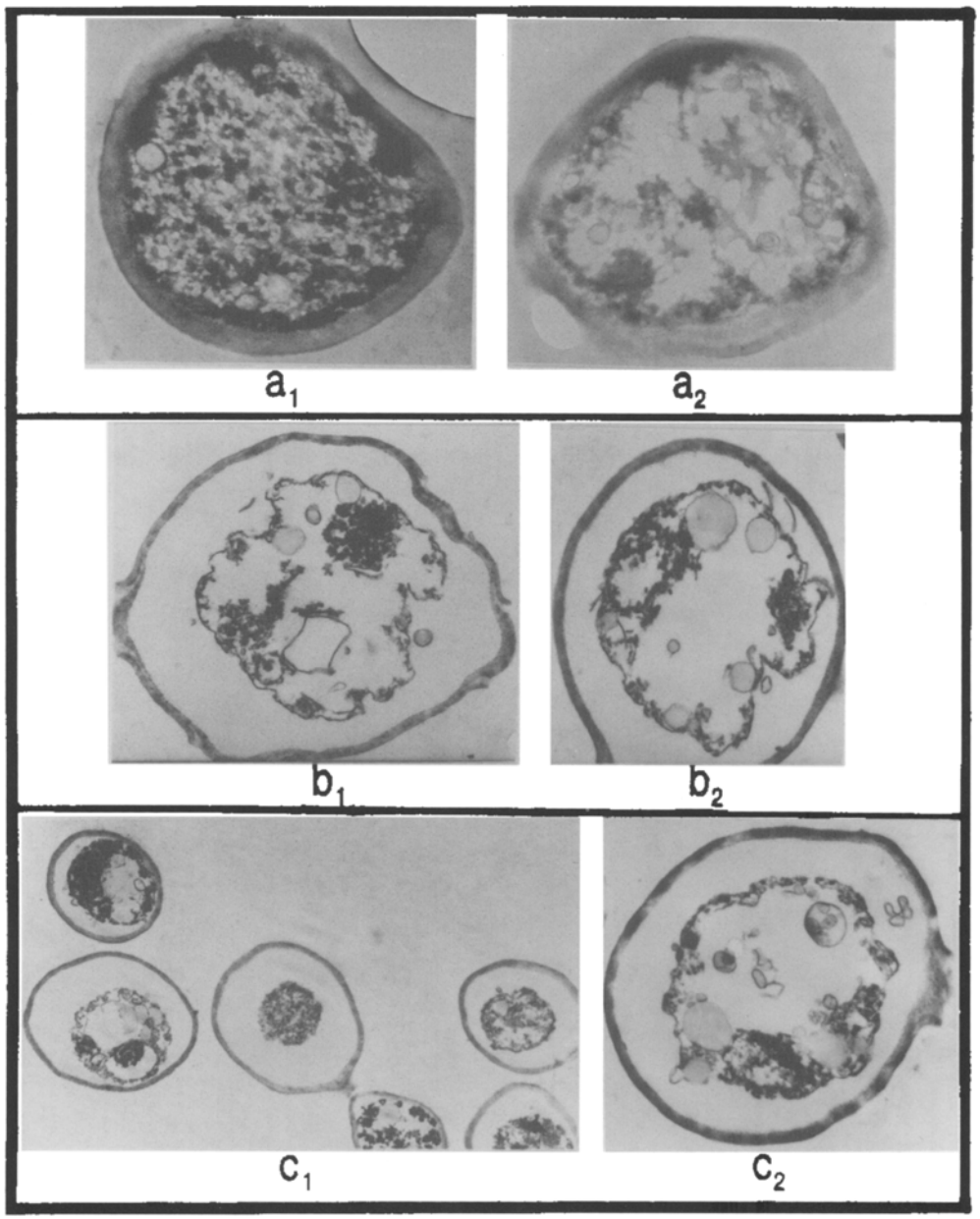

Fig. 4. Transmission electron micrographs of sections of *Candida stellata* 8008 after autolysis for: (a) 0 days; (b) 5 days; and (c) 10 days. Magnification 3150–9000.

(80% and 45%, respectively) of cell lipids during autolysis. In addition to losses in the phospholipids, we also recorded decreases in the concentrations of acylglycerols, sterols, sterol esters and free fatty acids (Table 6). Cells of *K. apiculata* gave notably less (15%) decrease in lipid content during autolysis but, nevertheless, all classes of lipids underwent some degradation. In another study on the autolysis of *S. cerevisiae* x2180 (Agagiotis and Fleet, unpublished data), we confirmed the general findings reported in Table 6, but also noted that, at certain stages during autolysis, the concentrations of mono- and di-acylglycerols increased slightly before decreasing. This observation would be consistent with the sequential enzymatic degradation of triacylglycerols to diacylglycerols, monoacylglycerols with the production of free fatty acids and glycerol [36]. Free fatty acids were readily detected in autolysates with palmitoleic, palmitic, oleic and stearic being the main acids found. Phospholipases have been implicated in yeast autolytic reactions [21,43] but the involvement of other lipolytic enzymes has not been investigated. Overall, our studies show that there are major changes to all lipid classes during autolysis and indicate the need for more specialized and quantitative study of their behavior.

The release of organic acids (Table 4) has not been reported in previous studies on yeast autolysis, although Peppler [35] has noted that organic acids represent about 1.6–4.8% of the dry weight of commercial yeast autolysates. There are some notable points about the data of Table 4. Less acid production occurred in the autolysates of *K. apiculata* than in those from

S. cerevisiae or *C. stellata*. For all the yeasts, propionic, succinic and, to a lesser extent, acetic were the main acids found. The absence of lactic acid from autolysates of *S. cerevisiae* but not from those of *K. apiculata* or *C. stellata* is noteworthy. The biochemical mechanisms by which the acids are produced during autolysis have not been reported, but, presumably, are related to the metabolism of sugars and lipids. In a practical context, the types and concentrations of acids produced would be expected to affect the sensory properties of the autolysate.

Cellular polysaccharides were weakly degraded during autolysis (Table 2), [6,26,30]. A stronger reaction seems to occur in *K. apiculata* than in the two other species. Principally, these polysaccharides are associated with the cell wall, since any glycogen reserves are considered to be metabolized during the first few hours of autolysis [4,27]. Both the chemical data of Table 2 and the electron microscopic observations (see later) suggest that the walls are little degraded during autolysis. The small amount of carbohydrate recovered in autolysates was mostly polysaccharide, an observation also recorded by Hough and Maddox [26]. According to Charpentier and Feuillat [10], this polysaccharide is mainly mannoprotein which is detached from the walls by the action of (1→3)-β-glucanases. The autolytic degradation of isolated cell walls of yeasts through the action of (1→3)-β-glucanases is well documented [16].

Our electron micrographs (Figs 2, 3, 4) as well as those reported elsewhere [6,11] have established that the cell wall retains its basic shape and integrity during autolysis. Presumably, the core (1→3)-β-glucans responsible for wall rigidity [16] are not degraded. Autolytic loss of mannoprotein would not affect wall integrity but would be expected to alter wall porosity which must increase to allow external passage of intracellular macromolecules [4,6,10,27]. Although wall integrity was not lost, autolysis was accompanied by extensive loss and disorganization of the intracellular contents especially for *S. cerevisiae* and *C. stellata* (Figs 2, 3) [5]. Such extensive intracellular changes were not noted in the electron micrographs of *K. apiculata* and support conclusions from some of the chemical data (e.g. less lipid degradation) that the autolytic processes within this species differ to varying extents from those which occur in *S. cerevisiae* and *C. stellata*.

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