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Mineral and Amino Acid Contents of Kinema, a Fermented Soybean Food Prepared in Nepal

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Five sun-dried Kinema samples were collected from the eastern hills of Nepal and their proximate composition, mineral contents and amino acid compositions were analyzed. The Kinema contained 6.0±0.3% (mean±s.d., dry matter base) ash, and potassium comprised about 30% of the ash prepared from the Kinema sample. The average value of the trichloroacetic acid-soluble nitrogen (TCA-N) content relative to the total nitrogen content of the Kinema samples was 35.3% (s.d.=6.6). The percent liberation of amino acids (ratio of the sum of the free amino acid contents to the sum of the total amino acid contents) of the Kinema samples ranged from 6.0% to 16.5% with an average value of 11.4% (s.d.=4.3). When these values were compared with those of the Thua-nao sample obtained in Thailand and the Natto sample prepared in Japan, a higher TCA-N relative to total nitrogen was observed in the Natto sample. The pattern similarities of free amino acid compositions between the samples were calculated and showed that the samples were classified into 2 groups, the soybean sample and the group of the fermented soybean samples. The positions of the Kinema samples were closer to the position of the Natto sample rather than the Thua-nao sample.

Keywords: Kinema, Thua-nao, Natto, mineral, amino acid

Kinema is a traditional non-salted fermented soybean food prepared in the eastern hills of Nepal, the Darjeeling hills and Sikkim in India (Karki, 1986; Tamang *et al.*, 1988). To make traditional Kinema (Karki, 1986; Tamang *et al.*, 1988), soybeans are washed, soaked, boiled until softened and cracked by pounding lightly. A small amount of firewood ash is often added. The role of the firewood ash is not yet clear. Karki (1986) mentioned that Kinema could be prepared in the laboratory without addition of firewood ash. It is considered that firewood ash may be added to increase the pH value of cooked soybeans to inhibit growth of other microorganisms such as fungus. The mixture is wrapped with banana leaves (Karki, 1986) or fresh fern leaves (Tamang *et al.*, 1988) and allowed to ferment for 1 to 3 days. The fermented soybean product (fresh Kinema) is often sun-dried and stored.

Karki (1986) investigated the chemical composition and microflora of Kinema. Sarkar *et al.* (1994) also investigated the proximate components and microflora of Kinema and have reported that *Bacillus subtilis* is the most dominant and it is the sole fermenting organism for Kinema production (Sarkar & Tamang, 1994). Karki (1986) and Tamang *et al.* (1988) have written that Kinema is very similar to Natto of Japan. However, nowadays, Natto is produced by a controlled fermentation process using a pure culture starter, while Kinema is allowed to undergo natural fermentation without adding a starter. Furthermore, firewood ash is not used in the Natto making process. According to Ueda (1989), the flavor of Natto is produced during the fermentation of soybeans with *B. subtilis* (*natto*), and the taste comes mainly from the hydrolyzate of the soybean proteins. However, sufficient information is not available on the minerals and protein hydrolysis of Kinema. Therefore, we collected Kinema samples in Nepal, analyzed their chemical compositions, particularly the minerals and amino acid compositions, and compared Kinema with Natto of Japan and Thua-nao, another Natto-like non-salted fermented soybean food prepared in Thailand (Sueana-Adth *et al.*, 1986), based on the influence of the addition of firewood ash and taste development.

Materials and Methods

Samples As shown in Table 1, sun-dried Kinema samples were collected from producing areas in the eastern hills of Nepal in pre-sterilized plastic bags in 1992. Among them, sample D1 was prepared at Hille located in these areas as follows:

Soybeans (1.5 kg) with brown hulls were washed, boiled for 4 h and cracked lightly using a wooden mortar and a wooden pestle. They were then placed in a bamboo basket

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Table 1. Sun-dried Kinema samples from the eastern hills of Nepal.

