simplify interpretation. A microorganism responding equally well to DL- and L-methionine might be helpful. This problem merits further investigation.

Stability of DL-Methionine Added to Feeds

The stability of DL-methionine in poultry diets has been investigated under several conditions, as shown in Table II. Broiler rations supplemented with 0.05 and 0.0625% DL-methionine were kept at room temperature for as long as 13 months without deterioration. In another experiment a commercial broiler mash containing 0.05% DL-methionine was stored for 14 months under the roof of a warehouse in different types of containers. During the summer months the heat in this warehouse occasionally reached 110° F. (43.3° C.), but no loss of added DL-methionine was noted.

The effect of higher temperatures was also studied. For this purpose a commercial diet was supplemented with 0.05% DL-methionine and samples were subjected to temperatures up to 100° C. for varying periods of time. The results of this experiment, shown in Table II, indicate that when the diet was heated to 50° C. for 5 days, no loss of DL-methionine occurred. But when the temperature was raised to 100° C., considerable loss took place after 3 days. As the diet itself became brown and decomposed, the loss of methionine is not surprising.

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CEREAL COMPONENTS

Free Amino Acids of Fresh and Aged Parboiled Rice

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Eighteen free amino acids were identified by filter-paper chromatography of adsorption-dialysis extracts of both fresh and oven-aged parboiled rice. Those in greatest initial concentration were alanine, aspartic acid, and glutamic acid; those in intermediate concentration were arginine, asparagine, glycine, leucine(s), lysine, proline, serine, valine, and one unidentified ninhydrin-reacting compound; those in lowest concentration were cystine, histidine, methionine, phenylalanine, threonine, tryptophan, and tyrosine. Decreases in size and intensity of amino acid spots from the aged rice indicated significant losses of amino acids during accelerated storage.

THE MARKED DARKENING OF PARBOILED RICE during accelerated storage (*5*) is believed to be the result of nonenzymatic browning reactions (*4*) in which amino groups and reducing sugars are known generally to take part. The parboiling process consists in steeping the rice in warm water, steaming under pressure, drying while still in the hull, and milling to remove hulls and bran. This treatment is known to increase the content of vitamins, minerals, phosphorus compounds, and reducing sugars in the milled product (*8*).

The present investigation of the content of—and changes in—free amino acids is part of a more comprehensive program to determine changes in parboiled rice during storage, and to develop means for accelerated testing of storage life. It has been established in prior work (*15*) that during storage both reducing sugars and total amino nitrogen decrease. The latter includes amino nitrogen from amino acids, the free amino groups of proteins, and possibly other compounds.

This report establishes the individual free amino acids present in the rice and shows that some of them decrease during storage. The results are preliminary to measurement of quantitative changes. No comparable determination appears to have been made on domestic varieties of rice. A report on amino acids of Indian parboiled rice appeared (*11*) when this work was nearly finished. A somewhat comparable investigation (*9*) was made in Japan on rice enriched with vitamin B_1 through a process which was essentially parboiling of white or brown rice instead of rough rice. The relation of these findings to the present results will be discussed following the experimental details.

Materials and Methods

The rice used was commercially parboiled Caloro, a short-grain variety, from the 1952 California crop. One portion was used as received, and a second was stored 28 days at 82° C. for accelerated aging.

To obtain the free amino acids, a 500-gram lot of each rice (ground to pass 20-mesh) was extracted by the adsorption dialysis technique of Hunter and coworkers (*6*). Each solution obtained was concentrated first under vacuum and then by storage over phosphorus pentoxide in an evacuated desiccator, to a volume of less than 5 ml. The concentrated solution was filtered, adjusted to 5 ml., and used for the chromatographic studies.

Amino acids were detected by two-dimensional ascending paper chromatography with the apparatus and techniques described by Hunter, Houston, and Owens (*7*). Chromatograms were run 16 hours at 27° C. on 9 × 9 inch sheets of either S&S 507 or S&S 589 (blue ribbon) papers.

Three solvent systems were utilized in these studies: (A) methanol-water-pyridine (80/20/4) (*12*), (B) phenol-water-ammonia (35/10, plus 0.3% ammonia in a beaker), and (C) *tert*-butyl alcohol–methyl ethyl ketone–water-diethylamine (40/40/20/4) (*12*). Proportions are by volume for solvent systems A and C, by weight for solvent system B. The solvent pairs employed were A-

B and A-C. The papers were developed by dipping in 0.5% ninhydrin in *n*-butyl alcohol, followed by air-drying.

