Distribution of Free Amino Acids in the Rice Kernel and Kernel Fractions and the Effect of Water Soaking on the Distribution

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The distribution of free amino acids within the milled-rice kernel was determined using the flours prepared consecutively by abrasive milling from the outer to the inner layers of the kernel. All free amino acids except arginine were present in the greatest quantities in the outermost layer of the 5%-milled kernel with a decreasing concentration gradient toward the center. Water soaking of the flours brought about increases in the contents of most amino acids and decreases in the glutamate and taurine contents. Taking into account the discriminative localization at the peripheral region of the milled-rice kernel and changes in contents during water soaking, glutamate, γ-aminobutyric acid (Gaba), and arginine can be assumed to contribute to the taste of cooked rice. Gaba content in the germ increased remarkably with soaking under a slightly acidic condition, which indicates the potential for rice germ to be included in the diet of people with hypertension.

INTRODUCTION

The distribution of the chemical constituents within the rice kernel has been extensively investigated (Normand et al., 1966; Houston, 1967; Hogan et al., 1968; Kennedy et al., 1974). Most components including proteins, fats, vitamins, and minerals have been found more abundantly in the outer than in the inner layers. By contrast, starch has been reported to be rich in the inner region. However, free amino acids, which play an important role in the tastes of various kinds of foods, have not been investigated for distribution within the rice kernel.

Although Okazaki et al. (1961) and Matuzaki et al. (1992) reported the correlation of the glutamate and arginine contents with the eating quality of rice, their findings have not been well accepted because the reported amino acid contents, being determined for the entire kernel and thus expressed as average values, were much lower than their thresholds.

Previously, we (Tajima et al., 1992) reported that flour prepared from the outermost layer of the milled-rice kernel was most tasteful and that water soaking, the process which in Japan has been traditionally practiced prior to rice cooking, brought about the formation of a considerable amount of oligosaccharides in the peripheral region through activating amylase(s). In this study, we determined the distribution of free amino acids within the rice kernel and examined the effects of water soaking on their contents for the purposes of better understanding the chemical nature of the rice kernel and of evaluating the contribution of free amino acids to the taste of rice.

MATERIALS AND METHODS

Rice. Koshihikari (*Oryza sativa* L. subsp. *japonica* Kato) was grown in an irrigated paddy field in 1992 and used throughout the study. This variety of rice is most popular in Japan for its excellent eating quality.

Fractionation of the Rice Kernel. Two hundred grams of the brown rice was milled to remove bran amounting to 5% by weight in a Model RMA-150 polishing machine (Yanagisawa Seiki Co., Ltd., Tokyo, Japan). The milled rice was further abrasively milled consecutively toward the center in a Model TM5 testing mill (Satake Co., Ltd., Hiroshima, Japan). The flours of the first two outermost fractions contained the particles resulting from the germ, which accounted for 3.2% of the weight of the brown rice. The flours and particles were separated from each other by passing through a 32-mesh screen. The flours thus prepared were fractionated according to the milling out-turn percent, which was calculated from the weight of the residual milled kernel divided by that of the brown rice. Fractionated flours were designated as follows: F1, 5–10%; F2, 10–14%; F3, 14–18%; F4, 18–23%; F5, 23–27%; F6, 27–100%. The remaining nucleus, F6, and the entire kernels were pulverized in a Retsch ultracentrifugal mill.

