ACKNOWLEDGMENT

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Radial Distribution of Amino Acids in the Milled Rice Kernel

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Radial distribution of all amino acids except Trp and Cys was determined for five varieties of rice. Consecutive fractions from the outer layer to the center of the rice kernel were obtained by abrasive milling. Five patterns of distribution of amino acid contents were observed. The patterns are as follows: (1) no change for Ile, Val, Arg, and Pro; (2) low in Gly; (4) low in the outer layer, increases in the middle layer, and no change in the central layer for Met and Ser; (5) the mirror image of (4) for Thr, His, Ala, and Asp. The data of Lys, the first limiting amino acid of rice proteins, suggest that the nutritive value of rice proteins may be reduced toward the middle layer and it may be elevated slightly in the kernel center.

The composition of rice proteins such as albumin, globulin, prolamin, and glutelin varies with the radial location in the kernel (Yoshizawa and Kishi, 1985). Moreover, it has been reported that there is a marked difference in the amino acid composition among their proteins (Juliano, 1985; Taira, 1962). These findings suggest that the amino acid composition may change with the portion of the rice kernel.

Previous studies (Hayakawa et al., 1985) have shown that there is a difference in the lysine content of milled rice between the outer layer and the inner layer, and the nutritive value of their layers depends on the limiting amino acid content. On the basis of elucidating the changes in the nutritive value of rice protein with processing, it is important to determine the radial distribution of amino acids. However, there has been very little work on the amino acid composition of consecutive layers of the kernel.

In the present study, we have examined the amino acid composition of each layer, obtained by abrasive milling of brown rice of five varieties, and have discussed the radial nutritive value of rice.

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MATERIALS AND METHODS

Rice Supplies. The brown rices of five varieties [Nipponbare, Koshihikari, Sasanishiki, Ohzora, Aoisora (*Oryza sativa* L. subsp. *japonica* Kato)] were prepared. These are the major varieties in Japan today. All rices were harvested in 1984 from Ibaraki prefecture, Japan.

Analytical Samples. A 5-kg sample of each brown rice was abrasively milled 25–35 consecutive times by passage through a Nakano type rice-whitening machine obtained from Sanyo Kosaku, Co., Ltd., Tokyo, Japan. The flour and particles produced from the kernels were collected after milling and sieved through a 32-mesh screen to remove the flour from the particles. The milling out-turn percent of rice was calculated from the weight of 1000 particles of milled rice divided by that of brown rice. The flour of 13–15 portions in 25–35 portions was subjected to the amino acid analysis and nitrogen determination.

Amino Acid Analysis and Nitrogen Determination. The samples (ca. 4 mg of protein) were analyzed for amino acid content by hydrolyzing for 22 h at 110 °C with 6 N HCl (containing 0.02% 2-mercaptoethanol) in evacuated

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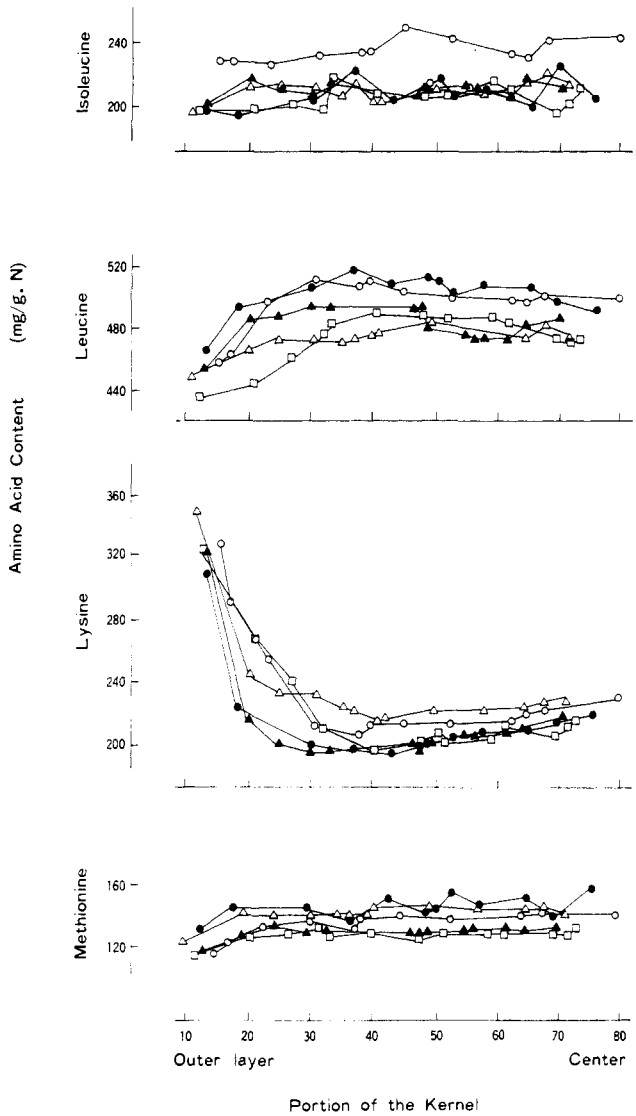

