Table III. Paper Chromatographic Behavior of Pepper Extract Constituents and Synthesized Analogs of Piperine

Spot No.	Identification	Approx. R_f	Ultraviolet Light, 366 mμ	Reaction when Sprayed with:	
				Lüdy-Tenger	Diazotized sulphanilic acid
1	Piperettine	0.1	Yellow spot	No reaction	No reaction
2	Piperine	0.24	Dark spot	Weak orange spot	No reaction
3	Unknown	0.41	Dark spot	No reaction	No reaction
4	Piperonylic acid piperidide	0.68	No reaction	Strong orange spot	No reaction
5	Unknown	0.9	Strong light blue fluorescence	No reaction	Blue spot
6	Methylene caffeic acid piperidide	0.9	Weak dark blue spot	No reaction	No reaction

On the basis of the shift of the ultraviolet maximum to a lower wavelength in the later column fractions and the apparent methylenedioxy content, it was anticipated that these fractions might have as their main constituents compounds such as piperonylic acid piperidide or methylene caffeic acid piperidide. The spectra of these compounds are given in Figure 2.

The paper chromatographic procedure showed four spots in ethanolic extracts of white and black pepper as indicated in Table III. None of the spots was identical to piperonylic acid piperidide nor methylene caffeic acid piperidide in spite of the similarity of the ultraviolet spectrum and the R_f value of methylene caffeic acid piperidide and fraction 8 illustrated in Figures 2 and 4 and Table III, respectively. Spot No. 5, the main component in fraction 8, at the R_f of methylene caffeic acid piperidide has a strong blue fluorescence in ultraviolet light and reacts with diazotized sulphanilic acid. Methylene caffeic acid piperidide, on the contrary, shows a

weak, dark blue color under the ultraviolet and does not react with diazotized sulphanilic acid. Although thin-layer chromatography resolves fraction 8 into several additional bands the evidence did not indicate that methylene caffeic acid piperidide was one of the components of this fraction.

A spot with the same R_f as piperettine was present in all peppers. Piperine and piperettine react with chromotropic acid and concentrated sulfuric acid on thin-layer chromatograms, whereas no spray reagent could be found for spot No. 3. Spot No. 3 is the main component of the fraction 5 obtained by column chromatography. Black and white pepper can also be distinguished by thin-layer chromatography and examination under ultraviolet light since some bands which are not present in any of the white peppers occur in black pepper.

Acknowledgment

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ples and the work of W. Skakum in the preparation of the samples. Thanks are also due to John McLean for placing at their disposal a sample of pure piperettine. The authors are also grateful to R. A. Chapman for his encouragement of this study.

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RICE QUALITY MEASUREMENT

Organic Acids of Rice and Some Other Cereal Seeds

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ANALYSIS of the organic acid mixtures in rice of different varieties and conditions was prompted by the belief that such metabolically active substances would act as key compounds for indicating other compositional varia-

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tion. Though present only as minor constituents, the organic acids are more susceptible to measurement of small changes than the major constituents, starch and protein. Hence, they might serve as sensitive indicators of quality changes.

Organic acids of plants (aside from acids in fats) are comprised chiefly of nonvolatile, nonnitrogenous aliphatic

acids commonly called plant acids (2). Their occurrence is widespread, and they play a central role in cellular metabolism. Reports on their presence, quantities, and changes have generally been for actively metabolizing tissue and usually for noncereal plants (2, 3). These acids are often accompanied by small amounts of aromatic acids which may act as growth regulators or germina-

Acids were extracted from rough rice (seven varieties), immature and stored mature rice, and milling fractions to determine possible relations to quality factors, and from comparison samples of wheat, rye, oats, corn, and barley. Organic acids separated into acetic, fumaric, succinic, oxalic, malic, citric, and several aromatic acids during silicic acid column chromatography and gave "fingerprints" of acid composition. Aromatics contained ferulic, vanillic, and p-coumaric acids. Fingerprints for rices were generally similar, and concentrations of total organic acids increased from long- to medium- to short-grain and waxy rice. Total extractable and organic acids were highest in bran and lowest in milled rice. They decreased during maturation but increased during storage—more strongly as moisture or temperature increased. Rice had lower acid content than other cereals. Citric was the predominant acid in rice and corn, malic in barley, oats, and wheat, and the two were almost equal in rye.

tion inhibitors (14, 22). In seeds, citric has frequently been the only acid reported. The term organic acids as used in this report includes those usually grouped as plant acids together with acetic and aromatic acids.

