are distributed in all of the foods investigated. It is interesting to point out that mushrooms and beans contained appreciable amounts of spermidine because mutagenicity of the nitrosamines formed from spermidine has been proved as described previously. To our knowledge, this finding of the presence of large amounts of spermidine in mushrooms was not reported in the literature.

From this study, it was concluded that the accurate measurements of di- and polyamines in foods can be achieved by using GLC after derivatization. Due to simplicity and reproducibility of the derivative formation reaction, and stability of the derivatives formed, the method reported herein appears to be adequate for both qualitative and quantitative analyses of di- and polyamines in foods, although requiring ion-exchange chromatographic purification.

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Isolation and Identification of Amino Acid Derivatives from Yeast

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N-Acetylalanine and *N*-acetyltyrosine were isolated from baker's yeast. Initially the α-amino acids were quantitiatively removed from a preextract of yeast by the copper–Sephadex method. Further separation was achieved with gas chromatography, ion-exchange chromatography, electrophoresis, and paper chromatography. The isolated compounds were identified by NMR spectroscopy and mass spectrometry, respectively.

A nonprotein fraction from *Saccharomyces cerevisiae* liberating amino acids upon hydrolysis has been described (Hoehn, 1974; Ruediger, 1963). A relationship between the unpleasant taste of yeast samples and the presence of this fraction was established (Hoehn, 1974). Low molecular weight compounds, which release amino acids upon hydrolysis (e.g., peptides and amino acid derivatives), have been shown to be important for taste in foods (Erikson and Fagerson, 1976; Kirimura et al., 1969; Schiffman et al., 1975; Solms, 1969). Especially peptides contribute to bitter, sweet, and sour sensations. They can also contribute to taste-enhancing properties.

Detailed analyses of this nonprotein fraction isolated from *S. cerevisiae* have not been published. This fraction has only been isolated from *Candida utilis* (Miettinen, 1951; Ruediger, 1963; Turba and Esser, 1955) and found to contain mostly peptides, mainly sour ones. There have also been a few reports of the presence of single amino acid derivatives in microorganisms (Hall et al., 1958; Okuhara and Harada, 1971; Nakanishi, 1978; Vandecasteele et al., 1973).

The purpose of this study was to analyze the low molecular weight components from yeast (*S. cerevisiae*) extracts which liberate amino acids upon hydrolysis. The analyses were conducted on the hydrophobic fraction of the extracts because of its particular importance in the taste (Ney, 1972; Wieser and Belitz, 1975).

MATERIALS AND METHODS

Sample. The yeast sample was a *S. cerevisiae* yeast (baker's yeast) obtained from Hefefabrik Hindelbank, Switzerland.

Extraction. A total of 35 L of a 30% baker's yeast suspension was disintegrated by passing the yeast slurry 3 times through a Manton-Gaulin homogenizer (Manton Gaulin Manufacturing Co.,) Inc., Everett, MA) and freeze-dried (Hoehn, 1974).

Five-gram samples of the dry material were extracted 3 times with 90 mL of $CHCl_3$–CH_3OH (2:1 v/v) and filtered. The filtrates were pooled and extracted with 18 mL of water (Harwalkar and Elliott, 1971). After separation, the water layer was filtered through an Amicon ultrafilter (M_r cutoff 1000; Amicon Corp., Oosterhout, The Netherlands). The freeze-dried sample (93 mg) was used for further separation.

Chromatography on Copper–Sephadex. A 5-mg extract, dissolved in 1 mL of borate buffer, was chromatographed on a copper–Sephadex G-25 column as described previously (Rothenbuehler et al., 1979), giving fractions I and II. Samples were percolated over small Dowex A-1 chelating ion-exchange columns in Na^+ form at pH 11 to remove copper.

GC–MS of Derivatives. An aliquot of fraction I was directly derivatized by using the procedure of Niederwieser

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and Steiner (1979) for analysis of peptides as O-trimethylsilylated trifluoroethyl oligoamino alcohols. The derivatives were separated on a 25-m SE-30 glass capillary column (LKB, Bromma, Sweden) by using a Carlo Erba Model 2101 AC gas chromatograph (Carlo Erba, S.p.A., Rodano, Italy) coupled over a platinum capillary directly to a Micromass F-16 mass spectrometer (Vacuum Generators Micromass, Winsford, Great Britain) linked to a DS 2020 data system (Vacuum Generators). Temperatures were as follows: injector, 300 °C; glass capillary column, 100–280 °C, program 4 °C/min; ion source, 215 °C. The helium flow rate was 2 mL/min; the injector split ratio was 1:4. Electron impact spectra were taken at 50 μA and 20 eV; scan time was 1 s. Chemical ionization spectra, with isobutane as a reactant gas, were taken at 200 μA and 50 eV, with a scan time of 1 s and a source temperature of 200 °C.