Figure 1. Distribution of Ile, Leu, Lys, and Met in milled rice. Rice varieties: ●, Nipponbare; ○, Koshihikari; △, Sasanishiki; ▲, Ohzora; □, Aoisora.

sealed tubes. The acid was evaporated in vacuo on a rotary evaporator and the residue dissolved in pH 2.2 citrate buffer and made up to a known volume. The hydrolyzed aliquots were analyzed on a Hitachi 835 amino acid analyzer with a column (15-cm length, 4-mm internal diameter) filled with 2619 ion-exchange resin. The sodium citrate elution buffers were prepared as follows: (a) pH 3.30, 0.2 N (Na^+), 13% ethanol; (b) pH 3.20, 0.2 N (Na^+), 2% ethanol; (c) pH 4.30, 0.2 N (Na^+); (d) pH 4.90, 1.2 N (Na^+), 0.5% benzyl alcohol.

Nitrogen content was measured by the Kjeldahl method.

Data Handling. The integrator output (nanomoles of each amino acid) served as the input for a computer program that can calculate for each amino acid micrograms/sample, microgram percent, and weight percent. Amino acid composition, not including Trp and Cys, is expressed as milligrams/gram of nitrogen (mg/g of N).

RESULTS AND DISCUSSION

The amino acid composition and nitrogen contents for five varieties of brown rice are shown in Table I. No marked differences in the amino acid composition of brown rice among five varieties were observed, whereas there were fluctuations in the nitrogen content among their varieties. The measured amino acid contents of brown rice are in good agreement with those found in the previous data (FAO, 1970; Juliano, 1985).

Table I. Amino Acid Composition (mg/g of N) and Nitrogen Contents (%) of Brown Rice

amino acid	varieties				
	Nipponbare	Koshihikari	Sasanishiki	Ohzora	Aoisora
Ile	231	221	223	191	195
Leu	514	452	464	465	472
Lys	231	244	248	244	238
Met	129	107	130	127	126
Phe	313	288	295	288	298
Tyr	328	266	295	341	327
Thr	214	206	191	217	218
Val	344	328	340	335	335
Arg	535	500	501	546	556
His	143	147	148	150	152
Ala	354	332	348	360	360
Asp	543	526	534	560	574
Glu	1024	952	959	1058	1083
Gly	283	277	290	296	297
Pro	318	280	273	285	304
Ser	306	281	288	316	322
N	1.24	1.12	1.02	1.11	1.20