Few published results are available for the acids of rice grain. Andrews and Viser reported 0.20% oxalic (dry basis) in rice (7); Gobis reported 0.29% citric acid (7). Harawasa and Saito, using paper chromatography, found malic, succinic, fumaric, citric, and α-ketoglutaric acids in stored unpolished (brown) rice (8); in freshly harvested samples, the malic content was very low. Mikkelsen has indicated that several aromatic acids present in rough rice act as germination inhibitors (13, 14). Munsey (16) reported the presence of coumaric acid.

The present work reports organic acid contents and differences among several representative domestic rice varieties, the distribution of these acids among rice milling fractions, and some effects of maturation and storage on the acid contents of the rice. In order to relate rice to the other cereal grains, there are included several measurements of acids made by the same techniques used in other common cereals.

Experimental

Materials. Rough rice, or paddy, of the short-grain Caloro and Colusa and the medium-grain Calrose varieties was newly dried seed-rice harvested in 1957 at the Biggs Rice Station, Biggs, California. The medium-grain Zenith and long-grain Texas Patna and Century Patna 231 varieties were high-quality rough rices grown in 1957 at the Rice-Pasture Experiment Station, Beaumont, Texas. Rough glutinous rice (waxy rice, sweet rice, Mochi Gome) was grown in 1957 at Dos Palos, Calif.

Rice milling products (hulls, brown or unpolished rice, bran, and white or milled rice) were prepared from the above Caloro rice in the McGill Sheller and Miller by the official USDA procedures (21). The bran so obtained

was a mixture of true bran with germ, polish, and some endosperm. True anatomical separation was not made.

For maturation studies, milled rice samples were prepared from Caloro and Calrose paddy, each of which had been harvested periodically during growth in 1957 from a single field at the Biggs Rice Station (11).

Milled rice was also prepared for study of storage changes from Caloro paddy which had been commercially dried from the 1955 California harvest to four moisture levels and held at four temperatures for 18 months (10).

Samples of other cereals for comparative tests were all freshly harvested from the 1957 crop. They were as follows: Elmar wheat, a white club wheat, grown in Oregon; Baart wheat, a soft white common variety, grown in California; Merced rye, grown in California; Indio oats, grown in California; Atlas 57 barley, grown in California; and Iowa 4572 corn, a standard open-pedigree hybrid, grown in Iowa.

All cereals and milling fractions were stored at 4° C. without grinding until needed. Just before use portions were ground in a Wiley Mill to pass a 40-mesh screen. Rice bran was used without grinding.

Extraction of Acids. A 20.00-gram sample of cereal product was extracted for 3 minutes in a high-speed electric blender with 400 ml. of water in the presence of 8 grams of Dowex-50 (H^+) to free the acids and facilitate extraction. The liquid was filtered through an 11-cm. hardened paper, and the moist residue was extracted twice more with 400-ml. portions of water.

Filtration of rice bran, wheat, and rye extracts required addition of small amounts of Celite to prevent clogging. Aliquants of the extracts were titrated with 0.01N sodium hydroxide to determine acid content. The three extracts were combined for further processing. Those not used immediately were treated with a few drops of chloroform and stored at 4° C.

Ion-Exchange Treatment. Solutions were passed through a column of Permutit

A (OH^-) to absorb the acids. A 200-ml. water wash removed nonacidic material. Acids were eluted with 50 ml. of 1N ammonium hydroxide followed by a 200-ml. water wash. About 10% excess of sodium hydroxide was added to provide sodium salts, and the solution was evaporated to a small volume at 30° to 35° C. in a rotary evaporator. Residual solution was transferred to a small sample container and evaporated to dryness at room temperature, usually under nitrogen.