Chromatography on SP-Sephadex C-25. Fraction I was dissolved in 50 mL of H_2O, the pH adjusted to 3.0, and the solution applied on a 45 × 2.5 cm SP-Sephadex C-25 column in H^+ form. The column was then eluted with 400 mL of H_2O and 800 mL of 0.5 M NH_3 at a rate of 100 mL/h. Ten-milliliter fractions were collected and their absorbance was measured at 254 nm; the fractions were further tested for pentoses and hexoses by the orcinol reaction and, after hydrolysis, for amino acids by the ninhydrin reaction. Three main fractions, IA to IC, were obtained.

Chromatography on Dowex 1 × 8. Fraction IA was dissolved in 20 mL of buffer, pH 9.4, and loaded onto a 60 × 1 cm Dowex 1 × 8 column, which was equilibrated with the same buffer. This buffer was composed of 80 mL of α-picoline, 60 mL of ethylmorpholine, and 40 mL pyridine in 4 L of H_2O, adjusted to pH 9.4 with glacial acetic acid (Schroeder, 1969). The column was sequentially eluted with 40 mL of pH 9.4 buffer, 120 mL of pH 8.4 buffer, and 160 mL of pH 6.4 buffer. These buffers were obtained from pH 9.4 buffer by adding appropriate amounts of glacial acetic acid. Finally, the column was rinsed with 240 mL of 0.5 N and 400 mL of 2.0 N acetic acid. The elution rate was 60 mL/h. Four-milliliter fractions were collected and analyzed with the ninhydrin test after hydrolysis. Four fractions, IA_1 to IA_4, were obtained.

Paper Electrophoresis. Fraction IA_4 was electrophoretically purified. Paper electrophoresis was performed with a Camag HE-5000 electrophoresis unit (Camag AG, Muttenz, Switzerland) on Whatman 3MM paper by using a buffer composed of pyridine–acetic acid–water (25:1:255), pH 6.4 (Clotten and Clotten, 1962). The sample was dissolved in 50 μL of pH 6.4 buffer and separated at constant voltage of 3000 V for 45 min. The spots were visualized under a UV lamp and identified by treatment with chlorine and subsequently with KJ-Starch reagent. The anionic compounds were eluted with 20 mL of 0.2 M NH_3 and dried in vacuo.

Paper Chromatography. Fraction IA_4 was separated by using Whatman 3MM paper and 1-butanol–acetic acid–water–pyridine (15:3:12:10) as a solvent system for paper chromatography (Clotten and Clotten, 1962). The chromatogram was characterized as described above, and the spots were eluted with 0.2 M NH_3 and dried.

HPLC. HPLC was performed with a Waters HPLC unit (Waters Associates, Inc., Koenigstein, West Germany) consisting of two pumps, model 6000, a programmer, 660, an injector, U6K, a detector Perkin-Elmer LC 55, and a column (300 × 4 mm i.d.) packed with 10 μm of octadecylsilyl particles (Nucleosil 10 C-18; Macherey & Nagel, Dueren, West Germany). A linear gradient was applied by using 0.05 M KH_2PO_4 (adjusted to pH 2 with H_3PO_4) as the initial eluent and CH_3OH as the final eluent at a rate of 3 mL/min (Moench and Dehnen, 1977). The gradient was terminated after 180 min. The elution profile was recorded at 220 nm.

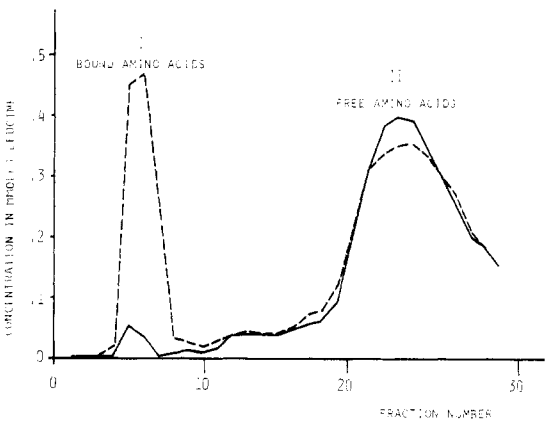

Figure 1. Chromatography of a yeast extract on copper–Sephadex G-25. 0.3 mmol Cu/g of Sephadex; borate buffer, pH 11.0. (—) Before hydrolysis; (---) after hydrolysis.

