**Table I. Photoproducts and Quantum Yields of Unsymmetrical PCB's in Cyclohexane**

| PCB | $\phi_r$ | Product | $T_r$, min | % yield[a] |
|---|---|---|---|---|
| 2,4,6-Trichloro-biphenyl (I) | 0.02 | 2,4-Dichloro | 1.25 | 85 |
| | | 4-Chloro | 0.85 | 15 |
| 2,4,5-Trichloro-biphenyl (II) | 0.05 | 3,4-Dichloro | 1.80 | 98 |
| | | 4-Chloro | 0.85 | 2 |
| 2,3,4,5-Tetrachloro-biphenyl (III) | 0.04 | 3,4,5-Trichloro | 3.60 | 95 |
| | | 3,4-Dichloro | 1.80 | 5 |
| 2,3,5,6-Tetrachloro-biphenyl (IV) | <0.01 | 2,3,5-Trichloro | 2.20 | 50 |
| | | 3,5-Dichloro | 1.50 | 50 |
| 2′,3,4-Trichloro-phenyl (V) | 0.02 | 3,4-Dichloro | 1.80 | 100 |

[a] Based on total product formation.

At the present time we are investigating the possibility that this effect may be transmitted from a para chlorine on ring 1 of a terphenyl to an ortho chlorine on ring 3, in order to elucidate the excited geometry of the terphenyl triplet.

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# Amino Acid Composition of Protein Isolates from *Saccharomyces fragilis*

The amino acid compositions of both the intact cells of the yeast, *Saccharomyces fragilis*, grown batchwise and continuously on crude lactose, and of extracted protein were determined. The composition of whole cells of *S. fragilis* grown under different conditions during batch and continuous cultivations was quite similar and the content of lysine and leucine was very high. The concentrations of amino acids in yeast protein isolates varied with different preparation methods. Yeast protein extracted with water and heat precipitated at 80°, pH 6.0, contained the greatest amount of essential amino acids. Methionine and tryptophan were apparently the most limiting amino acids in all protein isolates prepared from *S. fragilis*.

Determination of the biological value of proteins is important but in the case of several proteins, e.g. yeast protein, it has limited practical significance because such a protein is not expected to be the sole or major source of dietary protein. Within the foreseeable future yeast protein should be used for the supplementation of other proteins and complementing the amino acids supplied by cereals, etc. Thus, knowledge of the amino acid composition of novel proteins is very useful as an index of its nutritional value and of its optimum mode of utilization in combination with other foods.

The amino acid composition of proteins from microbial cells has been reviewed (Hedenskog and Ebbinghaus, 1972; Tannenbaum et al., 1966; Mitsuda et al., 1970). The concentration of most amino acids was higher than that of the original intact dried cells. Mitsuda et al. (1971) determined the E/T ratio (the amount of essential amino acids in milligrams per gram of total nitrogen) of various protein fractions from *Candida* yeast. They found that the E/T ratio of one protein fraction reached the level of animal protein. However, sulfur amino acids were limiting in those protein isolates. The amino acid composition of *Saccharomyces fragilis* compares well with that of brewers yeast and *Candida (Torula) utilis* grown on wood sulfite waste liquor (Amundson, 1966). Protein extracted from *S. fragilis* cells with trichloroacetic acid and ethyl alcohol had only a slightly higher concentration of amino acid compared to the intact cells and the content of sulfur containing amino acids was low (Wasserman, 1961). Chiao and Peterson (1953) unsuccessfully attempted to increase the sulfur amino acids in yeast by increasing the nitrogen, cysteine, choline, and threonine content of the medium. Nelson et al. (1960) studied the methionine content of 271 strains of yeast and found values of 0.4–1.7 g/16 g of nitrogen. In conjunction with other studies on yeast proteins we determined the amino acids of the intact cells of *Saccharomyces fragilis* and of various yeast protein preparations.