Column Chromatography. Acids were separated on columns of acidified Mallinckrodt's silicic acid for chromatography essentially according to Bulen, Varner, and Burrell's chloroform-butanol elution procedure (4). Collected 5-ml. fractions were treated with 5 ml. of water and one drop each of phenol red indicator and 2% sodium lauryl sulfate. Titration of the two-phase system was made with 0.01N sodium hydroxide with thorough shaking to ensure completion. Blank corrections were made for the solvent.

Acid Identifications. Emergence positions of eluted acids were compared with those of single and mixed known acids, and checked through admixture with known acids. Eluate residues were examined by one- and two-way paper chromatography, and confirmatory spot tests were made on the chromatographed acids. In addition, derivatives of acetic and oxalic acids were identified by chemical microscopy.

Results and Discussion

Acid Extractions. The method differs from previous extractions in the use of the solid, easily removable resin as an acidifier rather than a strong liquid acid. Presence of strong acid aids in freeing and extracting organic acids that may be present as salts. Effectiveness of the method depends, of course, on the completeness and reproducibility of acid extraction.

The presently detailed procedure was chosen after examination of numerous process variables. Sequential extrac-

Table I. Total Extracted and Organic Acids of Rice and Other Cereals (Meq./Kg. Dry Wt.)

Material	Total Acids Extracted[a]	Organic Acids Total[a]	Extracted acids, %
Rough rice—Caloro	67.3 ± 0.8 (2)	33.4 (4)	50
Zenith	68.1 ± 2.7 (3)	22.4	33
Calrose	68.5 ± 2.7 (4)	24.4 (4)	36
Colusa	73.0 ± 0.9 (2)	38.1 (2)	52
Texas Patna	73.7 ± 2.9 (3)	21.0 (3)	28
Century Patna 231	83.3 ± 1.0 (2)	21.6 (2)	26
Waxy	83.6 ± 0.7 (2)	27.2 (2)	33
Brown rice—Caloro	68.6 ± 1.0 (4)	29.5 (3)	43
Milled rice—Caloro, mature	17.1 ± 2.0 (4)	8.1 (2)	47
immature	36.3	15.3	42
Calrose, mature	12.8 ± 0.5 (2)	. . .	..
immature	22.7 ± 1.1 (4)	. . .	..
Rice bran and polish—Caloro	647.8 ± 3.1 (2)	135.4 (3)	21
Rice hulls—Caloro	57.8 ± 1.4 (2)	30.3 (3)	52
Rye—Merced	206.8	53.7	26
Wheat—Baart	181.2	66.4	37
Elmar	134.8 ± 1.4 (2)	42.4	31
Barley—Atlas	175.6	65.1	37
Oats—Indio	101.3	37.2	37
Corn—Iowa 4572	100.4	37.6	37

[a] Figures in parentheses indicate number of replications.

Table II. Organic Acids of Rough Rice (Meq./Kg. Dry Wt.)

| Rice | Acid Group | | | | | | |
	Aromatic	Acetic	Fumaric	Succinic	Oxalic	Malic	Citric
Colusa	1.18	2.65	4.21	2.56	10.06	5.56	11.87
Caloro	0.81	1.91	2.78	2.38	9.02	4.06	12.47
Waxy	0.58	2.07	2.25	1.90	7.25	3.04	10.12
Calrose	0.47	1.64	1.89	1.64	6.61	3.25	8.94
Zenith	0.60	1.69	2.34	2.26	2.74	3.77	8.98
C.P. 231	0.43	0.50	1.87	2.09	4.29	2.32	10.11
Texas Patna	0.63	1.70	2.15	2.05	3.50	3.23	7.71

Table III. Organic Acids of Caloro Rice Milling Fractions (Meq./Kg. Dry Wt.)

