reaction of β-mercatoacetaldehyde/ammonium sulfide. Ethyl 1-(ethylthio)ethyl disulfide was identified and has been reported in the volatiles generated from thermal decomposition of cystine in water (Shu et al., 1985).

Thiazoles were among the most important compounds identified in fried chicken and french-fried potato flavors. A total of 16 were identified in fried chicken flavor (Tang et al., 1983) and 50 in french-fried potato flavor (Carlin, 1983). Thiazoles possess potent sensory characteristics. Several of the thiazoles identified in our studies have odors that are reminiscent of fried food and could be important contributors to that aspect of fried chicken or french-fried potato flavor. Takken et al. (1976) proposed a mechanism for the formation of thiazoles involving aldehydes, hydrogen sulfide, and ammonia.

Reaction experiments between selected aldehydes and ammonium sulfide allowed us to identify 3-methyl-5-butyl-1,2,4-trithiolane and 3-methyl-5-pentyl-1,2,4-trithiolane in fried chicken flavor and 2-pentyl-3,5-dibutylpyridine in french-fried potato flavor. Other compounds identified such as the 1,3,5-dithiazines have potent sensory characteristics but have not yet been detected in food. The mass spectral data are listed for all compounds identified in the hope that they will assist researchers in the characterization of food flavors.

ACKNOWLEDGMENT

New Jersey Agricultural Experiment Station, Publication No. 10501-2-85, supported by State Funds. We thank Joan B. Shumsky for her secretarial aid.

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Received for review August 15, 1985. Revised manuscript received November 27, 1985. Accepted February 19, 1986.

Lipid Content and Fatty Acid Composition of Indica and Japonica Types of Nonglutinous Brown Rice

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The lipid content and fatty acid composition of Indica and Japonica types of nonglutinous brown rice were investigated on 20 cultivars each of both types. The Indica type, as compared with the Japonica type, gave significantly higher palmitic acid, stearic acid, and arachidic acid contents and lower lipid content and linoleic acid, eicosenoic acid, and lignoceric acid contents. As to to the relationship between fatty acid contents, there were the highest and negative correlations between oleic acid and linoleic acid in both types. The scatter diagram between both fatty acids could be divided into Indica and Japonica types.

In previous work, it was shown that the lipid content and fatty acid composition of brown rice were influenced by nonglutinous and glutinous types (Taira et al., 1981; Taira and Hiraiwa, 1982), cropping year (Taira et al., 1979a), and cropping season (Taira et al., 1979b). Rice can be classified into at least two groups, Indica type (*Oryza sativa* L. subsp. *indica* Kato) and Japonica type (*O. sativa* L. subsp. *japonica* Kato). As to the difference of chemical composition between both types of rice, Indica type, as compared with Japonica type, is higher in amylose content of nonglutinous starch (Hsieh et al., 1964; Juliano et al., 1964; Horiuchi and Tani, 1966). Little information, however, is available on the difference of the lipid content and fatty acid composition between both types of rice. Therefore, investigations were undertaken to study the lipid content and fatty acid composition of nonglutinous brown rice using Taiwanese cultivars of Indica and Japonica Types.

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METHODS AND MATERIALS

Mature grains of 20 nonglutinous cultivars each of Indica and Japonica types were collected from a field experiment conducted as second crop by the Chiayi Agricultural Experiment Station, Taiwan, in 1980. The transplanting time