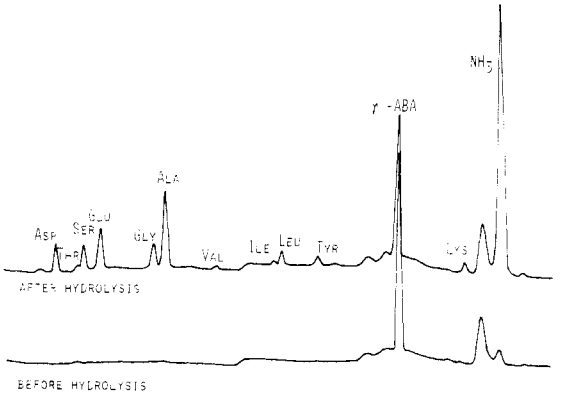

Figure 2. Amino acid analysis of the bound amino acid fraction (I) before and after hydrolysis.

NMR Spectra. The NMR spectra were recorded by using a Varian XL 200 spectrometer (Varian AG, Zug, Switzerland) at 200 MHz.

Ninhydrin Analysis. Ninhydrin analyses were performed before and after hydrolysis with a Technicon Auto Analyzer I.

Orcinol Analysis. Orcinol analyses were performed with a Technicon Auto Analyzer I (Trênel and Dellweg, 1969).

Amino Acid Composition. The amino acid composition was determined according to Werner (1976) with a Biocal BC201 amino acid analyzer (LKB-Biocal, München-Gräfelfing, West Germany).

RESULTS

The isolation of low molecular weight amino acid containing compounds from yeast is complicated by the presence of large amounts of free amino acids. Chromatography of such samples on copper–Sephadex columns showed that small amounts of bound amino acids (fraction I) can be completely separated from free α-amino acids (fraction II), as is shown in Figure 1. It can be seen from Figure 2 that fraction I is free of α-amino acids. Only γ-aminobutyric acid could not be separated by this method. The two chromatograms of the free amino acid fraction II are practically identical (Figure 3), which is a proof for the absence of bound amino acids in this fraction.

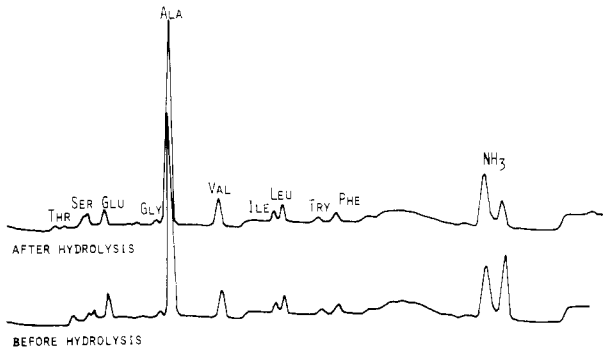

Figure 3. Amino acid analysis of the free amino acid fraction (II) before and after hydrolysis.

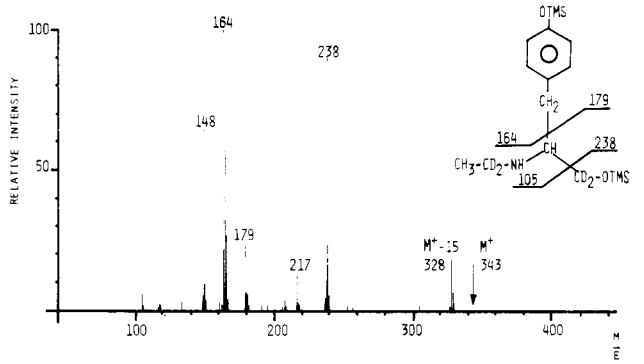

Figure 4. Electron impact mass spectrum of the deuterated derivative of yeast *N*-acetyltyrosine (as O-trimethylsilylated ethyl amino alcohol).

