γ rays and has reported that there was no significant difference between control and irradiated beans.

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LITERATURE CITED

Amuh, I. K. A., "Suppression of Mold Growth in Prepared Cocoa Beans by Means of Gamma Radiation", Technical Report, Ghana Atomic Energy Commission, Legon, 1974, unpublished data.

Bunting, R. H., "Fungi Occuring in Cocoa Beans", Department of Agriculture, Gold Coast Bulletin No. 16, Paper No. VIII, 1928, p 44.

Clegg, K. M., *J. Sci. Food Agric.* **7**, 40 (1956).

Deschreider, A. R., "Food Irradiation", proceedings of a symposium jointly organized by IAEA and FAO, Vienna, 1966, p 173.

Englis, D. T., Miles, J. W., *Anal. Chem.* **26**(7), 1214 (1954).

Jamieson, G. R., Reed, E., *J. Chromatogr.* **17**, 7 (1965).

Kodenchery, U. K., Nair, P. M., *J. Agric. Food Chem.* **20**(2), 282 (1972).

Loaharanu, S., "Irradiation of Beans and Cocoa Beans as Related to Insect Feeding and Organoleptic Properties, respectively". A lecture note discussed at the Inter-Regional Training Course in Food Irradiation, Rio de Janeiro, Brazil, Nov–Dec, 1975.

Nelson, G. D., *J. Biol. Chem.* **153**, 375 (1944).

Pearson, D., "The Chemical Analysis of Foods", 6th ed., Churchill, London, 1970, p 9.

Reineccius, G. A., Anderson, D. A., Kavanagh, T. E., Keeney, P. G., *J. Agric. Food Chem.* **20**(2), 199 (1972).

Rohan, T. A., *J. Sci. Food Agric.* **14**, 799 (1963).

Rohan, T. A., *J. Food Sci.* **29**, 456 (1964).

Rohan, T. A., Stewart, T., *J. Food Sci.* **31**, 206 (1966).

Van Kooij, J. G., "The Suitability of Irradiated Cocoa Beans for Processing", Progress report (Technical and Preliminary Research Report No. 67) read at the Joint FAO/IAEA Research Coordination Meeting on the Technological and Economic Feasibility of Food Irradiation, Feb 20–24, 1978, Accra, Ghana.

Amino Acid Composition of Whole Cells of Different Yeasts

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The amino acid composition of the intact cells of eight yeast species (*Saccharomyces cerevisiae, Candida utilis, Kluyveromyces fragilis, Saccharomyces uvarum, Schwanniomyces castellii, Saccharomycodes ludwigii, Pichia membranaefaciens, Lipomyces starkeyi*) was determined. High contents of threonine and lysine and a deficiency in methionine and cystine were apparently a characteristic of all the species considered. *Saccharomycodes ludwigii, Pichia membranaefaciens*, and *Lipomyces starkeyi* showed a very low protein content. Amino acid profiles and protein contents of *Saccharomyces uvarum* and *Schwanniomyces castellii* were comparable to those of *Candida utilis*.

Among the novel food sources presently being developed and studied, single-cell protein (SCP) from yeast holds a prominent role (Chen and Peppler, 1977). In fact, yeasts are highly efficient producers of protein from different carbon sources, show an elevated protein content ranging from 38.8 to 70.7% of dry weight (FAO, 1970), and have a reasonably high lysine content as well as sufficient amounts of threonine and tryptophan.

Though many studies have been done on the subject of food yeasts, these normally involve but a few species such as *Candida utilis, Saccharomyces cerevisiae*, and *Kluyveromyces fragilis*. The selection of these yeasts was not necessarily due to careful screening surveys. *Candida utilis*, for example, was isolated by chance as a contaminant in a German yeast factory and then used for its exceedingly rapid growth and ability to use a wide range of carbon sources. On the other hand, *Kluyveromyces fragilis* was chosen primarily because of its ability to utilize lactose from whey while *Saccharomyces cerevisiae* does not represent an actual choice, being itself an imposed, abundant byproduct of the alcoholic beverage industry.

In this work five additional yeasts were compared to the three above-mentioned species in relation to their protein contents and amino acid patterns.