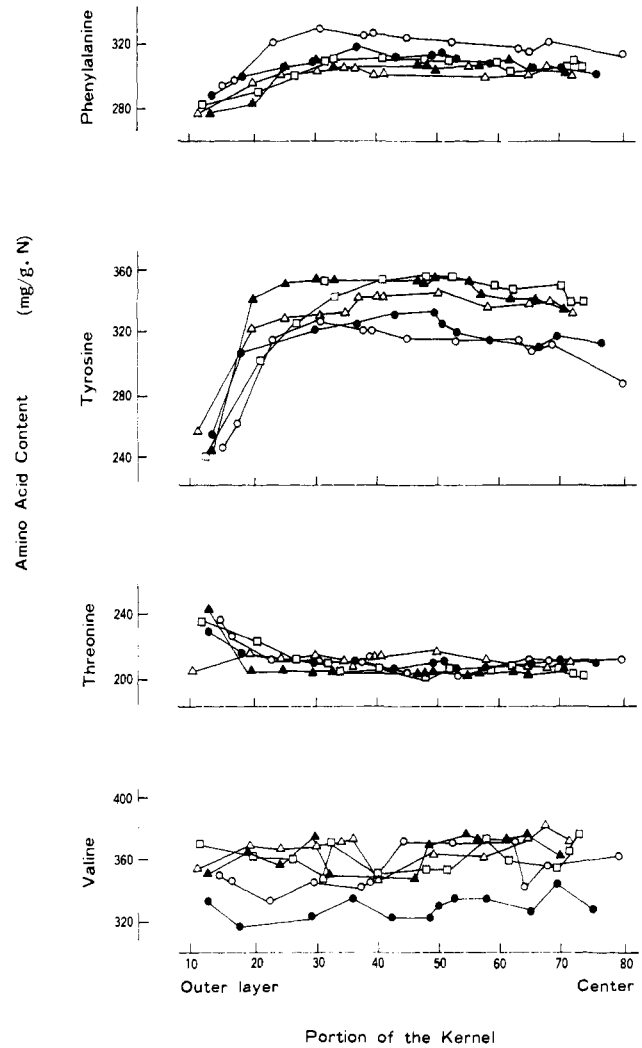

Figure 2. Distribution of Phe, Tyr, Thr, and Val in milled rice. Symbols for rice varieties are the same as those in Figure 1.

Distributions of amino acids from the surface to the center of the rice kernels are shown in Figure 1–4 plotted for 13–15 points for each rice flour fraction. The first point (10–15% of the kernel) contains the bran layer. Five patterns of amino acid contents in the radial distribution were found. The five patterns are as follows: (1) no change

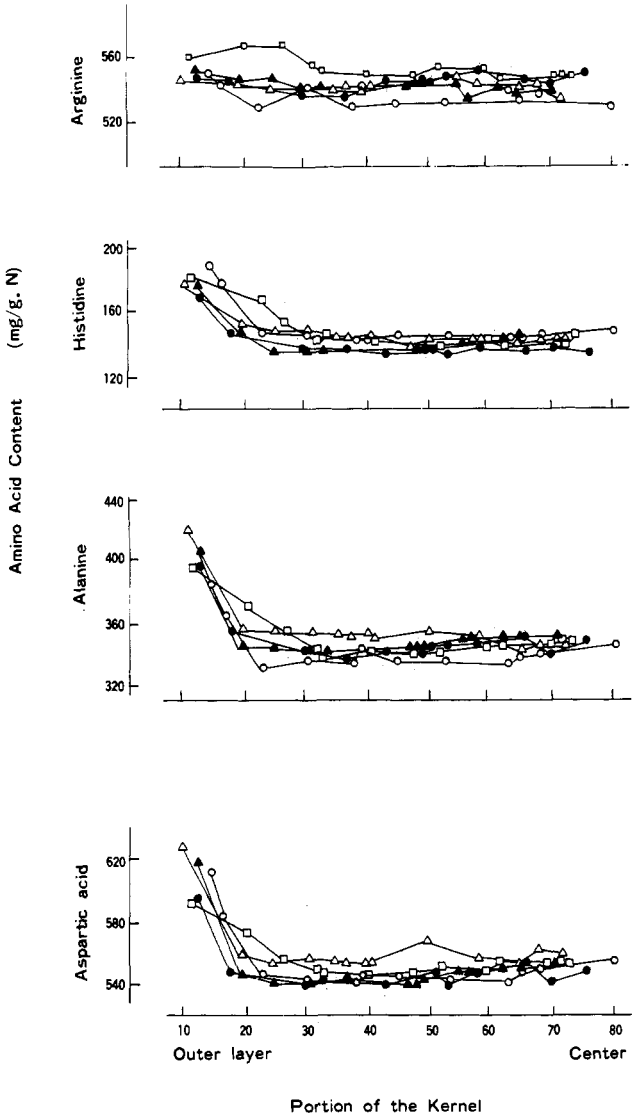

Figure 3. Distribution of Arg, His, Ala, and Asp in milled rice. Symbols for rice varieties are the same as those in Figure 1.