| Fraction[a] | Acid Group | | | | | | |
	Aromatic	Acetic	Fumaric	Succinic	Oxalic	Malic	Citric
Bran (3)	2.00	2.82	3.76	6.35	44.43	7.56	64.98
Hulls (3)	1.34	3.50	2.38	2.28	11.47	3.20	5.76
Brown rice (3)	0.43	0.61	1.52	2.43	7.22	2.79	14.73
Milled rice (2)	0.16	0.65	0.78	0.90	0.95	1.45	3.25

[a] Figures in parentheses indicate number of replications.

tions in the presence of the resin provided rapidly decreasing quantities of acid. This resembled Woodward and Rabideau's results with alcoholic extractions of plant material (23). As an example, successive extracts of 20.0 grams of rough rice by the present process removed 1.872, 0.408, 0.162, and 0.092 meq. of acid for a total of 2.534 meq. Three extractions usually removed about 95% of that obtainable with added repetitions, and avoided filtration troubles arising in continued operations. When 0.18 meq. of a mixture of acetic, citric, fumaric, malic, oxalic, and succinic acids was added to a rice sample, duplicate extractions recovered 0.18 meq. The recovery of added acids does not of course prove complete extraction of acids that may have occurred originally in the cereal.

Reproducibility of the results obtainable is illustrated by the variations shown in Table I, where values were obtained by two technicians at various times.

Total Extracted Acids of Rough Rice. A general trend appears for total extracted acids to increase from short- to medium- to long-grain and waxy rice. A definite varietal difference occurs; this is shown in the values for Zenith, Texas Patna, and CP 231, which were grown under the same conditions. Evidence is insufficient to show effects from cultural differences, though this is strongly suspected. Waxy rice, high in acidity, was grown under geographical conditions different from any of the other rices. Extractions of total acids from rices held at 4° C. for as long as 6 months showed no appreciable changes in amount.

Column Chromatography of Acids. Total acidity of solutions from the ion-exchange column included with organic acids other acidic material which was retained by the silicic column under the conditions used. Hence, the organic acid group was generally less than 50%
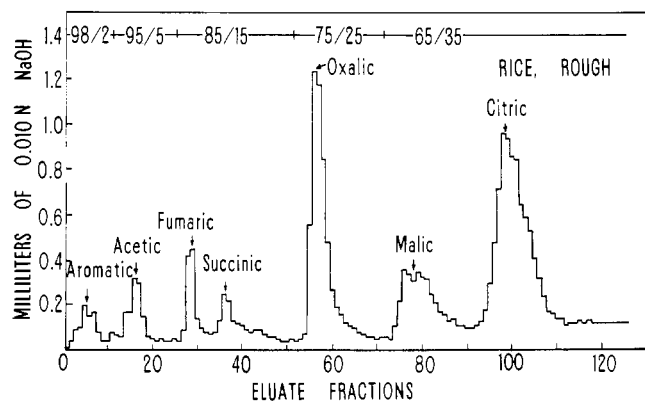

Figure 1. Representative titration of organic acids from Caloro rough rice (8.7 grams) as fractionated on a silicic acid column

Ratios at top of figure show proportions of chloroform-n-butyl alcohol mixture used as eluant. Acid names for the various peaks represent the predominant acid
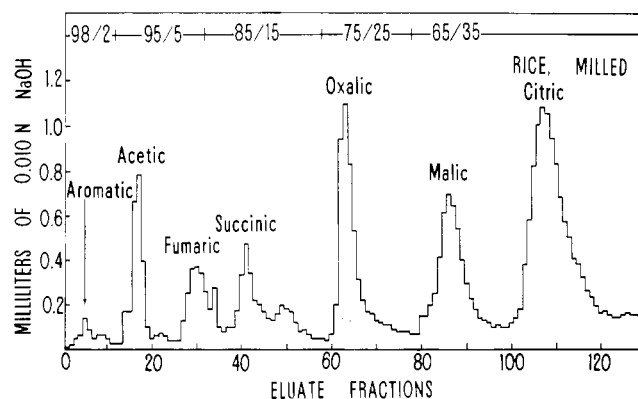

Figure 2. Titrations of organic acids from Caloro milled rice (39.2 grams)

Conditions as in Figure 1

of total acids (Table I). Inorganic, phytic, and uronic acids, known to occur in rice, would be included in the retained acidic material.