0021-8561/86/1434-0542$01.50/0

Table I. Heading Date, 1000-Kernel-Weight, Lipid Content, and Fatty Acid Composition of Indica and Japonica Types of Brown Rice

cultivar	heading date	1000-kernel-wt, g	lipid, % of dry wt	fatty acid[a] (wt % of total acids)										
				14:0	16:0	16:1	18:0	18:1	18:2	18:3	20:0	20:1	22:0	24:0
Indica type														
Taichung native No. 1	Sep 23	17.1	2.72	0.3	20.6	0.2	2.1	39.9	32.4	1.8	0.9	0.6	0.5	0.9
Tainung-sen No. 12	Sep 26	23.0	2.41	0.4	18.9	0.2	2.0	41.4	33.3	1.4	0.8	0.6	0.4	0.6
Kaosen No. 2	Sep 27	18.9	2.81	0.3	19.7	0.2	1.8	40.4	32.9	1.8	1.0	0.7	0.5	0.8
Chianung-sen-yu No. 19	Sep 29	18.1	2.76	0.2	18.9	0.2	2.0	38.3	35.6	1.6	1.0	0.7	0.5	0.9
Taichung-sen No. 10	Sep 29	20.2	2.71	0.3	18.2	0.2	2.1	42.5	32.4	1.5	0.9	0.6	0.4	0.8
Chianung-sen-yu No. 12	Sep 30	20.7	2.87	0.2	18.7	0.2	1.9	46.5	28.8	1.1	0.8	0.6	0.4	0.8
Chianung-sen No. 11	Sep 30	17.6	2.91	0.2	18.0	0.2	1.9	45.2	30.4	1.3	0.9	0.6	0.4	0.8
Chianung-sen-yu No. 14	Oct 3	19.4	2.68	0.3	19.9	0.2	1.8	42.8	31.4	1.3	0.8	0.5	0.4	0.6
Chianung-sen-yu No. 15	Oct 3	16.7	2.63	0.2	19.7	0.2	1.6	42.9	31.7	1.4	0.8	0.6	0.4	0.6
Chianung-sen-yu No. 17	Oct 3	19.7	2.63	0.2	19.1	0.2	1.9	41.3	33.3	1.4	0.9	0.6	0.4	0.8
Chianung-sen-yu No. 20	Oct 3	19.8	2.87	0.3	19.0	0.3	2.0	44.1	30.4	1.3	0.9	0.6	0.4	0.8
Taichung-sen No. 5	Oct 4	17.3	2.40	0.2	19.5	0.2	2.0	41.1	32.8	1.6	0.9	0.6	0.4	0.8
Kaosen No. 7	Oct 5	20.3	2.77	0.3	18.3	0.2	2.1	43.8	31.3	1.3	0.9	0.5	0.4	0.8
Chianung-sen-yu No. 23	Oct 5	19.8	2.44	0.2	19.1	0.3	2.1	43.1	30.8	1.3	1.1	0.6	0.5	1.0
Chianung-sen No. 6	Oct 5	17.1	2.63	0.3	18.4	0.2	1.8	44.5	30.5	1.5	0.9	0.7	0.5	0.8
Kaohsiung-sen-yu No. 104	Oct 6	20.7	2.72	0.3	18.4	0.2	2.2	45.3	30.0	1.3	0.8	0.5	0.4	0.8
Chianung-sen-yu No. 18	Oct 6	18.3	2.38	0.2	18.8	0.2	1.9	40.4	34.4	1.4	0.9	0.6	0.5	0.8
Chianung-sen-yu No. 25	Oct 6	23.5	2.65	0.3	18.6	0.2	1.8	43.1	31.9	1.4	0.8	0.6	0.5	0.9
Chianung-sen-yu No. 21	Oct 9	20.4	2.84	0.3	18.6	0.3	1.8	45.0	29.9	1.3	0.8	0.7	0.5	0.9
