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Jian Zhao · Graham H. Fleet

Degradation of RNA during the autolysis of *Saccharomyces cerevisiae* produces predominantly ribonucleotides

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Abstract Autolytic degradation of yeast RNA occurs in many foods and beverages and can impact on the sensory quality of the product, but the resulting complex mixture of nucleotides, nucleosides and nucleobases has not been properly characterised. In this study, yeast autolysis was induced by incubating cell suspensions of *Saccharomyces cerevisiae* at 30–60 °C (pH 7.0), and at pH 4.0–7.0 (40 °C) for 10–14 days, and the RNA degradation products formed during the process were determined by reversed-phase HPLC. Up to 95% of cell RNA was degraded, with consequent leakage into the extracellular environment of mainly 3′-, 5′- and 2′-ribonucleotides, and lesser amounts of polynucleotides, ribonucleosides and nucleobases. The rate of RNA degradation and the composition of the breakdown products varied with temperature and pH. RNA degradation was fastest at 50 °C (pH 7.0). Autolysis at lower temperatures (30 °C and 40 °C) and at pH 5.0 and 6.0 favoured the formation of 3′-nucleotides, whereas autolysis at 40 °C and 50 °C (pH 7.0) favoured 5′- and 2′-nucleotides. The best conditions for the formation of the two flavour-enhancing nucleotides, 5′-AMP and 5′-GMP, were 50 °C (pH 7.0) and pH 4.0 (40 °C), respectively.

Keywords Autolysis · Yeast · *Saccharomyces cerevisiae* · RNA degradation · Flavour nucleotides

J. Zhao (✉) · G. H. Fleet
Food Science and Technology,
School of Chemical Engineering and Industrial Chemistry,
The University of New South Wales, Sydney,
NSW 2052, Australia
E-mail: jzhao@csu.edu.au
Tel.: +61-2-69332968
Fax: +61-2-69332107

J. Zhao
School of Wine and Food Sciences, Charles Sturt University,
Wagga Wagga, NSW 2678, Australia

Introduction

Yeast autolysis is an enzymatic self-destruction that commences after cell death and is characterised by degradation of cellular constituents and subsequent release of the breakdown products into the extracellular environment. Although a naturally occurring event, autolysis of yeasts can be induced by exposing the cells to elevated temperatures (40–60 °C), salts, or organic solvents [2, 3]. The basic biology and chemistry of yeast autolysis have been well-studied and reviewed [2–8, 10], but much of the research has focussed on the autolytic breakdown of protein whereas the degradation of nucleic acids (DNA and RNA) has received much less attention.

Yeast autolysis has commercial significance as it occurs in many foods and beverages, including beer [21], wines [4–6, 19, 20, 31] and baker's yeasts [29, 30] where it may affect their sensory quality and commercial acceptability. Also, the process is used to produce yeast autolysates which are used in the food industry as flavour enhancers [7, 9, 10, 27]. Key components of yeast autolysates include nucleic acid degradation products, such as 5′-guanosine monophosphate (5′-GMP) and 5′-adenosine monophosphate (5′-AMP) [9, 22, 23]. 5′-GMP is an active flavour enhancer and 5′-AMP is a precursor for the well-known flavour enhancer 5′-inosine monophosphate (5′-IMP). Incidentally, the flavour-enhancing activity of 5′-GMP and 5′-IMP is more than 100 times greater than that of monosodium glutamate (MSG)—a widely used flavour enhancer [9, 22, 23].

In a previous communication [41], we reported that DNA was degraded during yeast autolysis by the action of endogenous DNases with consequent leakage into the autolysates of mainly 3′- and 5′-deoxyribonucleotides, and lesser amounts of polynucleotides. However, the DNA content of yeast cells is less than 1.5%, whereas the level of RNA is much higher and ranges from 5–15% on a dry weight basis [13]. Consequently, degradation of RNA could be a more significant source of nucleotides

including those with flavour-enhancing properties [9, 22, 23]. Previous studies have shown that RNA is degraded rapidly during autolysis [13, 33–36]. However, the dynamics of the reaction as influenced by conditions such as temperature and pH are not well-understood, and the composition of RNA degradation products (including ribonucleotides, ribonucleosides and bases) has not been reported. Such information may provide a better biochemical understanding of autolysis and, from a practical aspect, enable the process to be optimised to control the concentration of individual degradation products.

We report, here, the composition of RNA degradation products that are formed during the autolysis of *Saccharomyces cerevisiae*, and show ribonucleotides to be the main products and that the composition was significantly influenced by both temperature and pH.

Materials and methods

Yeast strain, cultivation and autolysis

Saccharomyces cerevisiae (haploid strain ×2180a) was obtained from the Department of Food Science and Technology, University of California, Davis, USA. The yeast was maintained by subculturing and storage at 4 °C on malt extract agar (Oxoid, CM59, Melbourne, Australia). Cells for autolysis experiments were grown in 5% glucose–0.5% yeast extract broth. Inoculum culture was grown in 100 ml of the medium at 25 °C for 24 h with orbital shaking at 200 rpm, and was used to inoculate the experimental culture (1 l in a 2-l conical flask) which was incubated at 25 °C for 48 h (end of exponential growth) with orbital shaking (200 rpm). Cells were harvested by centrifugation at 5,000 *g* for 10 min at 4 °C, washed three times with sterile 0.2 M phosphate–citric acid buffer, pH 7.0, and resuspended in the same buffer at either pH 4.0, 5.0, 6.0 or 7.0 to give suspensions containing 10^8–10^9 cells/ml. The precise biomass concentration in each suspension was determined by dry weight measurement (see below). To initiate autolysis, cell suspensions were incubated at either 30 °C, 40 °C, 50 °C or 60 °C for up to 14 days with orbital shaking (200 rpm). Samples (50 ml) of the autolysing cell suspensions were withdrawn at intervals and separated by centrifugation (5,000 *g* for 5 min at 4 °C) into cell pellet and autolysate (supernatant) fractions. The autolysate fraction was filtered through a 0.45-μ membrane (Millipore). The cell and autolysate fractions were analysed for RNA content. Autolysate fractions were also analysed for ribonucleotides, ribonucleosides and nucleobases. Three independent experiments were done for each autolytic condition and similar data and trends were found in each experiment. Aseptic conditions were maintained throughout the experiments and autolysing suspensions were checked by agar culture to ensure freedom from bacterial contamination.