Representative examples of the acid separations obtained are shown in Figures 1 to 4 for rough rice, milled rice, wheat, and rye. The peaks serve as a "fingerprint" of the organic acid mixture of the sample, and a single peak may contain more than a single acid. The peak group is hereafter referred to by the name of the major acid component; accompanying amounts of other acids are not indicated. For example, the acetic acid in peak 2 was accompanied by some pyruvic. The method of sample preparation and presentation was found to decompose pyruvic partially with resulting formation of acetic acid. This effect was less when the method of Zbinovsky and Burris (24) was used. The aromatic peak (No. 1) was shown to contain vanillic, ferulic, and p-coumaric acids. The peak on the trailing edge of the succinic group appeared to be due to trans-aconitic; some lactic may have been present. Oxalic was sometimes accompanied by glycolic, and the citric peak may have contained minor amounts of isocitric acid. More intensive investigation of a single material, as for barley by Wall and co-workers (22), undoubtedly would serve to identify additional acids.

Organic Acids of Rough Rice. Values for the separated organic acids of seven varieties of rough rice (Table II) showed expected general similarity, but two interesting trends occurred. Total organic acid contents were highest in short-grain and waxy, and lowest in long-grain rice. Moreover, the percentage of organic acids in total extracted acids decreased in essentially the same order. The long-grain rices showed lower proportions of the oxalic acid group than short-grain and waxy rice. Use of these differences might conceivably be developed as an aid in distinguishing varieties.

Acids of Milling Fractions of Rice. In Caloro rice, the greatest acid concentration by far was found (Table I) in the bran and polish removed in milling. Decreasing acid contents were shown by brown rice, hulls, and milled rice. A similar diminishing progression was shown by total organic acids, though brown rice and hulls were reversed. The values for individual acid groups (Table III) deviated considerably in some cases. For instance, the acetic group in hulls was higher than in other fractions. The high citric value in brown rice resulted from the high content

Table IV. Acidity in Calrose Rice during Maturation

Elapsed Time (Days)	Rice Moisture, % (Moist Basis)	Acidity of Milled Rice (Meq./Kg.)	
		Harvest-moisture basis	Dry-wt. basis
0	33.3	15.1	22.7
5	30.6	12.1	17.4
7	27.1	10.4	14.2
10	27.0	9.4	12.9
12	25.0	12.8	17.1
21	20.0	11.0	13.8
42	16.7	10.7	12.8

Table V. Organic Acids of Immature and Mature Milled Caloro Rice (Meq./Kg. Dry Wt.)

Rice	Acid Group						
	Aromatic	Acetic	Fumaric	Succinic	Oxalic	Malic	Citric
Immature[a]	0.31	3.48	1.39	1.28	1.99	3.20	3.63
Mature	0.16	0.65	0.78	0.90	0.95	1.45	3.25

[a] Harvested at 36.5% moisture content.

Table VI. Acidity in Caloro Rice after Storage (Meq./Kg. Dry Wt.)

Storage Temp., °F.	Total Acidity of Milled Rice[a] at 4 Moisture Levels (% Moisture, Wet Basis, in Parentheses)			
60	33.3 (12.3)	36.5 ± 1.6 (13.4)	42.2 ± 0.3 (14.0)	33.3 (15.3)
77	36.5 (12.3)	39.0 (13.4)	38.3 (13.9)	49.0 (15.7)
90	38.8 (11.9)	50.0 (13.4)	53.5 (14.0)	53.0 (14.9)
100	39.7 (9.5)	58.6 (12.4)	69.2 ± 0.4 (13.2)	...