Results and Discussion

Eighteen amino acids have been identified in both the fresh and aged parboiled rice samples: aspartic and glutamic acids, serine, glycine, asparagine, threonine, alanine, tyrosine, valine, leucine(s), phenylalanine, methionine, histidine, arginine, lysine, cystine, proline, and tryptophan.

The individual amino acids in the extracts were identified by comparing their R_f values with those of known amino acids (Table I). For confirmation, wherever possible, the substances on the chromatogram were identified by specific tests. In this manner the presence of tyrosine and histidine was confirmed by Pauly's reaction (*2*), arginine by the Sakaguchi reaction (*1*), proline by isatin (*3*), and tryptophan by Ehrlich's reagent (*3, 13*). Serine and threonine were confirmed by periodate oxidation as applied to paper chromatograms by Metzenberg and Mitchell (*10*). The presence of methionine and cystine was corroborated by the platinic iodide reagent of Toennies (*14*) and cystine alone by his sodium nitroprusside test.

In several instances spot identifications were effected by enhancement with known substances. The presence of threonine, tyrosine, valine, leucine(s), phenylalanine, methionine, and histidine was confirmed by this method. Tryptophan was apparently present in very small amounts, since it could not be detected by ninhydrin but could be detected by Ehrlich's reagent on chromatograms prepared from high concentrations of extract.

A substance whose identity has not been determined was observed when the solvent system A-B was used. This material was present in fairly high concentrations. In the solvent system A-C this substance was apparently obscured by the alanine spot.

From observations of the size and color density of the ninhydrin spots on a chromatogram, it is possible to classify roughly the amino acids in the order of their concentration. Aspartic acid, glutamic acid, and alanine are present in greater concentration than serine, glycine, asparagine, valine, leucine, arginine, lysine, proline, and the unidentified spot, and these in turn occur in greater concentrations than threonine, tyrosine, phenylalanine, methionine, histidine, cystine, and tryptophan.

Careful inspection of chromatograms prepared simultaneously from extracts of fresh and aged parboiled rice samples in solvents A-B and A-C revealed that, in general, the paper prepared from the aged sample of rice showed a decrease in amino acid concentration, as indicated by the lower color intensity and/or smaller area of all spots. The greatest changes occurred in asparagine, methionine, and cystine. Marked reduction of glutamic acid and alanine was also found.

Table I. R_f Values of Free Amino Acids in Extract of Parboiled Rice

	Solvent A, R_f × 100		Solvent B, R_f × 100		Solvent C, R_f × 100	
Amino Acid	Extract	Standard	Extract	Standard	Extract	Standard
Aspartic acid	36	35	18	18	6	5
Glutamic acid	52	53	28	29	6	5
Serine	42	42	37	36	22	23
Glycine	40	36	42	42	14	14
Asparagine	23	24	44	43	9	10
Threonine	56	52	51	50	51	49
Alanine	63	58	59	59	18	20
Tyrosine	53	57	61	63	32	31
Valine	80	73	79	80	39	35
Leucine	86	76	87	87	53	53
Phenylalanine	72	66	89	88	59	54
Methionine	68	65	82	81	43	39
Histidine	23	25	76	74	13	15
Arginine	9	13	89	90	2	3
Lysine	11	13	80	82	8	9
Unknown	33	..	78	..	..	..
Cystine	0	0	10	15	..	..
Proline	By isatin reaction only					
Tryptophan	By Ehrlich's reagent only					

A related study was made by Parihar (*11*), who used circular paper chromatography to detect the free amino acids in aqueous extracts of raw and parboiled Indian rice stored at 30° C. for periods up to 3 years. In general, these same acids were also detected by Parihar, except that glycine, serine, and asparagine were not found and cystine was detected only after a 2-year storage. Most free amino acids decreased during storage. Differences in materials, parboiling methods, and analytical techniques preclude closer comparisons.

Kondo (*9*) chromatographed free amino acids from aqueous-alcohol extracts of a B_1-enriched parboiled rice made from white or brown rice by a special acid treatment. Despite the difference in materials and parboiling processes, many of the acids of the present investigation were found. Kondo also reported two unidentified spots and the presence of glutamine, which was not detected by Parihar or by the present authors. Acids reported here but not found by Kondo are threonine, phenylalanine, methionine, cystine, proline, and tryptophan. All these except proline were present only in low concentrations in the present study. Changes found at the present test storage temperatures are not necessarily the same as those that would be found on prolonged storage at normal temperatures.

These investigations show which free amino acids commonly occur in parboiled rice of different natural origins and manufacturing processes, and indicate that losses result when rice is stored. Additional study is necessary to obtain quantitative data for determining the relationship of these losses to browning phenomena and to changes in nutritional values.

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