MATERIALS AND METHODS

Organisms and Growth Conditions. The species used in this study are listed in Table I. Basal medium was yeast nitrogen base (Difco) supplemented with 2% glucose. Cells were grown on a rotary shaker (150 rpm) at 28 °C to the end of the exponential phase of growth (usually 15–18 h of culture), collected by centrifugation, washed three times with distilled water, and freeze-dried.

Cell Analysis. Total nitrogen content was determined in a Merz apparatus, Model C (Heraeus Co., Hanau, West Germany). Total protein (true protein) was estimated by the method of Lowry et al. (1951) with bovine serum albumin (Sigma Chemical Co., St. Louis, MO) as a standard. Acid hydrolysis of freeze-dried cells was performed according to Puerser and Beuchler (1966). Amino acid profiles were determined by chromatography on an Optica S.A.S. Aminolyser (Milan, Italy) by the procedure of Mondino (1967). Tryptophan was estimated by the procedure of Opienska-Blauth et al. (1963) after hot alkali hydrolysis (Brown and Rose, 1969). Sulfur-containing amino acids were oxidized with performic acid to cysteic

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AA Composition of Whole Cells of Yeasts

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Table I. Nitrogen Components of Whole Cells of Various Yeasts (All Data Expressed as Grams per 100 g of Dry Cell Weight)

aa[b]	S. cerevisiae IMAT-PG 823[a]	C. utilis UCD C35	K. fragilis UCD 7158	S. uvarum IMAT-PG 1802	S. castellii UCD 587	S. ludwigii IMAT-PG 2675	P. membranaefaciens UCD 5722	L. starkeyi UCD 5155
Ile	5.7	5.2	5.2	6.0	5.4	4.1	3.9	2.6
Leu	3.8	3.5	3.8	4.2	3.6	2.7	2.8	1.9
Lys	2.4	1.7	1.8	2.3	1.9	1.7	1.6	1.1
Phe	2.6	2.1	2.4	2.7	2.0	1.6	1.6	1.3
Tyr	1.8	1.8	1.7	2.0	1.8	1.2	1.3	0.9
Thr	2.8	2.5	2.5	3.0	2.3	2.3	2.1	1.4
Trp	0.6	0.5	0.7	0.4	0.4	0.3	0.5	0.3
Val	2.8	2.7	3.0	3.0	2.8	2.0	2.2	1.2
Cys	0.6	0.9	0.9	1.1	0.8	0.6	0.7	0.7
Met	0.4	0.3	0.5	0.7	0.5	0.3	0.4	0.3
Asp	4.7	4.0	4.4	4.5	4.4	3.7	3.3	2.5
Ser	2.5	2.1	2.2	2.7	2.2	1.7	1.8	1.3
Glu	6.0	5.0	4.9	6.4	5.0	4.8	4.2	5.7
Gly	2.6	2.1	2.3	2.8	2.1	1.7	1.8	1.4
Ala	3.5	3.0	3.1	3.4	2.6	2.4	2.7	1.8
Pro	2.0	1.5	1.5	2.0	1.5	1.2	1.3	0.9
His	1.1	0.8	0.9	1.1	0.8	0.8	0.6	0.5
Arg	3.3	2.1	2.4	2.4	2.3	2.3	2.0	2.5
calcd PER[c]	1.5	1.3	2.2	1.8	1.6	1.7	1.6	1.2
total N (Kjeldahl)	8.7	7.8	9.0	9.3	7.8	5.7	7.3	5.6
crude protein (N × 6.25)	54.4	48.7	56.2	58.4	48.9	35.7	45.8	35.0
true protein (Folin)	48.0	44.0	47.0	49.3	43.0	32.5	36.0	25.5
sum of aa	49.2	41.8	44.2	50.7	42.4	35.4	34.8	28.3

[a] Acronyms UCD and IMAT-PG refer respectively to the collections of the Department of Food Science and Technology of the University of California at Davis and of the Istituto di Microbiologia Agraria e Tecnica of the University of Perugia, Italy. [b] Amino acid values are the means of at least five determinations on two separate hydrolysates. [c] According to eq 3 of Alsmeyer et al. (1974). PERC for lean beef = 2.9.

acid and methionine sulfone before hydrolysis (Moore, 1963).