[a] Milled to white rice after holding as rough rice for 18 months at the indicated temperature and moisture.

Table VII. Organic Acids of Stored Caloro Rice (Meq./Kg. Dry Wt.)

Storage Conditions		Acid Group in Milled Rice[a]							
H₂O (%, wet basis)	Temp. (°F.)	Aromatic	Acetic	Fumaric	Succinic	Oxalic	Malic	Citric	Total
..	.. [b]	0.16	0.65	0.78	0.90	0.95	1.45	3.25	8.14
13.4	60	0.32	7.12	3.02	0.62	3.36	1.06	3.97	19.47
13.2	100	0.76	3.17	6.16	1.75	7.36	2.18	9.98	31.36

[a] Stored 18 months under indicated conditions as rough rice from the 1955 harvest.
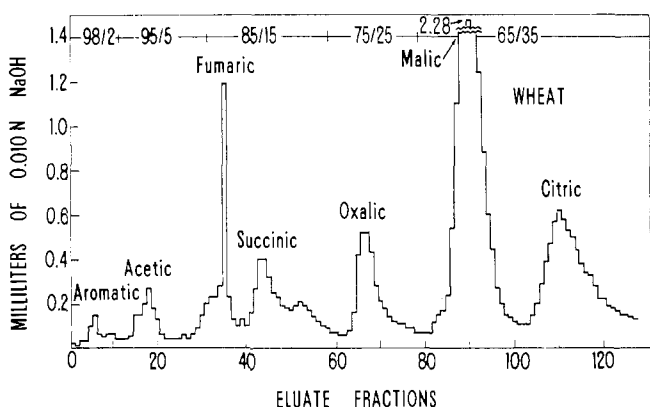[b] Freshly harvested from the 1957 crop.

Figure 3. Titrations of organic acids from Baart wheat (5.2 grams)
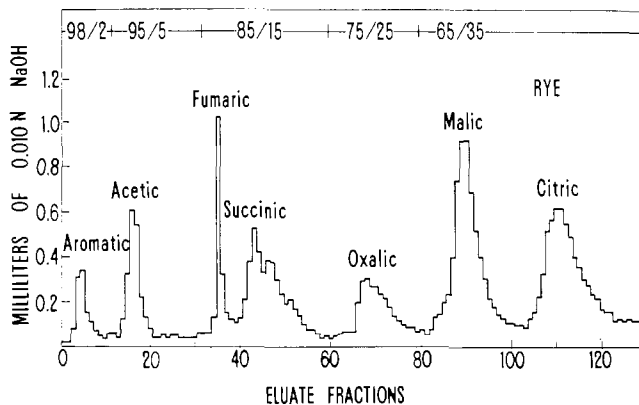
Conditions as in Figure 1

Figure 4. Titrations of organic acids from Merced rye (4.8 grams)

Conditions as in Figure 1

Table VIII. Organic Acids of Various Cereal Seeds
(Meq./Kg. Dry Wt.)

Cereal	Aromatic	Acetic	Fumaric	Succinic	Oxalic	Malic	Citric
Rye-Merced	2.63	4.42	4.07	9.35	5.57	14.15	13.54
Wheat-Baart	0.86	2.22	6.17	7.39	6.49	28.83	14.17
Wheat-Elmar	0.96	1.66	4.53	5.06	3.34	20.53	6.32
Bartley-Atlas	0.91	3.70	2.12	3.57	3.66	36.54	14.65
Oats-Indio	0.50	2.69	2.10	3.53	1.13	22.30	4.92
Corn-Iowa 4572	1.02	2.35	0.90	1.48	9.83	4.14	17.91

Table IX. Comparative Values for Oxalic, Malic, and Citric Acids in Various Cereals[a]
(Meq./Kg. Dry Wt.)