Chianung-sen-yu No. 22	Oct 10	21.4	2.58	0.3	19.5	0.3	1.8	44.7	29.4	1.2	0.8	0.6	0.4	0.8
mean	Oct 3	19.5	2.67	0.27	19.00	0.22	1.93	42.82	31.68	1.41	0.88	0.61	0.44	0.81
SD	4	1.9	0.16	0.06	0.66	0.04	0.15	2.14	1.73	0.18	0.08	0.06	0.05	0.09
Japonica type														
Kaohsiung-yu No. 1152	Sep 18	18.0	3.22	0.2	16.4	0.2	1.9	43.4	33.9	1.4	0.8	0.7	0.4	0.8
Taichung No. 187	Sep 23	18.9	2.76	0.3	16.2	0.2	1.7	40.9	36.4	1.4	0.9	0.8	0.4	0.9
Tainan No. 6	Sep 23	18.4	3.09	0.2	15.2	0.2	1.8	43.3	34.9	1.3	0.8	0.8	0.5	1.1
Si-pi 591134	Sep 24	21.9	2.66	0.2	15.5	0.2	2.1	42.8	34.8	1.3	0.9	0.7	0.5	0.9
C 247	Sep 24	23.6	2.90	0.2	15.8	0.2	2.0	49.2	28.7	1.2	0.8	0.7	0.4	0.8
Taipei No. 309	Sep 24	21.1	2.60	0.3	15.9	0.2	1.7	42.7	34.8	1.4	0.8	0.8	0.4	0.9
Taichung No. 65	Sep 24	20.4	2.71	0.3	15.2	0.2	2.0	43.0	35.0	1.3	0.9	0.7	0.4	0.9
Hsinchu No. 56	Sep 25	19.1	3.04	0.2	15.6	0.2	1.8	46.2	31.8	1.2	0.9	0.8	0.4	0.9
Tainung No. 61	Sep 25	21.5	2.65	0.2	14.9	0.2	1.7	48.8	29.6	1.4	0.8	0.9	0.5	1.0
Kaohsiung No. 140	Sep 26	17.6	3.03	0.2	15.9	0.2	1.7	42.9	34.6	1.3	0.8	0.8	0.5	1.0
Taitung No. 27	Sep 26	17.3	2.83	0.3	15.6	0.2	1.6	41.8	36.7	1.3	0.7	0.7	0.4	0.8
Taitung No. 29	Sep 26	19.8	2.65	0.4	16.4	0.3	1.7	41.6	35.5	1.4	0.7	0.7	0.4	0.9
Tainung No. 67	Sep 26	20.0	2.54	0.2	15.6	0.2	1.9	49.5	28.5	1.3	0.7	0.8	0.4	0.9
Chianan No. 8	Sep 28	19.1	3.24	0.2	16.0	0.2	1.9	44.4	33.2	1.2	0.9	0.7	0.4	0.9
Kaohsiung No. 135	Sep 28	18.0	3.58	0.2	15.9	0.2	2.1	43.9	33.6	1.3	0.8	0.6	0.4	0.9
Hualien No. 18	Sep 28	20.2	2.64	0.3	15.3	0.2	1.6	43.4	34.9	1.5	0.7	0.7	0.4	0.9
Si-pi 59140	Sep 30	21.5	2.72	0.2	15.5	0.2	1.9	44.9	33.0	1.2	0.9	0.8	0.4	0.9
C 243	Sep 30	22.0	2.86	0.3	15.9	0.3	1.9	42.5	35.4	1.4	0.8	0.6	0.4	0.7
Taitung No. 28	Sep 30	19.7	2.97	0.2	15.1	0.2	1.7	42.8	36.1	1.3	0.7	0.6	0.4	0.9
C 246	Oct 1	18.7	2.89	0.2	16.7	0.2	1.8	44.5	32.4	1.2	0.9	0.7	0.4	0.9
mean	Sep 26	19.8	2.88	0.24	15.73	0.21	1.83	44.13	33.69	1.32	0.81	0.73	0.42	0.90
SD	3	1.7	0.26	0.06	0.47	0.03	0.15	2.48	2.42	0.09	0.08	0.08	0.04	0.08
diff between types	**	ns	**	ns	**	ns	*	ns	**	ns	*	**	ns	**