RESULTS AND DISCUSSION

The true protein content of microbial cells cannot be derived by simply multiplying total nitrogen (Kjeldhal) by the factor 6.25. In fact there always is a considerable amount of Kjeldhal-reactive nitrogen in cellular fractions (5–20% dry weight) which does not contribute to true protein value (Kihlberg, 1972). In this study the overestimation of protein by this procedure ranged from 9 to 27%. As a result the expression of amino acid content as grams per 16 g of nitrogen or grams per 100 g of crude protein, normally used in nutritional studies, is rather misleading when applied to microorganisms. For these reasons we have reported the amino acid contents as percent of dry cell weight. The Folin procedure of true protein estimation in yeast biomass after hot alkali extraction is in good agreement with total amino acid values based on chromatographic analysis.

As to the selection of the yeast species studied, several factors were considered: due to its ability to utilize lactose, Schwanniomyces castellii was, for example, chosen as a possible alternative to Kluyveromyces fragilis. Saccharomyces uvarum and Saccharomycodes ludwigii were selected for their capability to utilize many sugars as well as for their larger cell size. Pichia membranaefaciens and Lipomyces starkeyi were included on the assumption that weak or nonfermenting organisms may give more efficient biomass yields. The latter species was selected also because of its ability to convert directly starch into SCP with a high yield (Spencer-Martins and Van Uden, 1977).

The nitrogen components of whole cells of the eight species considered are reported in Table I. S. uvarum showed a true protein content higher than C. utilis and K. fragilis. S. castellii profile was also comparable to that of C. utilis, but showed a slightly lower total amino acid

concentration than that of its counterpart K. fragilis. The three remaining species Saccharomycodes ludwigii, P. membranaefaciens, and L. starkeyi were characterized by lower true protein concentrations.

K. fragilis and S. uvarum showed the most favorable calculated protein efficiency ratio (PER) when compared to the rest of the species. The concentrations for each amino acid were, on the average, slightly higher than those of the traditional SCP yeasts C. utilis and S. cerevisiae. S. castellii amino acid profile was very similar to that of C. utilis; however, slightly lower values were observed when compared to K. fragilis. Amino acid contents of Saccharomycodes ludwigii and P. membranaefaciens were consistently lower than those of S. uvarum and K. fragilis as well as those of the SCP reference yeasts. L. starkeyi showed the lowest amino acid contents with values ranging about 50% of those of S. uvarum.

All the species considered showed very low contents of methionine and cystine, with the exception of S. uvarum whose sulfur-containing amino acid cell concentration appeared relatively higher. Data of the three reference organisms were also in good agreement with those published in the literature. In spite of these minor differences, the well-known deficiency in sulfur amino acids of the cell protein appeared to be a property related to yeasts as a group rather than to the species.

From the above observations it can be concluded that S. castellii, as far as protein content and amino acid pattern, can be used in alternative to K. fragilis for lactose-containing materials. However, a comparative study of the economics of their industrial production should be done.

S. uvarum large cell size accompanied by protein and amino acid contents comparable to those of C. utilis also suggests a deeper look into the economics of its production.

Saccharomycodes ludwigii and P. membranaefaciens did not appear to be good SCP producers due to their very

low protein contents. Similarly, the use of *L. starkeyi* as a converter of starch into SCP did not prove convenient for the same reason.

It should be recognized that SCP evaluated on the basis of N × 6.25 creates significant errors when comparisons are made with other protein sources due, primarily, to RNA contents. Also very confusing is the expression of amino acid content of SCP as grams per 16 g of nitrogen or grams per 100 g of protein, when the actual contents of total nitrogen and true protein are not given.