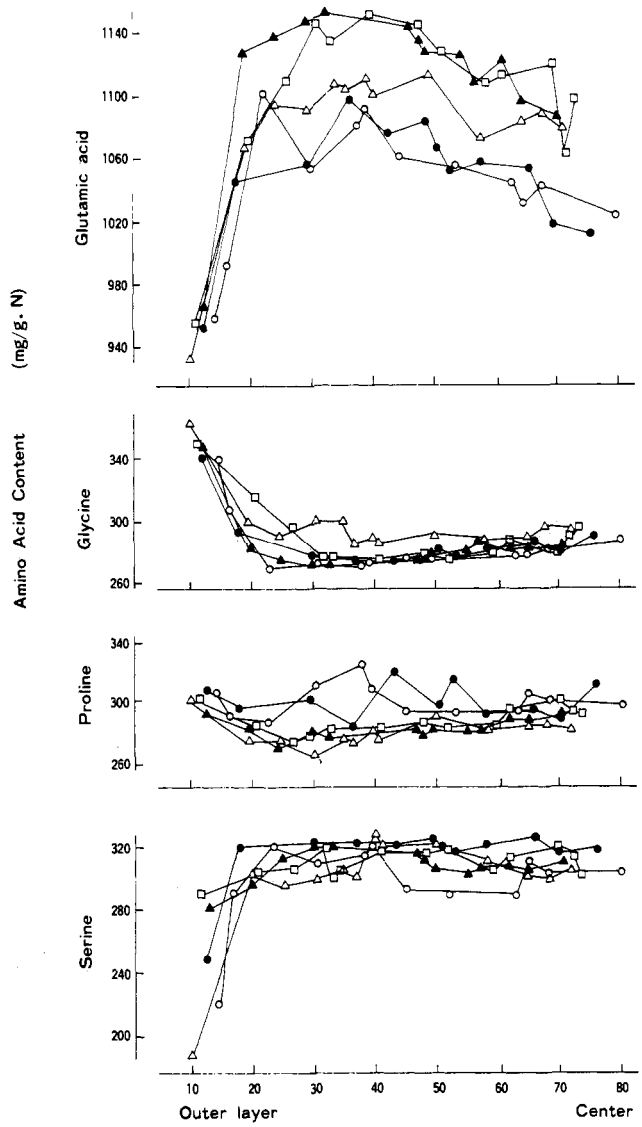

Figure 4. Distribution of Glu, Gly, Pro, and Ser in milled rice. Symbols for rice varieties are the same as those in Figure 1.

type (Ile, Val, Arg, Pro); (2) the type low in the outer layer, increasing in the middle layer, and decreasing slightly in the central layer (Leu, Phe, Tyr, Glu); (3) the type that is the mirror image of (2) (Lys, Gly); (4) the type low in the outer layer, increasing in the middle layer, and not changing in the central layer (Met, Ser); (5) the type that is the mirror image of (4) (Thr, His, Ala, Asp). However, there were no essential differences in the patterns between five varieties.

It has been known that percentages of glutelin and prolamin increase toward the middle layer of the rice kernel, while those of albumin and globulin decrease. Moreover, the albumin and glutelin exert a marked influence on the radical distribution of amino acids of rice because their proteins occupy the major portion of the kernel proteins (Yoshizawa and Kishi, 1985). The albumin and glutelin contain abundant amounts of lysine and glutamic acid, respectively (Juliano, 1985; Taira, 1962). From these findings it can be surmised that lysine content decreases toward the middle layer in contrast to the increase of glutamic acid content. The results of this study have substantiated the above inferences. Furthermore, we have found that lysine and glutamic acid contents show slight changes from the middle layer to the center.

The nutritive value of rice proteins is mainly influenced by lysine, the first limiting amino acid, content (FAO, 1973; WHO, 1973). The facts have also been pointed out by our previous studies using animals (Hayakawa et al., 1985). Taking into account the above information, the results obtained from this study suggest that the nutritive value of rice proteins varies in different layers; it may be high in the outer layer, low in the middle layer, and slightly higher in the center of rice kernel.

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Registry No. L-Ile, 73-32-5; L-Leu, 61-90-5; L-Lys, 56-87-1; L-Met, 63-68-3; L-Phe, 63-91-2; L-Tyr, 60-18-4; L-Thr, 72-19-5; L-Val, 72-18-4; L-Arg, 74-79-3; L-His, 71-00-1; L-Ala, 56-41-7; L-Asp, 56-84-8; L-Glu, 56-86-0; L-Gly, 56-40-6; L-Pro, 147-85-3; L-Ser, 56-45-1; N_2, 7727-37-9.