Further separation of the derivatized bound amino acids (fraction I) by gas chromatography yielded one important peak. Figure 4 represents the corresponding mass spectrum, the *O*-trimethylsilyl amino alcohol derivative of *N*-acetyltyrosine. The molecular ion of m/e 343 is not visible in the mass spectrum; it was determined in a parallel experiment by using chemical ionization. Typical for a Me_3Si derivative is the M − 15 fragment with m/e 328. The base peak with m/e 164 corresponds to the loss of a tyrosyl residue with a mass number of 179 from the M^+. Peak m/e 238 arises from the splitting off of the fragment with the mass number 105. m/e 148 and m/e 217 result from the loss of methane from the fragments with the mass number of 164 and 238. The result of this analysis is confirmed by the data from HPLC of fraction IA and an authentic sample of *N*-acetyltyrosine (Figure 5).

In another experiment, the fraction of bound amino acids (I) was chromatographed on a SP-Sephadex column. Three main fractions, IA to IC, were obtained (Figure 6). Table I presents the total content of bound amino acids in fractions IA and IB plus IC, obtained by amino acid analysis before and after hydrolysis. Most of the compounds occur as anions in fraction IA. Alanine and glutamic acid containing compounds are the predominant components in this fraction. Fraction IB consists mainly of inosine. Fraction IC contains γ-aminobutyric acid together with small amounts of bound amino acids. Figure 7 represents the result of the fractionation of fraction IA on a Dowex 1 × 8 anion exchanger, giving four major fractions, labeled as IA_1 to IA_4. Fraction IA_4 turned out to be the main fraction, containing mostly bound alanine and glutamic acid. Fraction IA_4 was further analyzed by paper electrophoresis and paper chromatography, giving two distinct spots. The two compounds were identified as *N*-acetylalanine and pyrrolidonecarboxylic acid by NMR spectroscopy. The spectra were identical with those of authentic samples.

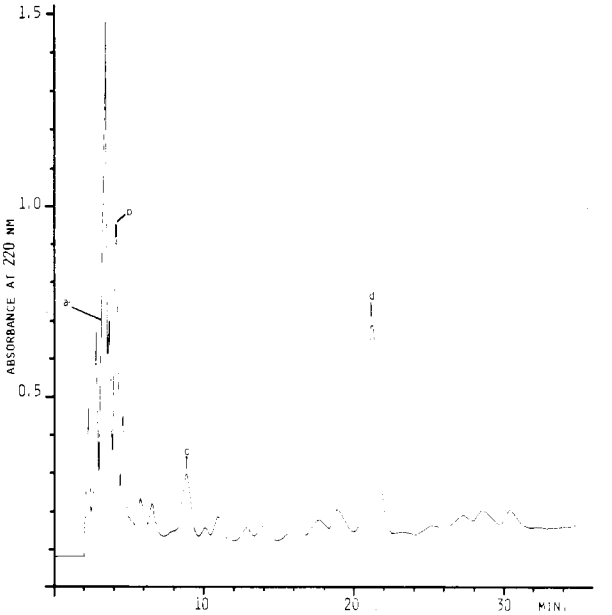

Figure 5. Chromatogram of HPLC of fraction IA. Peaks: (a) pyrrolidonecarboxylic acid; (b) *N*-acetylalanine; (c) inosine; (d) *N*-acetyltyrosine.

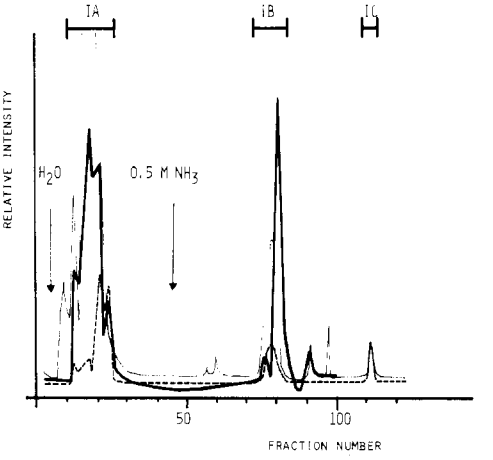

Figure 6. Chromatography of fraction I on a SP-Sephadex C-25 column. (---) Spectrum at λ = 254 nm; (—) ninhydrin test after hydrolysis; (—) orcinol test.

Table I. Amino Acid Analysis of the Covalently Bound Amino Acids in Fractions IA and IB Plus IC from Yeast

	bound amino acids, μmol/100 g of lyophilized yeast	
	fraction IA	fraction IB plus IC
Asp	42.7	
Thr	3.3	
Ser	9.5	6.3
Glu	107.2	8.2
Pro	5.8	6.5
Gly	21.9	6.3
Ala	163.5	11.0
Cys	4.5	
Val	2.9	1.6
Met	25.9	1.5
Ile	1.5	<1.5
Leu	22.8	<1.5
Tyr	22.6	<1.5
Phe	1.6	<1.5
γ-ABA	20.6	>150[a]
His	1.5	<1.5
Lys	9.0	3.1
Arg		<1.5

[a] Free amino acid.