Cell dry weight

Cell suspensions (1.0 ml) were centrifuged at 5,000 *g* for 5 min to sediment the cells which were washed twice with 5 ml of sterile distilled water and finally resuspended in 1.0 ml of distilled water. The suspension was poured onto a pre-weighed membrane of 0.45 μ pore size and dried at 60 °C in a vacuum oven until constant weight. Assays for cell dry weight were done in triplicate.

RNA assay

RNA in yeast cells and autolysates was determined by two methods: the traditional orcinol method [11] which is based upon the colourimetric determination of the pentose sugar (ribose) in RNA, and the guanine-HPLC method [32] which is based upon the HPLC determination of guanine in RNA. While both procedures gave similar data, the orcinol procedure suffered interferences from hexose sugars, such as glucose and mannose released from the hydrolysis of yeast cell walls. Only results from the guanine-HPLC procedure are presented here. Briefly, autolysing cell suspensions were separated into cell residue and supernatant (autolysate) fractions by centrifugation at 5,000 *g* for 5 min at 4 °C. The cell residues were washed twice with sterile de-ionised water and resuspended in 100 ml of 0.5 M $HClO_4$. Aliquots (2 ml) of the cell suspension were placed in teflon-capped test tubes and 0.5 ml of concentrated $HClO_4$ was added to give a final concentration of 2 M $HClO_4$. The tubes were incubated in a boiling water-bath for 60 min to hydrolyse the RNA and extract guanine. Following hydrolysis, the extract was cooled, neutralised with solid $NaHCO_3$ and filtered through a 0.45-μm membrane. The filtrate was retained for analysis of guanine by HPLC. The same procedure was used for extracting guanine from RNA materials in the autolysate. The detailed HPLC procedure is given elsewhere [32]. A standard curve of RNA was prepared from solutions of yeast RNA (Boehringer Mannheim 109223, Germany) analysed by the same procedure.

Assay of RNase activity

Autolysing cell suspensions (5 ml) were separated into cell pellet and autolysate fractions as described above. The cell pellet was washed once with 5 ml of phosphate-buffered saline, pH 7.3 (Oxoid BR0014) containing 0.16 M NaCl, 3 mM KCl, 8 mM Na_2HPO_3 and 1 mM NaH_2PO_3, and resuspended in 5 ml of the same solution. The suspension was mixed with 5 g of glass beads with a diameter of 0.45 mm, cooled to 4 °C in an ice-water bath, and homogenised in a Braun MSK cell homogeniser (B. Braun Biotech International, Melsungen, Germany) for 30 s to break the cells and release the RNases. This procedure was repeated three times and, at each interval, the mixture was

cooled in an ice-water bath to keep the temperature below 10 °C during the entire process. After homogenisation, the mixture was centrifuged at 5,000 *g* for 5 min at 4 °C, and the supernatant was carefully pipetted out and used for the assay of cellular RNase activity.

The autolysate fraction was ultrafiltered through an Amicon Micro-concentrator (Amicon, Sydney, Australia) to remove buffer salts and small molecular weight compounds released during autolysis. The micro-concentrator contained a membrane with a 10,000 Da molecular cut-off. The micro-concentrator containing the autolysate (2 ml) was centrifuged at 3,000 *g* for 60 min at 4 °C. Small molecules along with the buffer salts passed through the membrane while the larger molecules such as RNases were retained. This procedure concentrated the autolysate from an initial volume of 2–0.2 ml. The concentrated autolysate was readjusted to 2 ml with the addition of sterile distilled water, and used for the assay of RNase.

The RNase assay followed the procedure described by Lindblom [18]. The substrate consisted of yeast RNA (1.5 mg/ml, Boehringer Mannheim 109223, Germany) in 4.75 ml of 0.2 M acetate buffer, pH 5.6, to which 0.25 ml of enzyme solution was added. The mixture was incubated at 50 °C for 30 min and the reaction stopped by addition of 5 ml of ice-cold 0.4% uranylacetate in 12% perchloric acid, followed by cooling in an ice-water bath for 15 min. The mixture was centrifuged at 3,200 *g* for 30 min and the optical density of the supernatant was measured at 260 nm. A control was prepared by incubating the substrate alone under exactly the same conditions, with the enzyme sample being added after the reaction had been stopped. One unit of RNase activity was defined as the activity that produces acid soluble nucleotides to give an increase of 0.01 in absorbance at 260 nm under the described assay conditions.