Acid Group	Rough Rice	Corn	Rye	Wheat	Barley	Oats
Oxalic	2.7–10.1	9.8	5.6	3.3–6.5	3.7	1.1
	30.6[b] (1)	1.2 (12)			70–130 (9)	10–70 (9)
		9.1 (7)			0.05–0.06[c] (22)	
Malic	2.3–5.6	4.1	14.2	20.5–28.8	36.5	22.3
	Present (8)		6.0[d] (5)	Present (17)	82.2–87.5 (22)	
				6.0[d] (5)		
Citric	7.7–12.5	17.9	13.5	6.3–14.2	14.6	4.9
	45.3 (7)	31.2 (15)	4.7 (15)	1.5 (15)	4.7 (15)	1.6 (15)
	Present (8)	34.3 (6)	10.0 (19)	7.4–7.7 (20)	8.9–11.1[e] (22)	9.7 (18)
		42.2 (7)	10.8 (18)	12.2 (18)	10.3 (19)	21.9 (7)
		15.6[d] (5)	14.0–14.3 (20)	Present (17)	10.5–11.3 (20)	
			35.9 (6)	Trace (5)	10.9 (18)	
			2.3[d] (5)		35.9 (6)	

[a] Present values are given first; recorded values include (in parentheses) the reference number in the Literature Cited.
[b] Milled rice.
[c] Includes oxalic plus glycolic acids.
[d] Reported on moist weight.
[e] Includes citric plus isocitric acids.

in the bran layer (about 0.4% of the bran). The decrease in the oxalic group from bran to milled rice was remarkably large. Aromatic acids were concentrated in the bran and hulls, where they could be most effective as germination inhibitors (14). The major acid group in most cases was citric, followed by oxalic, malic, and succinic.

Changes of Rice Acidity during Maturation. Milled rice from the periodically harvested Calrose series exhibited total acid values (Table IV) which decreased with maturation, on either dry or as-harvested basis, until the moisture content fell to about 27%; thereafter, there was little change. This moisture corresponded closely with the maturity at which the majority of quality and milling properties reached maximum or minimum values (11).

Separate organic acid values for mature Caloro milled rice were lower in each case (Table V) than for the immature rice on a dry-weight basis. The largest change occurred for the acetic group and the least for citric; on a harvest-moisture basis there was actually an appreciable increase for citric acid.

Changes of Rice Acidity during Storage. Total acid (Table VI) and organic acid content (Table VII) of milled Caloro rice were greater after 18 months of storage as rough rice than in fresh rice. Increases in total acids

were larger as the temperatures or moistures during holding were higher, and ranged from double to fourfold the initial values. Individual acid groups increased from 1½ to nearly 8 times the values for fresh rice, with greatest relative increases in the fumaric and oxalic groups. Acetic increased over 10-fold at lower temperatures, but apparently disappeared in part at a higher temperature.

Total and Organic Acids of Other Cereals. To compare rice with other cereals, determinations of total acids (Table I) and organic acids (Table VIII) were made on several common grains. Both total and organic acids were higher in all those measured than in rice; corn and oats showed the next lowest amounts. The difference in results for the two white wheats—even more than the spread among rices—emphasizes possible varietal or cultural spread in a single grain and the need for additional data to permit firm generalizations.

The relatively low organic acid content of rice probably is related to the almost universal use of ammonium nitrogen in a fairly heavy fertilization practice. Plants supplied with nitrogen in the ammonium form contain much lower concentrations of organic acids than those supplied with nitrate nitrogen (2). This is suggested also to cause lowered concentrations of keto-acids.

Distribution of plant acids differed markedly among the several grains. Like rice, corn had citric as the predominant acid, followed by the oxalic and malic groups. Rye had about equal amounts of citric and malic and a remarkably high proportion of aromatic acids. Wheat, oats, and barley contained a preponderance of malic, followed by citric and succinic. Oats were particularly low in oxalic group acid. The presence of different acids of the Krebs or tricarboxylic acid cycle as the major component in the various grains suggests interesting possible metabolic differences.