[a] Fatty acids are expressed as the ratio of number of carbons to the number of double bonds. Key: ns = not significant; * = significant at the 5% level; ** = significant at the 1% level.

was July 23. The heading dates are shown in Table I. Amounts of fertilizer per hectare were as follows: N, 60 kg; P_2O_5, 80 kg; K_2O, 80 kg as basal dressing; N, 30 kg each as twice top dressings. The grain samples were dehulled by using conventional seed-cleaning equipment and ground to pass a 0.5-mm-diameter sieve. Lipid was extracted from the ground samples on a Butt-type extractor with diethyl ether as a solvent. Fatty acids in the lipid were determined by gas chromatography after transesterification to their methyl ester by the boron trifluoride method as outlined by the Association of Official Analytical Chemists (1975). Esters were separated by using a Shimadzu GC-6APF chromatograph equiped with a FID and 3 mm × 3 m glass column packed with Unisol 3000 Uniport C, 80–100 mesh (Gasukurokogyo Co., Ltd.). The column temperature was 240 °C, and the carrier gas was nitrogen at a flow rate of 40 mL/min. Compound retention times and areas were automatically recorded by means of a Shimadzu Chromatopac C-R2A. Standard methyl ester fatty acid mixtures were separated under identical conditions to identify the compounds and to calculate the response factors of the acids. Moisture content was determined by heating the ground samples for 1 h at 135 °C, and lipid contents were reported on a dry basis of grain samples.

RESULTS AND DISCUSSION

The lipid contents and fatty acid compositions of the Indica and Japonica types of brown rice are shown as mean values of duplicated data in Table I. The significant test of difference between both types for lipid and fatty acid contents, 1000-kernel-weight, and heading date have been carried out by the Student's t-test. The results of the test are also shown in Table I.

The Indica type, as compared with the Japonica type, gave significantly lower lipid content. The Indica type/Japonica type ratio of the mean value was 0.93.

As for the fatty acid composition, the Indica type, as compared with the Japonica type, was significantly higher

Table II. Correlation Coefficients of Lipid Content with Fatty Acid Content and Fatty Acid Content Pair of Indica and Japonica Types of Brown Rice[a]

	14:0	16:0	16:1	18:0	18:1	18:2	18:3	20:0	20:1	22:0	24:0
						Indica Type					
lipid	0.046	−0.218	0.038	−0.048	0.384	−0.394	−0.101	−0.096	0.107	−0.086	−0.039
14:0		0.009	0.087	0.126	0.096	−0.142	0.084	−0.366	−0.096	−0.036	−0.029
16:0			0.043	−0.208	−0.475*	0.201	0.454*	0.046	0.001	0.118	−0.201
16:1				−0.017	0.337	−0.461*	−0.384	0.123	0.170	0.102	0.366
18:0					−0.110	0.096	0.086	0.347	−0.368	−0.098	0.480*
18:1						−0.942**	−0.774**	−0.458*	−0.265	−0.382	−0.122
18:2							0.643**	0.380	0.227	0.300	0.061
18:3								0.434	0.429	0.476*	0.154
20:0									0.334	0.452*	0.512*
20:1										0.623**	0.372
22:0											0.597**
						Japonica Type					
lipid	−0.446*	0.234	−0.161	0.333	−0.097	0.070	−0.322	0.161	−0.401	−0.042	0.017
14:0		0.197	0.629**	−0.406	−0.551*	0.535*	0.583**	−0.313	−0.154	−0.343	−0.277
16:0			0.304	0.055	−0.266	0.091	−0.062	0.175	−0.220	−0.385	−0.360
16:1				−0.056	−0.287	0.249	0.332	−0.260	−0.341	−0.167	−0.394
18:0					0.259	−0.317	−0.426	0.462*	−0.325	0.000	−0.200
18:1						−0.979**	−0.361	−0.018	0.373	0.067	0.078
18:2							0.379	−0.069	−0.382	−0.046	−0.064
18:3								−0.481*	0.008	0.059	−0.062
20:0									0.200	0.098	0.089
20:1										0.448*	0.581**
22:0											0.652**

[a] Key: * = significant at the 5% level; ** = significant at the 1% level.

in palmitic, stearic, and arachidic acid contents and lower in linoleic, eicosenoic, and lignoceric acid contents. The Indica type/Japonica type ratios of the mean value were 1.21 in palmitic acid, 1.09 in arachidic acid, 1.05 in stearic acid, 0.94 in linoleic acid, 0.90 in lignoceric acid, and 0.84 in eicosenoic acid.