Amino Acid Analysis. Amino acids were extracted from 1.6 g of the flours with 4.0 mL of an 8% trichloroacetic acid solution in test tubes (2 × 12.5 cm) under shaking (100 strokes/min; 4-cm amplitude) at 30 °C for 1 h. For experiments on changes in free amino acid contents during water soaking, 1.6 g of the flours was suspended and incubated in 3.2 mL of deionized water. At appropriate periods 0.8 mL of a 40% (w/v) trichloroacetic acid solution was added to the suspensions to terminate the enzyme(s). The suspension was filtered through a 0.45-μm membrane filter (Advantec Co., Ltd., Tokyo, Japan). The filtrate was analyzed on a Hitachi L-8500 amino acid analyzer with a column (6-cm length, 4.6-mm i.d.) filled with P/N 855-3501 ion-exchange resin (Mitubishi Kasei Co., Ltd., Tokyo, Japan). The lithium citrate elution buffers were prepared as follows: (a) pH 2.8, 3% ethanol; (b) pH 3.7, 3% ethanol; (c) pH 3.6, 10% ethanol; (d) 0.2 M lithium hydroxide. Norleucine was used as an internal standard.

RESULTS AND DISCUSSION

Distribution of Free Amino Acids within the Rice Kernel. Distribution of free amino acids within the rice kernel, as well as in the entire kernels, is presented in Table 1. The germ contained a greater amount of free amino acids than did any other part of the kernel. Among the fractions prepared consecutively by abrasive milling of the 5%-milled kernel, the outermost layer, F1, contained all of the amino acids but arginine most abundantly, and a decreasing concentration gradient for each amino acid was observed toward the center of the kernel. For arginine, a concentration peak was observed between the F2 and F3 layers. Total content of free amino acids in F1 was 5.7 times as much as the content determined for the entire milled kernel. The relative ratio of the amount of each amino acid in F1 to the one in the entire milled kernel ranged from 2.2 for taurine to 8.9 for asparagine. Glutamate and aspartate accounted for 36 and 20%,

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0021-8561/94/1442-1122$04.50/0

Table 1. Distribution of Free Amino Acids in Rice Kernel (Milligrams per 100 g)

fraction	portion of kernel (%)	Tau	Asp	Thr	Ser	Asn	Glu	Gln	Pro	Gly	Ala	Val	Met	Ile	Leu	Tyr	Phe	Gaba	Lys	His	Arg	total
F1	5–10	6.4	59.7	4.8	18.1	23.1	109.8	2.2	5.6	4.1	22.5	4.1	5.2	2.2	3.5	2.8	2.6	12.9	4.0	3.0	5.7	302.3
		(0.2)[a]	(0.8)	(0.1)	(0.2)	(0.4)	(1.5)	(0.1)	(0.1)	(0.0)	(0.3)	(0.1)	(0.0)	(0.3)	(0.2)	(0.1)	(0.1)	(0.3)	(0.1)	(0.0)	(0.1)	(4.2)
F2	10–14	5.3	43.8	3.8	13.2	14.2	82.1	1.7	3.7	3.1	17.4	3.3	4.4	2.0	3.3	2.5	2.3	9.8	3.2	1.8	7.1	228.0
		(0.3)	(0.5)	(0.1)	(0.1)	(0.2)	(1.2)	(0.2)	(0.1)	(0.1)	(0.2)	(0.0)	(0.1)	(0.0)	(0.1)	(0.0)	(0.3)	(0.3)	(0.1)	(0.1)	(0.1)	(2.8)
F3	14–18	4.4	28.2	2.5	8.6	8.0	52.6	1.2	3.0	2.2	11.8	2.4	3.3	1.7	2.8	1.8	2.0	7.1	2.5	1.6	7.0	154.7
F4	18–23	3.7	15.5	1.7	5.7	5.2	32.9	0.7	1.4	1.6	7.9	1.6	2.9	1.6	2.8	0.8	1.8	4.3	1.5	0.8	3.1	97.4
F5	23–27	3.0	12.7	1.2	4.1	4.7	23.0	0.7	1.7	1.2	5.7	1.1	1.8	1.0	1.7	1.0	0.6	3.3	0.9	0.6	2.1	72.1
F6	27–100	2.5	1.9	tr[b]	0.7	ND[c]	3.9	tr	ND	0.3	0.8	tr	0.8	tr	0.5	tr	0.3	0.8	tr	tr	tr	14.1
milled rice	5–100	2.9	8.9	0.8	2.9	2.6	16.3	0.6	1.5	1.0	3.9	0.6	1.6	0.7	1.2	0.7	0.7	3.0	0.6	0.6	1.6	52.7
brown rice	0–100	3.5	16.7	1.0	3.6	3.6	21.0	0.7	1.6	1.1	4.9	0.7	1.8	0.8	1.3	0.8	0.8	3.8	0.7	0.6	1.6	70.6
bran	0–5	8.5	72.5	5.6	20.8	28.2	132.2	2.3	6.6	5.1	25.8	4.8	6.9	2.6	3.8	3.2	2.6	15.9	5.4	2.9	5.6	361.4
germ	–[d]	7.9	114.9	11.2	30.5	23.6	232.0	2.4	8.4	7.6	40.4	9.3	9.3	5.2	7.7	5.4	4.1	25.4	14.1	6.9	28.6	594.9
		(0.5)	(2.7)	(0.4)	(0.7)	(0.5)	(3.9)	(0.1)	(0.3)	(0.2)	(1.0)	(0.6)	(0.5)	(0.3)	(0.3)	(0.2)	(0.2)	(2.4)	(0.3)	(0.4)	(0.5)	(6.1)