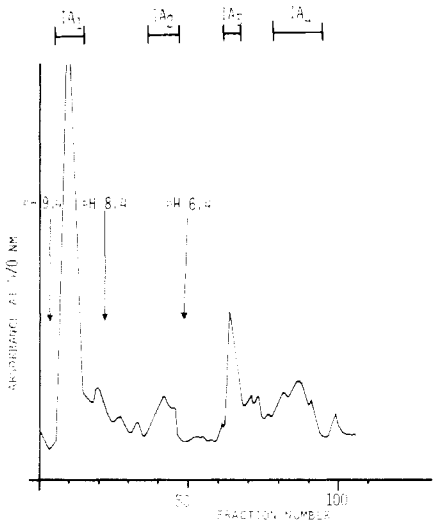

Figure 7. Chromatography of fraction IA on a Dowex 1 × 8 column.

Fraction IA was also analyzed by HPLC (Figure 5). The results confirm the presence of the above-described compounds. Compounds a–d, shown in Figure 5, are responsible for the release of glutamic acid, alanine, glycine, and tyrosine during hydrolysis of yeast samples (compare Figure 2 and Table I).

DISCUSSION

The present paper shows that *N*-acetyl amino acids represent a considerable proportion of the hydrophobic, low molecular amino acid containing compounds in *S. cerevisiae*. *N*-Acetylalanine represents about a third of this fraction (Table I). *N*-Acetyltyrosine occurs to a smaller extent. This is the first report of its presence in this microorganism. Pyrrolidonecarboxylic acid and inosine were isolated and probably were the compounds responsible for glutamic acid and glycine release during hydrolysis. Our analyses show also the presence of additional bound amino acids in smaller amounts (Figure 2 and Table I).

Acetyl amino acids are known to occur in microorganisms and in the central nervous system of different animals and man (Auditore and Wade, 1971; Hall et al., 1958; Okuhara and Harada, 1971; Tallan et al., 1956; Nakanishi, 1978). *N*-Acetlalanine and *N*-acetylglycine were found to be formed from glucose by *Candida tropicalis* (Okuhara and Harada, 1971). Schreier and Drawert (1977) found *N*-acetylalanine in the flavor of wine, apparently due to added yeast. Other authors (Miettinen, 1951; Ruediger, 1963; Turba and Esser, 1955) mentioned compounds in *Candida utilis* which released alanine, glycine, and glutamic acid during hydrolysis. From their experiments they concluded acidic peptides occur as intermediates in protein synthesis. During our investigations there was no indication of significant amounts of low molecular weight, hydrophobic peptides in yeast extracts.

In vitro and in vivo experiments with microorganisms and mammals showed that *N*-acetylamino acids are enzymatically formed from the correspondent free amino acids (Goldstein, 1959; Paik and Kim, 1964; Zenk and Schmitt, 1965). The metabolic importance and functional importance of these compounds are still obscure. Auditore and Wade (1971) discuss the possible role as regulator of acetyl group concentration in the cell or as neurotransmitter in the central nervous system.

N-Acetyl amino acids are known to have taste properties (Schiffman et al., 1975). *N*-Acetyl-L-alanine, as an example, has a sour taste; *N*-acetyl-L-tyrosine tastes bitter at neutral pH. The isolated amino acid containing compounds, together with *N*-acetylglutamic acid, known to occur in yeast (Hall et al., 1958), could contribute to the characteristic taste of baker's yeast.

There is little known about *N*-acetyl amino acids in connection with food (Nakanishi, 1978; Vandecasteele et al., 1973). Vandecasteele et al. (1973) produced fermentatively *N*-acetyllysine by *Corynebacterium A20* in an attempt to use this amino acid derivative as a lysine source in food. *Corynebacterium glutamicum* is known to produce *N*-acetylglutamine, depending on the conditions in the medium (Nakanishi, 1978). These and other examples (Berlinguet and Laliberte, 1970; Stadtman et al., 1952) show that the amount of *N*-acetyl amino acids present in the cell is strongly influenced by the conditions prevailing in the system and that the amount can be manipulated, depending on the actual purpose.

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