Separation and quantification of RNA degradation products

RNA degradation products in autolysates were separated and quantified by two HPLC procedures [39, 40]. Briefly, nucleotides were separated by reversed-phase ion-pairing HPLC using a Waters μBONDAPAK C18 10 μ stainless steel column (30 cm×3.9 mm I.D). Elution was performed with a gradient system consisting of a mixture of eluent A (50 mM K_2HPO_4, 5 mM Waters PIC A, pH 5.45) and eluent B (100% methanol). Elution was commenced with eluent A, and the ratio of eluent B was increased from 0% at the start to 10% over 10 min. Injection volume was 20 μl and flow rate was 1.5 ml/min. Detection was done with a Waters (Model 440) UV-detector set at 254 and 280 nm.

Nucleosides and bases were separated by reversed-phase HPLC using a Waters Resolve C18 5 μ Radial-Pak cartridge (100 mm×8 mm I.D) equipped with a Waters RCM-100 cartridge holder. Elution was performed with a gradient system consisting of eluent A (0.02 M K_2HPO_4, pH 6.30) and eluent B [60% methanol in water (v/v)]. Elution was commenced with eluent A and the ratio of eluent B was increased from 0% at the start to 40% over 18 min. The injection volume was 20 μl and the flow rate was 3.0 ml/min. Detection was done as described already.

Separated compounds were quantified by comparing their peak areas with standard curves which were obtained by injection of individual nucleotides, nucleosides and bases (US Biochemical Corporation, Cleveland, OH, USA) into the HPLC systems.

Assays for RNA, RNase activity and RNA degradation products were done in triplicate with the mean values and standard error of means being reported.

Results

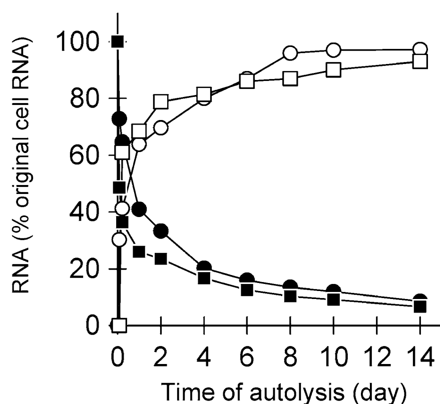
Changes in RNA content

The initial RNA content of the yeast cells was about 7% of the cell dry weight, which decreased by about 95% over a period of 14 days of autolysis (Fig. 1). The decrease occurred mostly during the first 2 days. For example, more than 30% of the original cell RNA was lost after 5 h of autolysis at 30 °C. After 2 days, less than 35% of the RNA was found remaining inside the cells.

The decrease in cell RNA was affected by autolysis temperature, with higher temperatures giving a more rapid decline (Fig. 1a). After 5 h of autolysis, RNA content of the cells had decreased by about 35% at 30 °C, 43% at 40 °C, 60% at 50 °C and 64% at 60 °C (data for autolysis at 40 and 50 °C not shown). Approximately 90% decrease in cell RNA had occurred after autolysis for 8 days at 60 °C, 10 days at 50 °C and 40 °C and 14 days at 30 °C (Fig. 1a).

The decrease of cell RNA was affected by pH (Fig. 1b). Within the first 24 h, the greatest decrease in RNA was observed at pH 7.0, where about 66% of the RNA was lost. Corresponding values for pH 4.0, pH 5.0 and pH 6.0 were 55, 64 and 57%, respectively (data for autolysis at pH 5.0 and 6.0 not shown). Thereafter, the decrease in cell RNA was most rapid at pH 4.0, followed by pH 5.0 and pH 7.0, while the decrease of RNA at pH 6.0 was the slowest. About 90% reduction in cellular RNA had occurred after autolysis for 8 days at pH 4.0, 10 days at pH 5.0 and 7.0 and 14 days at pH 6.0 (Fig. 1b).

The RNA released from the cells was recovered in the autolysates as its degradation products, which were found to be 4–8% greater than predicted by the initial amount of RNA in the cells (Fig. 1). As explained later in the Discussion section, this was due to contributions from the pool of cellular nucleotides and DNA that were released into the autolysates.



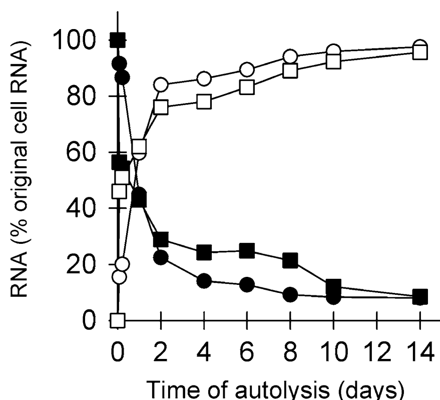


Fig. 1 Changes in RNA content in the cells and autolysates of *Saccharomyces cerevisiae* during autolysis at different temperatures (**a**) and pH values (**b**). For (**a**), cell RNA at: (*filled circle*) 30 °C, (*filled square*) 60 °C; RNA material in autolysate at: (*open circle*) 30 °C, (*open squre*) 60 °C. For (**b**), cell RNA at: (*filled circle*) pH 4.0, (*filled square*) pH 7.0; RNA material in autolysate at: (*open circle*) pH 4.0; (*open square*) pH 7.0

RNase activity

RNase activity was detected in autolysing yeast cells (Table 1). In general, the activity increased until day 5 and then declined, except for autolysis at 60 °C where the activity in the cells progressively decreased with time and gave the lowest values. Initially, RNase activity could not be detected in the autolysates due to its inhibition by the presence of low molecular weight (<10,000 Da) compounds. When these compounds were removed by ultrafiltration, using membranes with a molecular cut-off of 10,000 Da, RNase activity was then detected in the autolysates. The activity increased until day 5, and then declined. The activity, also, was sensitive to the higher temperature of 60 °C.