## EXPERIMENTAL PROCEDURE

*Saccharomyces fragilis* was grown both in batch and continuous cultures as described (Vananuvat, 1973; Vananuvat and Kinsella, 1975b). Batch culture was performed using a deproteinized whey medium containing 2% lactose, under the following conditions: pH 5.0; temperature, 30°; aeration, 1 vol of air/vol of culture medium per min; agitation speeds of 600 and 700 rpm. Continuous culture was carried out at pH 5.0; temperature, 30°; aeration 1 vol of air/vol of culture medium per min; lactose concentrations in the deproteinized whey medium of 2, 4.7, and 5.9%; dilution rates of 0.10, 0.18, and 0.23/hr. Yeast harvesting, extraction of protein from yeast cells, and preparation of four types of yeast protein isolates were done according to Vananuvat and Kinsella (1975a). Sample numbers 1 through 4 corresponded to proteins extracted from *S. fragilis* with sodium hydroxide (0.4%) and subsequently

**Table I. Comparison of the Amino Acid Composition of Whole Cells of *Saccharomyces fragilis* during Batch and Continuous Cultivation, with That of *Saccharomyces cerevisiae* (g of Amino Acid per 16 g of N)[a]**

| | *S. fragilis* | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|
| | Batch cultivation | | Continuous cultivation | | | | | | | |
| Amino acids | A, 8 hr | B, 8 hr | C | D | E | F | G | *b* | *c* | *S. cerevisiae*[c] |
| Essential | | | | | | | | | | |
| Lys | 8.76 | 8.30 | 8.63 | 8.17 | 8.02 | 8.56 | 8.03 | 11.1 | 11.14 | 6.9 |
| Thr | 4.10 | 4.15 | 4.54 | 4.39 | 4.60 | 4.45 | 4.17 | 6.5 | 5.57 | 4.6 |
| Val | 5.57 | 5.70 | 6.20 | 6.06 | 6.39 | 6.02 | 5.97 | 7.8 | 5.72 | 4.9 |
| Met | 1.52 | 1.39 | 1.46 | 1.45 | 1.47 | 1.51 | 1.48 | 1.6 | 1.57 | 1.3 |
| Ile | 4.22 | 4.29 | 4.41 | 4.26 | 4.34 | 4.55 | 4.33 | 6.0 | 5.05 | 4.3 |
| Leu | 7.22 | 7.14 | 7.31 | 6.98 | 7.12 | 7.65 | 7.22 | 9.6 | | 6.0 |
| Phe | 3.47 | 3.65 | 3.63 | 3.46 | 3.64 | 3.85 | 3.86 | 5.1 | 5.05 | 3.0 |
| Nonessential | | | | | | | | | | |
| His[d] | 0.90 | 0.70 | 0.66 | 0.75 | 0.74 | 0.60 | 0.79 | 4.0 | 3.98 | 2.5 |
| Arg | 5.33 | 5.76 | 7.33 | 5.93 | 6.09 | 5.13 | 4.78 | 7.4 | 7.37 | 5.4 |
| Asp | 9.56 | 9.67 | 10.12 | 9.52 | 9.95 | 10.03 | 10.11 | | 10.40 | 7.2 |
| Ser | 4.67 | 4.63 | 4.83 | 4.50 | 4.92 | 5.05 | 4.87 | 7.0 | 5.21 | 4.2 |
| Glu | 16.69 | 16.35 | 15.26 | 15.19 | 15.72 | 13.40 | 13.41 | | 15.24 | 10.9 |
| Pro | 3.70 | 3.70 | 3.80 | 3.77 | 3.82 | 3.82 | 3.62 | | | 4.0 |
| Gly | 5.23 | 5.05 | 5.25 | 5.06 | 4.82 | 5.10 | 4.77 | | 4.24 | 4.0 |
| Ala | 6.23 | 6.62 | 7.69 | 7.68 | 8.67 | 8.26 | 7.55 | | 7.21 | 5.5 |
| Cystine | 1.83 | 1.99 | 1.69 | 1.65 | 1.69 | 1.46 | 1.53 | | | |
| Tyr[d] | | | | | | | | 3.4 | 4.57 | 3.3 |