[a] Standard deviations in parentheses, $n = 4$. [b] tr, values lower than 0.3 mg/100 g of the flour. [c] ND, not detectable. [d] Germ was collected from the flours of F1 and F2 fractions as described under Materials and Methods.

respectively, of the total content of free amino acids in F1. Glutamate, a well-known taste compound, was present in the milled-rice kernel at a concentration (16.3 mg/100 g) below its reported threshold (26 mg/100 g in water; Obara, 1973) when measured as an average content in the entire kernel. In F1, however, its concentration, even taking into account the dilution (about 2.3-fold) at the time of cooking, exceeded the threshold. Thus, at the surface of the milled-rice kernel, where one makes direct contact with one's tongue to taste, glutamate very likely contributes to the taste of cooked rice. This result is in accord with the findings reported by Okazaki et al. (1968) and Matuzaki et al. (1992) that there was a correlation between the glutamate content and the eating quality of rice in spite of its low concentration in the entire milled-rice kernel.

Effects of Water Soaking on Amino Acid Composition. During soaking of the flours of each fraction in water, changes in free amino acid contents were observed with variations in patterns and extents depending on types of amino acids. Representative data for the fluctuation of the free amino acid contents in F1 are presented in Figure 1. Most amino acids increased during soaking in water. Especially, a remarkable increase was observed for the γ-aminobutyric acid (Gaba) content; it reached 70 mg/100 g after a 4-h incubation at 30 °C. As our plain sensory test tentatively indicated that Gaba had a glutamate-like taste accompanied by sweetness with a threshold of roughly 30 mg/100 g in water, it might be possible that through water soaking Gaba exceeds its threshold to affect the taste of rice. In a 4-h incubation, arginine also exceeded its reported threshold (10 mg/100 g, Obara, 1973); by contrast, the glutamate content decreased to 42% of the initial value to become about twice its threshold. These results, together with our earlier findings (Tajima et al., 1992) of oligosaccharide formation during water soaking, coincide well with the Japanese traditional rice cooking. It recommends moderate water soaking prior to cooking: for 1–2 h in summer or for 4–8 h in winter.

These accumulations and declines of free amino acids are apparently due to enzyme actions triggered by the addition of water, which was evidenced by the fact that the addition of 10 mM Hg^{2+} to the soaking water stopped or retarded these changes (data not shown). As can be seen in Figure 2, the largest alteration in the free amino acid content was observed in F1, and the extent of the alteration decreased successively from the outer to the inner layers. In F6, changes were negligible. These results indicate that enzymes responsible for the formation and/or degradation of free amino acids are discriminatively localized at the peripheral region.