Composition of RNA degradation products

Tables 2 and 3 show the general composition of RNA degradation products after autolysis for 1 and 10 days, while Fig. 2 shows their concentrations in autolysates obtained under a typical set of conditions (40 °C, pH 7.0). Ribonucleotides were by far the most predominant components for all autolysis conditions (except 60 °C), accounting for 65–98% of the products. Comparatively, the levels of ribonucleosides and nucleobases were much lower, accounting for only 3–14% and 0.7–5% of the products, respectively. The levels of oligonucleotides (plus some di- and trinucleotides) were also low for most autolysis conditions except 60 °C (pH 7.0), where they were the prevalent components, accounting for 62–67% of the products.

The composition of RNA degradation products varied with autolysis temperature, especially at 60 °C. There was much less degradation of RNA into nucleotides and nucleosides at this temperature than at 30–50 °C. At 30 °C, there was slightly less degradation of RNA into these products than at 40 and 50 °C (Table 2). The influence of pH on autolysis was less pronounced. Similar degradation of RNA into nucleotides and nucleosides was evident at pH 4.0–7.0, but the data did not show clear influence of pH on the general composition of the degradation products (Table 3).

Table 1 RNase activity during the autolysis of *Saccharomyces cerevisiae*

Source of RNase activity	Autolysis Time (days)	Autolysis conditions							Maximum SEM[a]
		pH 7				40 °C			
		30 °C	40 °C	50 °C	60 °C	pH 4	pH 5	pH 6	
Cellular	1	31.1	36.4	32.3	35.8	26.0	31.6	24.3	6.1
	5	47.0	47.6	39.3	26.0	46.5	55.3	42.9	7.3
	10	23.6	28.3	19.5	9.1	26.1	29.5	32.4	2.4
Autolysate[b]	1	11.0	27.2	18.9	15.2	21.4	21.8	18.4	2.0
	5	36.7	24.4	30.1	24.2	35.7	36.2	39.1	4.1
	10	23.5	18.1	26.3	7.0	23.0	26.4	28.6	1.7

One unit of RNase activity was defined as the activity that produces acid-soluble nucleotides to give an increase of 0.01 in absorbance at 260 nm under the described incubation conditions (see Materials and methods)

[a]Maximum standard error of means for data in the row
[b]Activity determined after ultrafiltration of autolysates using membranes with a molecular cut-off of 10,000 Da

Table 2 Composition of RNA degradation products in autolysates of *S. cerevisiae* after autolysis for 1 and 10 days at different temperatures, pH 7.0

Degradation product	Composition (percentage of total degradation products)								Maximum SEM[a]
	30 °C		40 °C		50 °C		60 °C		
	Day 1	Day 10	Day 1	Day 10	Day 1	Day 10	Day 1	Day 10	
Ribonucleotides	81.9	86.6	94.7	86.1	88.8	98.3	34.2	28.3	4.6
Ribonucleosides	7.1	7.7	8.7	9.5	8.8	8.8	3.4	3.3	0.6
Bases	1.6	3.5	2.9	4.0	1.4	1.8	0.9	1.0	0.4
Others[b]	9.4	2.2	ND	0.4	1.0	ND	61.5	67.4	3.2

ND not detected

[a]Maximum standard error of means for data in the row

[b]These included oligonucleotides and di- and trinucleotides calculated as: Total RNA materials − (ribonucleotides + ribonucleosides + bases)

Table 3 Composition of RNA degradation products in autolysates of *S. cerevisiae* after autolysis for 1 and 10 days at different pH values, 40 °C

Degradation product	Composition (percentage of total degradation products)								Maximum SEM[a]
	pH 4.0		pH 5.0		pH 6.0		pH 7.0		
	Day 1	Day 10	Day 1	Day 10	Day 1	Day 10	Day 1	Day 10	
Ribonucleotides	64.7	88.2	68.4	75.7	72.2	82.3	94.7	86.1	5.1
Ribonucleosides	14.3	11.8	4.6	10.1	4.7	9.0	8.7	9.5	1.2
Bases	0.7	0.5	2.9	5.1	0.7	2.0	2.9	4.0	0.6
Others[b]	21.7	ND	24.1	9.1	22.5	6.7	ND	0.4	1.4

ND not detected

[a]Maximum standard error of means for data in the row

[b]These included oligonucleotides and di- and trinucleotides calculated as: Total RNA materials − (ribonucleotides + ribonucleosides + bases)

Ribonucleotides

Tables 4 and 5 show the concentration of ribonucleotides after autolysis for 1 and 10 days. 3′-Ribonucleotides were by far the most predominant compounds for all autolysis conditions studied, accounting for 50–94% of total ribonucleotides. The levels of 5′- and 2′-isomers were much lower in comparison, representing only 3–21% and 3–29% of total ribonucleotides, respectively.

The composition of ribonucleotides in the autolysates varied with temperature (Table 4). Autolysis at 40 and 50 °C was more conducive to the formation of 5′-nucleotides than at 30 and 60 °C. At day 10, for example, the concentration of 5′-nucleotides at 40 °C was more than fourfold higher than that at 30 °C. The formation of individual 5′-ribonucletides was also affected by temperature, and the highest concentrations of 5′-AMP and 5′-GMP were found in autolysates produced at 50 °C and 40 °C, respectively.