[a] Batch cultivation as in Experimental Procedure, i.e. 2% lactose, pH 5, 30°, and aeration rate of 1 vol of air/vol of culture medium per min. A and B denote agitation speeds of 600 and 700 rpm, and duration of incubation of 7 and 8 hr, respectively. Continuous cultivation as in Experimental Procedure, pH 5.0, 30°, aeration 1 vol of air/vol of culture medium per min; C = 2% lactose at dilution rate of 0.1 per hr; D = 2% lactose at 0.18 per hr; E = 2% lactose at 0.23 per hr; F = 5.9% lactose at 0.18 per hr; G = 4.7% lactose at 0.18 per hr. [b] Amundson (1966). [c] Wasserman (1961). [d] Histidine and tyrosine were partially destroyed by performic acid oxidation.

precipitated with acid, pH 4 (no. 1), or heat (80°) at pH 6 (no. 2) and protein extracted with water and then precipitated with acid, pH 4 (no. 3), and heat (80°) at pH 6 (no. 4). Details of extraction methods used in obtaining these proteins were described in a preceding paper (Vananuvat and Kinsella, 1975a).

Amino acids analyses were performed according to the procedures of Moore and Stein (1954) and Moore (1963) following performic acid oxidation of the proteins. A Beckman amino acid analyzer (Model 120C) was used. Norleucine (K&K Lab, Inc., Plainview, N.Y.) and α-aminoguanidinopropionic acid (AGPA) were used as internal standards. Results are expressed as grams of amino acid per 16 g of nitrogen. Tryptophan was quantified according to procedure K of Spies and Chambers (1949). L-Tryptophan (Nutritional Biochemicals Corp., Cleveland, Ohio) was used to prepare a standard curve. The extent of tryptophan destruction during hydrolysis was corrected for by determining the loss of added tryptophan when subjected to the same analytical procedures.

## RESULTS AND DISCUSSION

The amino acid composition of whole cells of *S. fragilis* grown under different conditions during batch and continuous methods was compared with data available from previous studies on this and other yeasts (Table I). These data reveal a marked similarity in amino acids of *S. fragilis* irrespective of culture conditions.

The concentrations of valine, lysine, and leucine were much higher than those of the other essential amino acids in intact cells of *S. fragilis*. Because of the previous lack of information concerning cystine we routinely did analyses on performic acid treated proteins to quantify the sulfur amino acids, which are frequently limiting in food proteins. Methionine and cystine were quite limited in these cells. The low concentration of methionine in *S. fragilis* cells is in agreement with data from the previous studies of *S. fragilis* and other yeasts (Enebo, 1968; Miller, 1968; Wasserman, 1961). The histidine was apparently low because the pretreatment of protein with performic acid partially destroyed this amino acid (Moore, 1963). The average contents of histidine, tyrosine, and tryptophan in these cells were 4, 4.5, and 0.9 g per 100 g of cell protein, respectively. These values are averages of two determinations of pooled samples determined by hydrolysis under nonoxidizing conditions.

The amino acids of the yeast grown under batch conditions at 700 rpm agitation speed were slightly higher than those grown at 600 rpm. The concentration of amino acids was similar in the cells continuously cultured at the dilution rates of 0.23 and 0.10 but both were slightly higher than those grown at the 0.18 per hr dilution rate. Yeasts grown on 5.9% lactose possessed higher concentrations of amino acids than yeasts grown on 4.7% lactose at the same dilution rate of 0.18 per hr.

The slightly lower concentration of amino acids obtained in the present study compared to those reported by Amundson (1966) and Wasserman (1961) may arise from the different culture conditions employed in this study. Surazynski et al. (1968) observed that growth conditions affected the concentration of amino acids, especially histidine, in *S. fragilis* grown in aerated and nonaerated media. Wasserman (1961) indicated that the protein fraction of this organism has the same amino acid composition as long as the yeast remain healthy, regardless of the composition of medium. However, we found that both total nitrogen and protein in cells of *S. fragilis* varied with culture conditions (Vananuvat and Kinsella, 1975a,b).