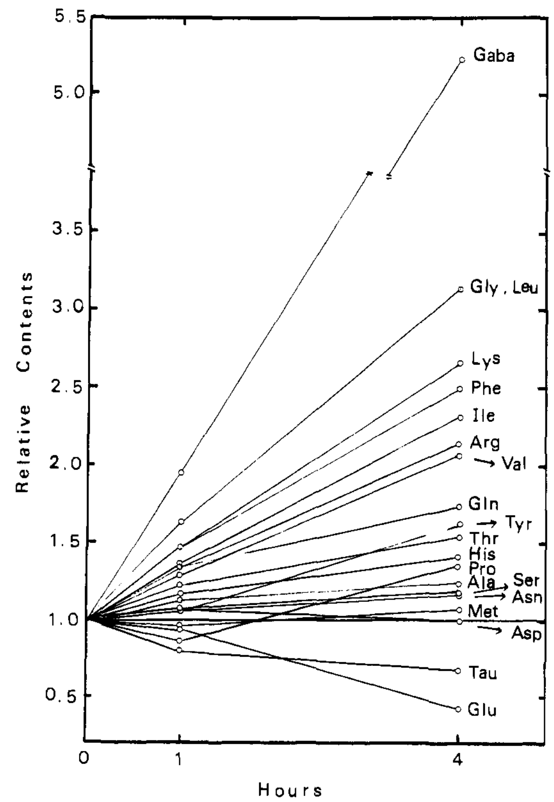

Figure 1. Changes in the amino acid contents of F1 flour during soaking in water at 30 °C. Contents after a 1- or 4-h soaking are indicated as relative contents to the initial ones.

Accumulation of Gaba in the Germ during Soaking in Water. Besides the possible contribution to the taste of rice, Gaba in rice is worth further investigating since it has been proved to be effective for lowering the blood pressures of mammals including dogs, rabbits, pigs, and cats (Stanton, 1963). Ohmori et al. (1987) clearly showed that green tea enriched with Gaba by anaerobic treatment (Tsushida et al., 1987) worked effectively to keep the blood pressure of spontaneously hypertensive rats (SHR) at a normal level. In Japan, Gaba-enriched tea has been produced on a commercial basis for people with hypertension. The remarkable accumulation of Gaba in rice was most clearly demonstrated in the germ. As shown in Figure 3, Gaba accumulation proceeded very rapidly at an early

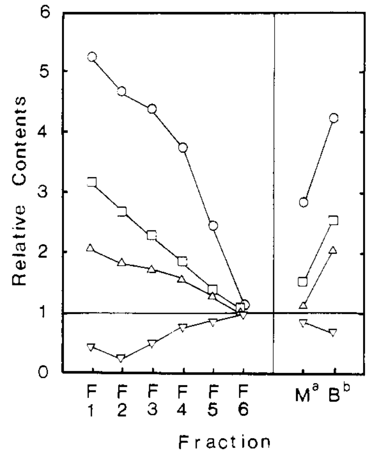

Figure 2. Changes in the Gaba (○), glycine (□), valine (△), and glutamate (▽) contents of the kernel fractions during water soaking at 30 °C. Contents after a 4-h soaking are indicated as relative contents to the initial ones. M[a], milled rice; B[b], brown rice.

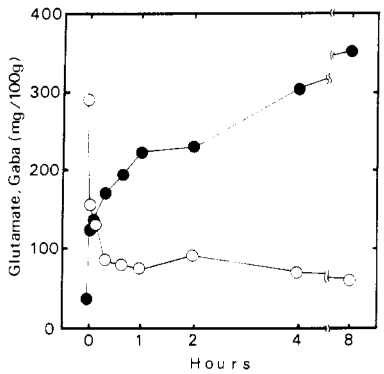

Figure 3. Changes in glutamate (○) and Gaba (●) contents of the germ during soaking in water at 40 °C.