Autolysis at higher temperatures gave lower concentrations of 3′-ribonucleotides. Of the four 3′-ribonucleotides, 3′-GMP was the most abundant for all autolysis temperatures, while 3′-UMP was the least abundant except for autolysis at 60 °C, where 3′-AMP was present at the lowest concentration. The concentration of 2′-ribonucleotides was highest at 50 °C, followed by 40 °C and 30 °C, and lowest at 60 °C. Of the four 2′-ribonucleotides, 2′-GMP was by far the most prevalent, while 2′-AMP was the least abundant in most cases.

The formation of 5′-ribonucleotides was highest at pH 7.0, and least at pH 5.0 (Table 5), but for individual components within this group, pH and time of autolysis gave no consistent trends. For example, the highest concentrations of 5′-AMP, 5′-GMP and 5′-UMP were found at pH 6.0 (day 1), pH 4.0 (day 10) and pH 7.0 (day 10), respectively. The production of 3′-ribonucleotides was greatest at pH 5.0. The predominant 3′-nucleotide was 3′-CMP at day 10, pH 4.0 (71.2 mg/ml), followed by 3′-UMP at day 1, pH 5.0 (61.8 mg/ml) and 3′-GMP at day 10, pH 6.0 (60.5 mg/ml). The production of 2′-ribonucleotides was greatest at pH 7.0, followed by pH 6.0, and least at pH 5.0. 2′-GMP was the most predominant 2′-nucleotides at pH 4.0–6.0, while 2′-UMP was more prevalent at pH 7.0.

The concentrations of ribonucleosides and nucleobases in the autolysates were also determined (data not shown). Uridine was the most abundant ribonucleoside under most of the autolysis conditions studied, followed by adenosine. The concentrations of the other two

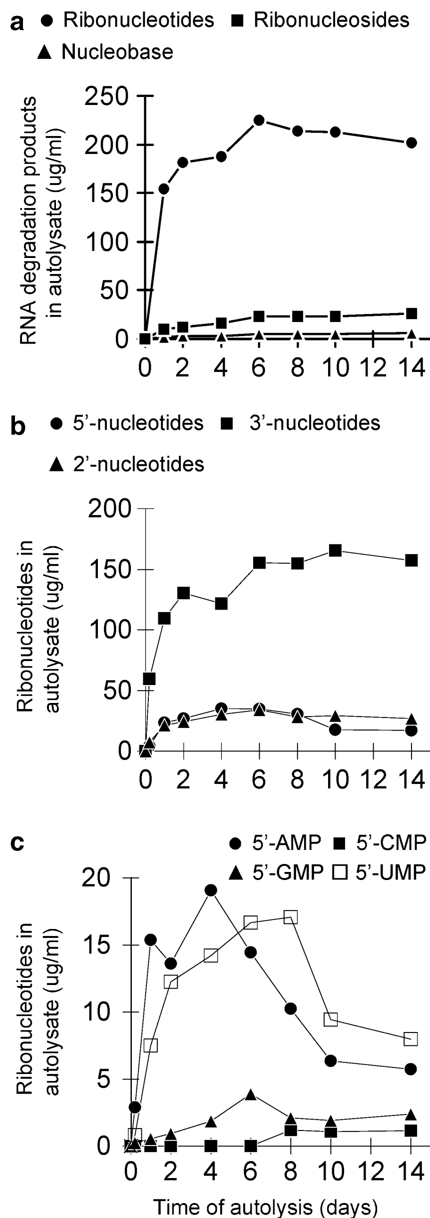


Fig. 2 Changes in the concentration of RNA degradation products in the autolysates of *S. cerevisiae* during autolysis at 40 °C, pH 6.0. 4.2 mg/ml of cells (dry weight) was used for autolysis

ribonucleosides, cytidine and guanosine, were much lower in comparison. Production of ribonucleosides was greatest at 50 °C, followed by 40 °C and 30 °C, and least at 60 °C. In general, higher pH values (6.0 and 7.0) favoured the formation of uridine while lower pH values (4.0 and 5.0) gave more of the other three nucleosides.

Of the four purine and pyrimidine bases, uracil was the most predominant for all the autolysis conditions. The concentrations of the other three nucleobases were much smaller in comparison. Autolysis at lower temperatures (30 °C and 40 °C) gave higher concentrations of uracil than at higher temperatures (50 °C and 60 °C), while autolysis at pH 5.0 produced more of this base than at the other pH conditions.

Discussion

In a previous report [41], we described the general kinetics of cell death, protein release and nucleic acid degradation during autolysis of *S. cerevisiae* at different pH and temperature conditions. Briefly, it was found that yeast cells undergoing autolysis lost their viability within 48 h with a resultant release of protein and nucleic acid materials into the extracellular environment. The present study confirmed that degradation of RNA is a key reaction of yeast autolysis which is enhanced with an increase in temperature between 30 °C and 60 °C. This result is broadly consistent with our previous findings on the autolytic degradation of RNA in three yeast species [12] as well as several reports in the early literature on baker's yeasts [13, 33–36]. Autolytic breakdown of cell RNA was confirmed by agarose gel electrophoresis of RNA extracted from autolysing cells (electrophoretograms not shown). The RNA materials that leaked into the autolysates were also examined by gel electrophoresis, but large RNA fragments were detected only in autolysates produced at 60 °C (pH 7.0). They appeared as a diffuse band with molecular sizes less than 600 bp (which converts to approximately 180,000 Da). This observation was consistent with polynucleotides predominating in autolysates at 60 °C (Table 2) and was likely due to the reduced thermal stability of RNases at this temperature as shown in Table 1.