**Table II. Comparison of Amino Acid Composition of Protein Isolated from *S. fragilis* Grown under Continuous Cultivation**

| Amino acids | Yeast protein isolates,[a] g of AA per 16 g of N | | | |
|---|---|---|---|---|
| | 1 | 2 | 3 | 4 |
| Essential | | | | |
| Lys | 6.86 | 8.54 | 7.91 | 9.75 |
| Thr | 3.95 | 4.73 | 4.38 | 4.95 |
| Val | 4.37 | 5.34 | 4.96 | 5.57 |
| Met | 1.20 | 1.51 | 1.38 | 1.46 |
| Ile | 3.33 | 4.00 | 3.84 | 4.03 |
| Leu | 7.25 | 9.11 | 8.18 | 9.43 |
| Phe | 3.09 | 3.89 | 3.48 | 3.90 |
| Trp | 0.36 | 0.45 | 0.41 | 0.47 |
| Nonessential | | | | |
| His | 0.59 | 0.72 | 0.64 | 0.87 |
| Arg | 3.75 | 4.57 | 4.35 | 4.70 |
| Asp | 10.17 | 11.99 | 11.17 | 12.70 |
| Ser | 5.11 | 6.19 | 5.62 | 6.33 |
| Glu | 10.62 | 12.65 | 10.86 | 12.42 |
| Pro | 3.46 | 4.16 | 3.88 | 3.42 |
| Gly | 4.27 | 4.66 | 4.62 | 4.98 |
| Ala | 5.51 | 6.62 | 6.03 | 7.18 |
| Cystine | 1.05 | 1.17 | 1.31 | 1.31 |
| Tyr[b] | (3.42) | (3.42) | (3.42) | (3.42) |

[a] Yeast protein isolates: 1 and 2 were extracted with 0.4% NaOH and precipitated with acid (pH 4) and heat (80°, pH 6); 3 and 4 represent water extracts precipitated with acid (pH 4) and heat (80°, pH 6), respectively. Cell protein recoveries were 65, 54, 26, and 33%, respectively (Vananuvat and Kinsella, 1975a). [b] From Wasserman (1961).

The amino acid compositions of the four types of protein isolates from *S. fragilis* were determined (Table II). The amino acids varied with different preparation methods. The isolates prepared from alkaline extracts of the ruptured cells had lower concentrations of all amino acids than the water-soluble proteins precipitated by identical methods. This is consistent with the report of Hedenskog and Ebbinghaus (1972) who found that various amino acids were degraded by alkali during extraction. Samson et al. (1971) also observed that the protein extracted from coconuts with water contained higher amounts of lysine, arginine, and glutamic acid than did corresponding alkaline extracts.

Precipitation of proteins by mildly heating (80°) the soluble protein extracts at pH 6 resulted in improved amino acid recoveries in both the alkaline and aqueous extracts of *S. fragilis* cells.

The concentration of most amino acids in the protein precipitated by heat from the water extracts of *S. fragilis* was relatively higher than that of the whole cells. Studies on other organisms showed similar results (Hedenskog and Ebbinghaus, 1972; Mitsuda et al., 1970; Tannenbaum, 1968). This was because the amino acid analysis of the intact yeast cells was not based on the composition of true yeast protein but rather on the total cell nitrogen which included nitrogen from protein and nonprotein materials as discussed in a preceding paper (Vananuvat and Kinsella, 1975a,b).

The total essential amino acid content of the yeast protein (no. 4) was 44, compared to 52 (grams of amino essential acids per 100 g of protein) for egg protein. The lower biological value is attributed to the limiting amounts of methionine and tryptophan in these yeast proteins. Sulfur-containing amino acids occur in low concentrations in most yeast proteins (Hedenskog and Ebbinghaus, 1972; Tannenbaum, 1968; Mitsuda et al., 1971) and a challenge to future research is to select yeast strains which produce more sulfur-containing proteins.

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