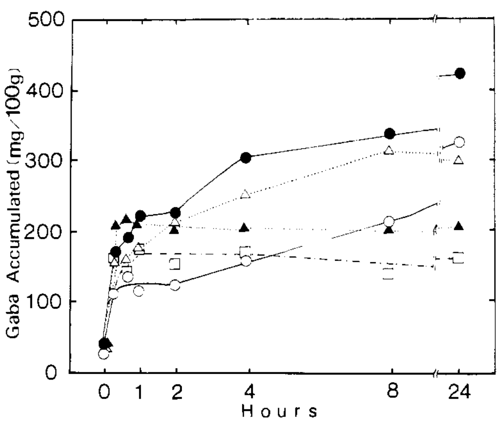

Figure 4. Influence of temperature on Gaba accumulation in the germ. The flour from the germ was incubated in water at 30 (○), 40 (●), 50 (△), 60 (▲), and 70 °C (□). Gaba contents were determined on a amino acid analyzer as described under Materials and Methods.

stage of incubation with a loss of glutamate. After 20 min, glutamate stopped decreasing, despite Gaba showing a progressive increase over that period, suggesting a supply of glutamate, probably through proteolysis. The optimal temperature and pH for Gaba accumulation were 40 °C and 5.5, respectively (Figures 4 and 5). In 20 min of incubation at 60 °C Gaba accumulation had been completely arrested, and at pH 8 it was very sluggish. As proved by Streeter et al. (1972) with radish leaves and by Wallace et al. (1984) with soybean leaves, an excess of glutamate decarboxylase over Gaba-pyruvate trans-

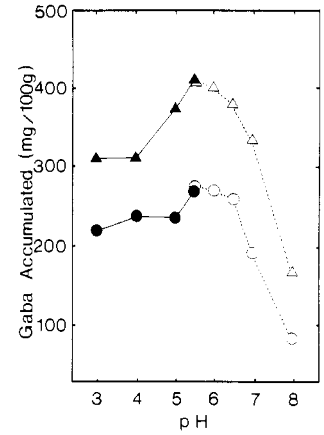

Figure 5. Influence of pH on Gaba accumulation in the germ. The flour from the germ was incubated at 40 °C in 0.1 M phosphate buffer (○, △) and citrate buffer (●, ▲) for 1 (○, ●) and 4 (△, ▲) h.

aminase is presumably responsible for the Gaba accumulation in the germ of rice. Its large accumulation under a slightly acidic condition and small accumulation under a basic condition are consistent with pH-activity responses reported (Streeter, 1972) for glutamate decarboxylase (optimum pH, 5.9) and Gaba-pyruvate transaminase (optimum pH, 8.9) from radish leaves. Gaba has been reported to accumulate in many plants under stresses including anaerobiosis (Streeter and Thompson, 1972), molybdenum deficiency (Possingham, 1957), abrupt transfer to a low temperature, and mechanical damage (Wallace et al., 1984). Kishinami (1987, 1988) observed a significant amount of Gaba accumulation in rice cells cultured under normal conditions with addition of ammonium, but not in intact rice plants. Accumulation of a large quantity of Gaba in rice grain was reported for the first time in this study. Whether or not it was induced by the mechanical damage of milling remains unclear.

The amount of Gaba in the germ after a 4-h incubation at 40 °C and pH between 5.0 and 6.5 reached twice the amount contained in Gaba-enriched tea (about 170 mg/100 g of dry weight), which indicates the possibility that the rice germ can be used as a source of Gaba. For estimating more precisely the contribution of free amino acids to the taste of cooked rice and the function of Gaba in the germ, the effects of heating in addition to those of water soaking have next to be investigated.

ACKNOWLEDGMENT

We are grateful to Ms. Sanae Kodoi for excellent technical assistance. This work was supported in part by a grant-in-aid (Super Rice Program) from the Ministry of Agriculture, Forestry and Fisheries of Japan.

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Received for review October 11, 1993. Accepted March 14, 1994.[⊗]

[⊗] Abstract published in *Advance ACS Abstracts*, April 15, 1994.