The RNA lost from the yeast cells was recovered in autolysates as degradation products. However, as measured by the guanine-HPLC procedure, the recovery was 4–8% more than that predicted from the initial RNA content in cells. This was attributable to interferences by DNA degradation products and the pool of free nucleotides of the yeast cell (e.g. ADP and ATP) released into the autolysates. Measurement of RNA by the guanine-HPLC method is based upon determining its guanine content. DNA degradation products and some of the free nucleotides of the cell contain guanine and, therefore, would be included in the measurement. DNA degradation products and the free nucleotides were estimated to constitute about 2–3% [12, 41] and 5% [34] of the nucleic acid materials in autolysates, respectively.

Cells of *S. cerevisiae* contain an array of nucleic acid-hydrolysing enzymes, including endo-ribonucleases, exo-ribonucleases, nucleotidases and nucleosidases (reviewed by Zhao [38]), all of which could be involved in the autolytic degradation of RNA [1, 23]. Endo-nucleases hydrolyse the phosphodiester bond between adjacent ribose units within the polymer to produce poly- and oligo-ribonucleotides. Exo-nucleases hydrolyse this bond in a step-wise manner from one end of the polymer to produce mono-ribonucleotides. For both types of enzymes, there are sub-classes that hydrolyse at either the 5′ or 3′ side of the phosphate, generating ribonucleotides with either a 3′- or a 5′-phosphate group, respectively. For the endo-nucleases, the base type may

Table 4 Concentration of ribonucleotides in autolysates of *S. cerevisiae* after autolysis for 1 and 10 days at different temperatures, pH 7.0

Degradation product	Concentration in autolysate (μg/mL)								Maximum SEM[a]
	30 °C		40 °C		50 °C		60 °C		
	Day 1	Day 10	Day 1	Day 10	Day 1	Day 10	Day 1	Day 10	
Total 5′-ribonucleotides	3.9	9.1	16.1	37.2	25.8	27.5	9.0	11.1	2.4
5′-AMP	1.1	3.3	1.3	3.0	17.7	19.6	5.9	10.1	1.0
5′-CMP	1.5	2.3	0.8	1.2	ND	1.9	1.5	ND	0.2
5′-GMP	1.2	3.5	2.5	14.0	2.7	3.5	ND	0.2	0.6
5′-UMP	ND	ND	1.9	19.0	5.4	5.4	1.6	0.9	0.6
Total 3′-ribonucleotides	94.6	140.5	93.6	108.5	80.3	95.5	34.8	28.1	8.8
3′-AMP	32.3	29.5	34.7	28.3	13.9	17.3	3.0	3.3	2.0
3′-CMP	7.9	31.0	18.7	26.2	7.5	16.8	3.2	4.3	1.6
3′-GMP	47.7	68.2	33.1	46.1	47.0	62.1	22.1	16.1	3.5
3′-UMP	6.7	11.7	7.0	8.1	11.9	16.3	5.9	4.5	0.7
Total 2′-ribonucleotides	22.5	30.1	28.2	38.8	38.9	62.0	14.5	17.0	5.2
2′-AMP	1.6	ND	3.7	1.8	1.3	6.5	ND	ND	0.4
2′-CMP	2.9	3.6	2.1	3.8	5.9	5.8	1.2	1.6	0.4
2′-GMP	16.0	23.3	8.5	14.5	31.8	53.8	10.8	11.6	3.6
2′-UMP	2.0	3.9	13.9	15.8	ND	ND	2.5	3.8	0.8

ND not detected
[a]Maximum standard error of means for data in the row

Table 5 Concentration of ribonucleotides in autolysates of *S. cerevisiae* after autolysis for 1 and 10 days at different pH values, 40 °C

Degradation product	Concentration in autolysate (μg/mL)								Maximum SEM[a]
	pH 4.0		pH 5.0		pH 6.0		pH 7.0		
	Day 1	Day 10	Day 1	Day 10	Day 1	Day 10	Day 1	Day 10	
Total 5′-ribonucleotides	14.5	33.4	5.1	10.1	23.4	18.8	16.1	37.2	2.7
5′-AMP	7.2	11.7	0.4	0.7	15.4	6.4	1.3	3.0	0.4
5′-CMP	1.4	2.5	ND	1.4	ND	1.1	0.8	1.2	0.2
5′-GMP	4.9	17.2	2.7	3.9	0.6	1.9	2.5	14.0	1.1
5′-UMP	1.1	2.2	2.1	4.1	7.5	9.4	1.9	19.0	1.0
Total 3′-ribonucleotides	55.7	147.2	153.0	183.8	109.4	165.5	93.6	108.5	12.3
3′-AMP	10.9	29.2	31.7	36.6	52.0	52.7	34.7	28.3	3.5
3′-CMP	25.2	71.2	32.8	41.1	15.1	18.1	18.7	26.2	3.9
3′-GMP	9.3	28.7	26.8	47.8	26.2	60.5	33.1	46.1	2.3
3′-UMP	10.3	18.1	61.8	58.3	16.1	34.1	7.0	8.1	2.6
Total 2′-ribonucleotides	4.9	17.4	4.4	7.6	21.1	29.2	28.2	38.8	2.5
2′-AMP	ND	ND	ND	0.4	ND	ND	3.7	1.8	0.3
2′-CMP	ND	4.6	ND	ND	1.3	1.8	2.1	3.8	0.3
2′-GMP	4.9	12.8	4.4	7.2	17.1	22.7	8.5	14.5	1.0
2′-UMP	ND	ND	ND	ND	2.7	4.7	13.9	15.8	0.9

ND not detected
[a]Maximum standard error of means for data in the row

determine which phosphodiester linkage is cleaved. Ribonucleotidases act on the nucleotides to liberate phosphate and the nucleoside. Finally, nucleosidases hydrolyse the nucleosides to liberate nucleobases and ribose [1, 23]. As a consequence of the number and specificity of the enzymes involved, there is likely to be a substantial diversity in the products of RNA degradation, and this is reflected in the products shown in Tables 2, 3, 4, 5.