In regard to the variation of lipid content of brown rice, it was reported that the earlier the cropping season, the more the value in nonglutinous Japonica type and that the value showed significant positive correlation with daily mean temperature during ripening (Taira et al., 1979b). Further, it was recognized that glutinous grain had more lipid than nonglutinous grain in brown rice (Taira et al., 1981; Taira et al., 1982) and milled rice (Taira et al., 1982) and that glutinous milled rice, as compared with nonglutinous milled rice, contained more nonstarch lipid that corresponded to the lipid of our experiment (Choudhury and Juliano, 1980). Indica type, as compared with Japonica type, was the earlier heading date (Table I), or cropping season in this study, and had higher amylose content of noglutinous starch (Hsieh et al., 1964; Juliano et al., 1964; Horiuchi and Tani, 1966). Accordingly, the difference in lipid content between Indica type and Japonica type may be due to in part the effect on temperature during ripening and also amylose contents of the starch.

As for the fatty acid composition of nonglutinous brown rice of Japonica type, it was reported that the earlier the cropping season, the more the myristic, palmitoleic, stearic, oleic, and arachidic acid contents and the less the linoleic and linolenic acid contents and that daily mean temperature during ripening showed significantly positive correlations with myristic, palmitoleic, stearic, oleic, and arachidic acid contents and negative correlations with linoleic and linolenic acid contents (Taira et al., 1979b). In spite of the earlier heading date, Japonica type, as compared with Indica type, showed higher linoleic acid content and lower stearic acid content and showed no difference in palmitoleic, oleic, and linolenic acid contents in this study. Further, palmitic acid content was not affected by cropping season (Taira et al., 1979b) but showed significant difference between Indica and Japonica types and the highest Indica type/Japonica type ratio of the mean value among the fatty acids in this study. On the basis of the results, it is presumed that Indica and Japonica types differ in fatty acid composition of brown rice and further that bran and milled rice may differ in fatty acid composition between both types.

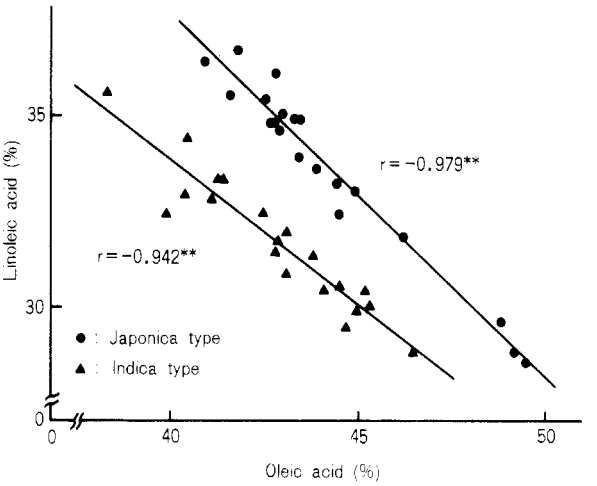

Figure 1. Relationship between oleic acid and linoleic acid contents of Indica and Japonica types. Indica type: $y = -0.76x + 64.26$. Japonica type: $y = -0.96x + 75.85$.