LITERATURE CITED

Alsmeyer, R. H., Cunningham, A. E., Happich, M. L., *Food Technol.* **28**, July 34 (1974).
Brown, C. M., Rose, A. H., *J. Bacteriol.* **97**, 261 (1969).
Chen, S. L., Peppler, H. J., *Dev. Ind. Microbiol.* **19**, 79 (1977).
Food and Agriculture Organization, "Amino Acid Content of Foods and Biological Data on Proteins", Nutr. Data No. 24, F.A.O., Rome, Italy, 1970.
Kihlberg, R., *Ann. Rev. Microbiol.* **26**, 427 (1972).
Lowry, O. H., Rosenbrough, N. J., Farr, A. L., Randall, L. J., *J. Biol. Chem.* **193**, 265 (1951).
Mondino, A., *J. Chromatogr.* **30**, 100 (1967).
Moore, S., *J. Biol. Chem.* **238**, 235 (1963).
Opienska-Blauth, T., Charezinski, M., Berbec, H., *Anal. Biochem.* **6**, 69 (1963).
Puerser, D. B., Beuchler, S. M., *J. Dairy Sci.* **49**, 81 (1966).
Spencer-Martins, I., van Uden, N., *Eur. J. Appl. Microbiol.* **4**, 29 (1977).

Influences of Irrigation Regimens on Phytate and Mineral Contents of Wheat Grain and Estimates of Genetic Parameters

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To determine the effects of irrigation on the phytate and mineral contents of the grain and to estimate the magnitude of genotype–environment interaction, eight Iranian and two foreign wheat (*Triticum aestivum* L.) varieties were grown under dryland and irrigated conditions for 2 years. Grain yield, phytate P (PP), total P (TP), and PP as percent of TP were reduced while plant height, nonphytate P (NPP), and Ca were increased under dryland conditions. Genotypic variances were low while environmental variances comprised high proportions of total variability in the Iranian wheats. The genotype–environment interaction variance was low for plant height, Fe, TP, and yield and high for Mg and Zn.

Between 40 and 94% of the total P in wheat grain is shown to be constituted of phytic acid (O'Dell et al., 1972; Abernethy et al., 1973; Nahapetian and Bassiri, 1975, 1976; Bassiri and Nahapetian, 1977). Considerable research has been conducted to determine the relationships between phytate P (phytic acid) and other chemical constituents within the wheat grain (Reinhold, 1971, 1975a,b; Halsted et al., 1972; Berlyne et al., 1973; Reinhold et al., 1973a,b; Nahapetian and Bassiri, 1975, 1976; Bassiri and Nahapetian, 1977).

Excess phytate in the diet (predominantly bread of high phytate content) in villages of Iran decreases the availability of Ca, Zn, Fe, and Mg, thus causing deficiencies of these minerals and symptoms associated with them (Reinhold, 1971, 1972, 1975a; Reinhold et al., 1973a).

One way to decrease the amount of phytic acid in the diet is the production of wheat varieties having genetically low phytic acid content of the grain. Through breeding programs, new varieties should be made available which are as good as or better than the present varieties but with low phytate.

A breeder should know the magnitude of some genetic parameters such as genotypic, environmental, and their interaction variances and heritability of each character prior to the start of breeding for improved varieties. There are no reports on the importance of genotype–environment effects on the grain contents of phytic acid and minerals in Iranian wheat varieties. The present experiment was conducted to determine (1) the interrelationships between phytate and some mineral constituents in the wheat grain as affected by irrigation regimens in 2 years and (2) the contribution of genotype, environment, and their interaction to the total variation when each irrigation regimen in each year is considered a separate environment.

MATERIALS AND METHODS

Two foreign (Penjamo and Tobari) and eight Iranian (Derakhshan, Harbash, Jawanjani, Jolgeh, Kalheidari, Koohrang, Ommid, and Roshan) varieties of wheat (*Triticum aestivum* L.) were grown in two adjacent experimental fields at the College of Agriculture Experiment Station, Shiraz University, Shiraz, Iran, in two successive years. The soil characteristics at the site of the experiments are previously reported (Bassiri and Nahapetian, 1977). The fields were kept fallow the year prior to plantings and were not fertilized during the course of the experiment.

Plantings were made during mid-November of each year in a randomized complete block design with four replications in each field. Within each year one field was designated as dryland (irrigated only after sowing) and the other as irrigated (irrigated after sowing and whenever necessary). The amounts and dates of natural precipitation and irrigation are reported earlier for the first year (Bassiri and Nahapetian, 1977) and shown in Figure 1 for the second year.

Each plot consisted of six 5-m rows at 50-cm distance but only the four middle rows were harvested in late May or early June. The grains were weighed and analyzed for

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