The predominance of ribonucleotides suggests that endo-nucleases and exo-nucleases were principally responsible for the degradation of RNA. A similar observation was made by Ohta et al. [25] about the autolysis of *Candida utilis*. However, at the higher temperature of 60 °C and some pH values (pH 5.0 and 6.0), increased concentrations of poly- or oligonucleotides were found, indicating lesser contributions from the exonucleases. Higher proportions of ribonucleosides were found with autolysis at 30–50 °C, suggesting that nucleotidases may be more active at these temperatures. Nucleosidases appear not to be very active in autolytic degradation of RNA since we could detect only low concentrations of nucleobases.

The predominance of 3′-ribonucleotides in RNA degradation products of *S. cerevisiae* (Table 5) is consistent with observations of RNA degradation in other yeasts [24, 25]. Nakao et al. [25] studied the autolysis of *S. miso* and found that 3′-ribonucleotides were the pre-

dominant isomers at acidic conditions (pH 4.0–5.0) whereas 5′-ribonucleotides were more predominant at alkaline conditions (pH 9.0). In our study, the proportion of 3′-ribonucleotides in the total RNA degradation products was higher when autolysis was conducted at pH 4.0 and 5.0 than at pH 7.0. These results suggest that activities of different RNA degrading enzymes are influenced by environmental factors such as pH. Manipulation of these factors may allow autolysates with desired amounts of particular ribonucleotides to be produced.

The predominance of 3′-ribonucleotides in the degradation products has practical implications. Only 5′-nucleotides (e.g. 5′-GMP) have flavour-enhancing properties [9, 22, 23]. If autolysis is employed to produce flavouring materials, the predominance of 3′-isomers would mean that most of the raw material (RNA) is wasted. In this regard, it might be necessary to identify and use inhibitors that specifically inhibit 3′-nucleotide-forming RNases, and this topic is worthy of further study.

The autolytic formation of 2′-ribonucleotides was unexpected. A possible pathway for the formation of these isomers is through 2′,3′-cyclic mononucleotides as the intermediates, which could then be converted to either 3′- or 2′-ribonucleotides. RNases producing 2′,3′-cyclic nucleotides have been found in *S. cerevisiae* [28], and 2′-ribonulceotides have been reported in beer [26], but it is not known if these products were from yeast metabolism or from autolytic RNA degradation. 2′-Ribonucleotides were identified by matching their retention times with those of standard 2′-ribonucleotides. However, it remains a possibility that these peaks were actually 2′,3′-cyclic mononucleotides. So it is necessary to obtain further evidence about the presence of 2′-ribonucleotides in autolysates, probably by co-eluting standard 2′-ribonucleotides with corresponding 2′,3′-cyclic mononucleotides. Until such study is done, the formation of 2′-isomers of ribonucleotides during the autolytic degradation of RNA can be regarded only as tentative.

The presence of ribonucleosides and nucleobases means that some nucleotides were further degraded during autolysis. The same secondary degradation has also been reported by other researchers [25, 37]. The enzymatic activity responsible for this secondary degradation of RNA was not measured in our study and so remains a subject for further investigation. Understanding the mechanisms of these reactions is of practical value, as further degradation of nucleotides into nucleosides or bases means that some of the flavour-enhancing products are lost.

In summary, RNA was degraded during autolysis of yeast with consequent production of predominantly ribonucleotides, and lesser amounts of poly- and oligoribonucleotides, ribonucleosides and bases. Judging from the diversity of the products formed, a wide array of RNA-degrading enzymes must be involved in the reactions. Research is needed to identify the roles of specific enzymes in the reactions and factors that affect their relative activities. Such information would help to control the composition of nucleic acid degradation products in yeast autolysates as commercially produced for the food industry.