Published data for comparison with present results are limited essentially to citric, malic, and oxalic acids. Acetic acid has been reported present in wheat (17), and fumaric and succinic acids in wheat (17) and rye (5). Available values for citric, malic, and oxalic acids are compared in Table IX with present results. The most striking point here is the wide variation in reported contents resulting from varietal, cultural, and investigative differences. The oxalic acid content of barley and oats estimated by Holton and Noll (9) from paper chromatograms appears high by an order of magnitude, as does Andrewss and Viser's value (7) for oxalic acid in rice.

Comparison of the present results on a single barley with the elegantly detailed data of Wall and co-workers (22) for another single variety is difficult because of experimental, cultural, and varietal differences in the two investigations. The slightly lower organic acid contents presently found could well result from varietal and cultural differences. There is considerable similarity in the distribution of plant acids. The present 56% of malic in the total organic acids compares with 60% reported by Wall et al., and the 5.5% in the succinic group with 6.0 to 6.5% succinic. Present citric plus isocitric, however, amounts to 22% instead of 10 to 13% found by them. In view of the experimental differences, the proportional agreement is considered good.

The variation of organic acid composition in the rice kernel with changes in variety, maturity, storage, and cultural practice—as well as the compositional differences among the individual cereals—suggests that more extensive measurements of plant acids may be fruitful in cultural, quality, metabolic, and genetic studies of the cereal grains.

Marian Long, Elizabeth McComb, and E. F. Potter for analytical assistance.

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CORN FLOUR SUPPLEMENTATION

The Enrichment of Lime-Treated Corn Flour with Proteins, Lysine and Tryptophan, and Vitamins

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The results indicate that the nutritive value of lime-treated corn can be improved significantly both in quantity and quality by adding any one of the following quantities of protein-rich materials of either animal or vegetable origin: fish flour 3%, meat flour 5%, whole egg flour 3%, casein 5%, skim milk powder 8%, soybean protein 8%, soybean flour 8%, cottonseed flour 9%, pepitoria flour 9%, and torula yeast 3%. The improvement in the protein quality of lime-treated corn was higher with the animal products and is due to the contribution made by the supplements in providing lysine, tryptophan, and isoleucine, amino acids limiting in lime-treated corn. A highly significant correlation ($r = 0.874$) was found between PER and the lysine content of the supplement. It was estimated that the greatest improvement in PER of lime-treated corn can be obtained by adding a supplement providing around 0.6 gram of nitrogen, 0.25 gram of lysine, 0.045 gram of tryptophan, 0.20 gram of isoleucine, and 0.075 gram of methionine. The nutritive value of lime-treated corn is also improved by the addition of vitamins, particularly riboflavin.

THE per capita average daily corn consumption in rural communities of the Central American countries has been reported to vary from 185 to 423 grams (*7, 8*). These quantities provide 15 to 34 grams of protein, which are equivalent to 32 to 49% of the daily protein intake which varies from 40 to 66 grams per adult. In several communities in Guatemala, corn contributes up to 74% of the daily protein intake. The average quantities of animal protein consumed are relatively low, varying from 7 to 20 grams per person daily (*8*).

The tortilla is the most important form in which corn is consumed, but its preparation involving alkaline cooking, washing with water, and baking reduces significantly the concentrations of thiamine and riboflavin (*4, 6*). Although the cooking improves the protein quality slightly (*3, 16*), this improvement is of relatively small practical significance.

Since the quality of corn protein is so extremely poor (*7, 10, 14, 17*), and since protein malnutrition frequently occurs in areas of the world where corn is the most important staple food, it would be highly desirable if corn could be cheaply enriched to provide more and better quality protein as well as other essential nutrients in short supply in local diets.

Because commercial corn flours are already being produced in Central America (*3*), their enrichment with protein and vitamins could be easily accomplished. This paper reports the results of studies designed to find the minimum quantity of protein-rich foods of animal and vegetable origin needed to obtain a maximum improvement in the quality of corn protein. The possibilities of adding synthetic vitamins are also explored.

Materials and Methods

A commercial lime-treated corn masa prepared as described previously (*6*) was brought to the laboratory still moist