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Detecting Sugar Beet Syrups in Orange Juice by D/H and $^{18}O/^{16}O$ Analysis of Sucrose

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Sucrose from pure orange juices and beet sugar syrups was isolated by preparative HPLC. A portion was converted to sucrose octanitrate, to remove the readily exchangeable hydroxyl hydrogens. D/H ratios were then determined on the carbon-bound hydrogen atoms by mass spectrometric stable-isotope ratio analysis. The mean D/H ratios in orange sucrose were significantly elevated compared to those in beet sucrose, the δ(D) values being −27 and −143/mil respectively. $^{18}O/^{16}O$ ratios were determined for underivatized sucrose from both soures. With a single exception, $\delta(^{18}O)$ values were higher in orange sucrose than in beet sucrose. From these data, a discriminatory formula has been developed describing a 99.99% confidence ellipse about pure orange juices. The availability of this method to identify orange juices as pure or adulterated with beet sugar will provide a disincentive for the economic adulteration of orange juice.

Variations in the natural abundance of stable isotopes in plant components provide the means whereby adulteration of food products can be identified. Carbon isotopes have been especially useful in this regard. Most plants that are cultivated for food utilize the C_3 photosynthetic mode, with two major exceptions being the plants sugar cane and corn, which use the C_4 mode. C_4 plants and their metabolites are substantially enriched in ^{13}C compared to C_3 plants. Analysis of $^{13}C/^{12}C$ ratios thus readily identifies food products derived from C_3 plants that have been adulterated by the undeclared addition of low-cost sugar cane and corn-derived syrups. These adulterants bring no unique components to the mixture, and their sugar profiles are within the range found in most juices. So conventional nonisotopic analytical approaches cannot discriminate between pure and adulterated samples. Application of carbon isotope analysis by enforcement agencies has largely discouraged the addition of C_4 plant derived cane or high-fructose corn syrups to maple syrup (Hillaire-Marcel et al., 1977), apple juice (Doner et al., 1980), orange juice (Nissenbaum et al., 1974; Doner and Bills, 1982), honey (Doner and White, 1977), and fermented beverages (Bricout, 1982).

Carbon isotope analysis is not useful for identifying C_3 plant food products adulterated with inexpensive invert syrups prepared from the C_3 plant sugar beet, and no other useful procedures are available. This is now the major form of orange juice adulteration in the United States. The market value of orange juice is about $2 billion in the United States alone, so the economic impact of such adulteration is significant. Water in orange juice has higher deuterium and oxygen-18 concentrations than sugar beet invert syrup watter. This observation has permitted development of an isotopic method for detecting beet invert syrups added to orange juice (Brause et al., 1984). However, the availability of fourth-stage condensate water from orange juice concentrate production, enriched in oxygen-18 and deuterium, that can be added to orange juice along with sugar beet invert syrup has rendered this method of detecting adulteration ineffective.

We describe here a method that permits identification of orange juice samples to which have been added sugar beet invert syrups. The method is based on D/H and $^{18}O/^{16}O$ ratios in sucrose.

EXPERIMENTAL SECTION

Preparative HPLC of Sucrose from Orange Juice and Beet Invert Syrups. Prior to HPLC of orange juice, 25 mL of single-strength juice or 5 g of concentrate diluted to 10 mL with water was deionized by passing through a column of Amberlite MB-3 resin (50 mL). The column was rinsed with 50 mL of water, and the column eluant was lyophilized and dissolved in water to give 500 mg/mL solutions, which consisted primarily of the orange juice sugars fructose, glucose, and sucrose. The deionization step was unnecessary for the beet invert syrup samples (about 70° Brix). For these, 1.2 g of syrup was diluted with 1.8 mL of water. Of the diluted beet syrups or deionized orange juice samples 500 μL was injected onto an Aminex Q-15S (Ca^{2+} form) column (2.2 × 30 cm), which was then eluted at 2.0 mL/min with water at 85°C. Eluted peaks were monitored by refractive index detection, and the first peak (sucrose, retention time 15 min) was collected and

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