References

1. Adams RLP, Knowler JT, Leader DP (1993) The biochemistry of the nucleic acids, 11th edn. Chapman & Hall, London
2. Arnold WN (1981) Autolysis. In: Arnold WN (ed) Yeast cell envolopes: biochemistry, biophysics and ultrastructure. CRC, New York, pp 129–137
3. Babayan TL, Bezrukovv MG (1985) Autolysis of yeasts. Acta Biotechnol 5:129–136
4. Charpentier C, Feuillat M (1993) Yeast autolysis. In: Fleet GH (ed) Wine microbiology and biotechnology. Harwood Academic, Chur, pp 225–242
5. Charpentier C, Dos Santos AM, Feuillat M (2004) Release of macromolecules by *Saccharomyces cerevisiae* during ageing of French sherry wine “Vin jaune”. Int J Food Microbiol 96:253–262
6. Connew SJ (1998) Yeast autolysis. A review of current research. Aust N Z Wine Ind J 13(1):61–64
7. Dziezak JD (1987) Yeast and yeast derivatives: definitions, characteristics, and processing. Food Technol 41(2):104–125
8. Farrer KTH (1956) The autolysis in yeasts. Food Sci Abstr 28:1–12
9. Günter M (1999) Flavour modifiers. In: Ashust PR (ed) Food Flavorings, 3rd edn. Aspen, Gaithersburg, pp 367–406
10. Halasz A, Lásztity R (1991) Use of yeast biomass in food production. CRC Press, Boca Raton
11. Herbert D, Phipps DJ, Strange RE (1971) Chemical analysis of microbial cells. In: Norris JR, Ribbons DW (eds) Methods in microbiology, vol 5B. Academic, London, pp 209–344
12. Hernawan T, Fleet GH (1995) Chemical and cytological changes during the autolysis of yeasts. J Ind Microbiol 14:440–450
13. Hough MD, Maddox IS (1970) Yeast autolysis. Proc Biochem 5:50–52
14. Huang YT, Kinsella JE (1986) Phosphorylation of yeast protein: reduction of ribonucleic acids and isolation of yeast protein concentrate. Biotechnol Bioeng 28:1690–1698
15. Joslyn MA (1955) Yeast autolysis. Chemical and cytological changes involved in autolysis. Wallerstein Lab Commun 18:107–119
16. Joslyn MA, Vosti DC (1955) Yeast autolysis. Factors influencing the rate and extent of autolysis. Wallerstein Lab Commun 18:191–210
17. Kinsella JE (1986) Functional proteins from yeast nucleoprotein for food uses: method for isolation. In: Knorr D, Dekker M (eds) Food biotechnology. Marcel Dekker, New York, pp 363–391
18. Lindblom M (1977) Properties of intracelullar ribonuclease utilised for RNA reduction in disintegrated cells of *Saccharomyces cerevisiae*. Biotechnol Bioeng 19:199–210
19. Martinez-Rodriguez AJ, Polo MC (2000) Characterization of the nitrogen compounds released during yeast autolysis in a model wine system. J Agric Food Chem 48:1081–1085
20. Martinez-Rodriguez AJ, Carrascosa AV, Polo MC (2001) Release of nitrogen compounds to the extracellular medium by three strains of *Saccharomyces cerevisiae* during induced autolysis in a model wine system. Int J Food Microbiol 64:155–160
21. Masschelein CA (1986) Centenary review: the biochemistry of maturation. J Inst Brew 92:213–219
22. Nagodawithana T (1992) Yeast-derived flavors and flavor enhancers and their probable mode of action. Food Technol 46(11):138–140
23. Nagodawithana T (1993) Enzymes associated with savory flavor enhancement. In: Nagodawithana T, Reed G (eds) Enzymes in food processing, 3rd edn. Academic, New York, pp 401–421
24. Nakao Y, Imada A, Wada T, Ogata K (1964) Degradation of nucleic acids and their related compounds by microbial enzymes. Part XVI. Excretion of mononucleotides by yeasts. Agric Biol Chem 28:151–159

25. Ohta S, Maul S, Sinskey AJ, Tannenbaum SR (1971) Characterisation of a heat-shock process for reduction of the nucleic acid content of *Candida utilus*. Appl Microbiol 22:415–421
26. Qureshi AA, Prentice N, Burger WC (1979) Quantitation of potential flavouring compounds in worts and beers by HPLC. Inst Am Soc Brew Chem 37:154–160
27. Stam H, Hoogland M, Laane C (1998) Food flavours from yeast. In: Wood BJ (ed) Microbiology of fermented foods, vol 2, 2nd edn. Blackie Academic & Professional, London, pp 505–542
28. Stevens A (1985) Pyrimidine-specific cleavage by an endoribonuclease of *Saccharomyces cerevisiae*. J Bacteriol 164:57–62
29. Suomalainen H (1975) Some enzymological factors influencing the leavening capacity and keeping quality of baker's yeast. Eur J Appl Microbiol 1:1–12
30. Takakuwa M, Watanabe Y (1981) Degradation of cellular phospholipids and softening of pressed baker's yeast. Agric Biol Chem 45:2167–2173
31. Todd BE, Fleet GH, Henschke PA (2000) Promotion of autolysis through the interaction of killer and sensitive yeasts: potential application in sparkling wine production. Am J Enol Vitic 51(1):65–72
32. Todd B, Zhao J, Fleet GH (1995) HPLC measurement of guanine for the determination of nucleic acids (RNA) in yeasts. J Microbiol Methods 22:1–10
33. Trevelyan WE (1977) Induction of autolytic breakdown of RNA in yeast by addition of ethanol and by drying/rehydration. J Sci Food Agric 28:579–588
34. Trevelyan WE (1978) Effect of procedures for the reduction of the nucleic acid content of SCP on the DNA content of *Saccharomyces cerevisiae*. J Sci Food Agric 29:903–908
35. Vosti DC, Joslyn MA (1954a) Autolysis of baker's yeast. Appl Microbiol 2:70–78
36. Vosti DC, Joslyn MA (1954b) Autolysis of several pure culture yeasts. Appl Microbiol 2:79–90
37. Watanabe S, Osawa T, Yamamoto S (1968) Studies on enzymatic action for nucleic acid components in microorganisms. II. Excretion of nucleotides from yeast cells and action of surfactant. J Ferment Technol 46:538–544
38. Zhao J (1995) Degradation of nucleic acids during the autolysis of *Saccharomyces cerevasiae*. PhD Thesis. The University of New South Wales
39. Zhao J, Fleet GH (1996) Separation of 20 isomers of ribonucleotides and deoxyribonucleotides by reversed-phase ion-pairing high-performance liquid chromotography. J Chromotogr A 732:271–275
40. Zhao J, Todd BE, Fleet GH (1994) Separation of ribonucleotides, ribonucleosides, deoxyribonucleotides, deoxyribonucleosides and bases by reversed-phase high-performance liquid chromotography. J Chromotogr A 673:167–171
41. Zhao J, Fleet GH (2002) Degradation of DNA during the autolysis of *Saccharomyces cerevisiae*. J Ind Microbiol Biotechnol 30:175–182