Correlation coefficients of lipid content with fatty acid content and fatty acid content pair of Indica and Japonica types of brown rice are shown in Table II. For both types, there were significant positive correlations between eicosenoic acid–behenic acid and eicosenoic acid–lignoceric acid and significant negative correlations between oleic acid–linoleic acid. The coefficients between oleic acid–linoleic acid showed the highest in both types. As to the relationship between fatty acid contents in cereals, it was reported that there were the highest and negative correlations between oleic acid and linoleic acid in Japonica type rice (Taira et al., 1979a,b), Japanese barnyard millet *Echinochloa crus-galli* Beauv. var. *frumentacea* Wight

(Taira, 1983a), foxtail millet *Setaria italica* Beauv. (Taira, 1984), pearl millet *Pennisetum americanum* (L.) K. Shum. (Jellum and Powell, 1971), Job's tears *Coix lacryma-jobi* L. var. *ma-yuen* Stapf (Taira et al., 1985), and corn *Zea mays* L. (Jellum, 1970). Figure 1 shows the relationship between oleic and linoleic acid contents of Indica and Japonica types. The scatter diagram could be divided into Indica and Japonica type groups. In the case of the same oleic acid content between both types, it was shown that Indica type was lower than Japonica type in linoleic acid content. According to the diagram, it is supposed that both groups may be caused by the difference between Indica and Japonica types in heading date or temperature during ripening. In the previous study of brown rice of 24 Japonica type cultivars in normal and lower temperature cropping years, however, the scatter diagram showed as one group (Taira, 1983b). Consequently, it is presumed that both groups in the scatter diagram are due to the difference between Indica and Japonica types.

Registry No. Palmitic acid, 57-10-3; stearic acid, 57-11-4; arachidic acid, 506-30-9; linoleic acid, 60-33-3; eicosenoic acid, 28933-89-3; lignoceric acid, 557-59-5; oleic acid, 112-80-1.

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Received for review August 22, 1985. Revised manuscript received January 3, 1986. Accepted January 27, 1986.

Relationship of Disulfide Bonds to the Maintenance of the Active Secondary Structure of Alfalfa (*Medicago sativa*) Leaves Protease Inhibitor

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The polypeptide inhibitor of the alfalfa leaves serine protease contains four disulfide bridges and a high (40–50%) α-helix secondary structure content for an M_r of 6300. The progressive reduction of the four disulfide bridges results in a progressive loss of both the α-helix secondary structure content and the biological activity.

Most of the plant polypeptides with antiproteolytic activity have two common structural peculiarities: i.e. a random-coil structure and a high content of disulfide bridges. Furthermore, all the so far described leaf inhibitor polypeptides show activity only toward nonplant proteases (Ryan, 1981a).

We have recently described (Gonnelli et al., 1985) in alfalfa leaves a novel type of polypeptide showing high specificity and efficiency toward a neutral protease purified from the same source, giving 100% inhibition when the enzyme active site/polypeptide molar ratio is 1. It has four disulfide bonds per M_r of 6300 and an organized secondary structure with a α-helix content ranging between 40 and 50%.

Even though the reduction of disulfide bonds renders the plant polypeptides more susceptible to both thermal denaturation and proteolytic degradation by plant sulfhydryl enzymes they have no particular relevance on the mechanism of inhibition. Plunkett and Ryan (1980) have shown that the potato inhibitor I fully retains its original inhibitory activity after reduction and alkylation of the single monomeric disulfide bond. It has been postulated (Plunkett and Ryan, 1980) that the combined action of S–S bond reduction and the subsequent proteolysis could be important in the in vivo regulation of plant proteolytic activity.

The high content of α-helix secondary structure is a particular feature of alfalfa protease inhibitor polypeptide. To our knowledge (Laskowski and Sealock, 1971) only pancreatic trypsin inhibitor shows some α-helix secondary structure.

Here we report that the progressive reduction of the four disulfide cross-links of the alfalfa protease inhibitor polypeptide brings out a parallel loss of α-helix secondary structure and that this fact per se causes the loss of biological activity.

EXPERIMENTAL SECTION

Materials. The alfalfa leaves protease was purified according to Tozzi et al. (1981); its specific leaf polypeptide inhibitor was purified according to Gonnelli et al. (1985). All the other chemicals were of the highest commercially available quality.

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0021-8561/86/1434-0545$01.50/0