Sample code	Locality	Hull of soybean
D1	Hille	Brown
D2	Hille	Brown
D3	Hille	Brown
D4	Hille	Brown
D6	Shirdhuwa	Yellow

lined with banana leaves which had been dusted with a small amount of firewood ash followed by occasional dusting with a small amount of ash and covered with banana leaves. The bamboo basket was covered with a jute bag and left beside an earthen fire oven to keep it warm. After 3 days of fermentation, the fermented soybeans (fresh Kinema) were spread on a bamboo tray lined with newspapers and sun-dried for 2 days.

One sample of soybeans with brown hulls was also collected at Hille. The sample of sun-dried Thua-nao chips (12 cm in diameter, about 16 g apiece) was collected from Mae Hong Song located in the northern part of Thailand in 1992 (Okada, 1993) and stored in a deep freezer. The Natto sample (Takano Foods Co., 85 g in a package) was purchased at a supermarket in Japan and stored in a refrigerator.

Analytical methods

Preparation of samples for chemical analysis The Kinema samples, soybean sample and the Thua-nao sample were homogenized in liquid nitrogen using a homogenizer (Iwatani Co. model IFM 140). The Natto sample was lyophilized and homogenized. These homogenized sample powders were transferred to plastic bags and stored at −20°C.

Determination of moisture The moisture contents of the powdered samples were calculated by drying at 130°C for 1 h (Tsutsumi, 1982a). The moisture in the fresh Natto sample was determined according to the Methods of Natto Research (Society for study of Natto, 1990) by 2-h drying at 130°C.

Determination of nitrogen Total nitrogen was determined by the Kjeldahl method. Trichloroacetic acid-soluble nitrogen (TCA-N) was determined by homogenizing the powdered sample (100 mg) in 5 ml 5% TCA and leaving it overnight. After centrifugation at 15,000 rpm (20,000×g) for 15 min, the supernatant was filtered through No. 2 paper, and the nitrogen in 2 ml of the filtrate was determined by the Kjeldahl method. Ethanol-soluble nitrogen (EtOH-N) was determined by mixing the powdered samples (0.5 g) and hot water (10 ml), boiling for 2 min, homogenizing and centrifuging at 15,000 rpm (20,000×g) for 15 min. Ethanol (99.5%, 15 ml) was added to the supernatant (4 ml) and the mixture was allowed to stand overnight in a refrigerator. After centrifugation at 15,000 rpm (20,000×g) for 15 min, the nitrogen in 5 ml of the supernatant was determined by the Kjeldahl method.

Determination of ash Ash contents were calculated by heating the samples (0.5 g) at 550°C for 3 h.

Determination of lipids Lipid contents were determined by ether extract using a Soxhlet fat extraction apparatus for 18 h (Tsutsumi, 1982b).

Mineral analysis The ash after heating the sample (0.5 g) at 550°C for 3 h was dissolved in 5 ml 20% HCl. The solution was evaporated to dryness on a hot plate at a temperature of 100–110°C and in an oven at 100°C for 1 h. The minerals in the dried residue were dissolved in about 10 ml 1% HCl and the solution was heated on a hot plate at a temperature of 100–110°C for 3–4 times. The solution was made up to 100 ml with 1% HCl. Potassium, magnesium, copper, iron, manganese, zinc and sodium in the solution were determined with a model AA781 atomic absorption spectrophotometer (Nippon Jarrell-Ash Co.) using wavelengths of 404.4 nm, 285.2 nm, 324.8 nm, 248.3 nm, 279.5 nm, 213.9 nm and 589.0 nm, respectively. Phosphorus and calcium were determined with a model VOP-1 inductively coupled plasma atomic emission spectrometer (Kyoto Koken Co.) using wavelengths of 213.618 nm and 393.366 nm, respectively.

Amino acid analysis Total amino acid compositions were determined with a model L-8500 amino acid analyzer (Hitachi Co.) after hydrolysis with redistilled constant-boiling HCl (Wako Pure Chemical Industry Co.) at 110°C for 22 h in sealed evacuated tubes. Free amino acids in the extract used for ethanol-soluble nitrogen determination were determined with the same amino acid analyzer.

Pattern similarity The pattern similarities of amino acid patterns among samples were calculated according to the methods of Tamura and Osawa (1969) and Tamura (1978) using the following equation.

$$S_{(A,B)}=\cos\theta=\frac{\sum_{i=1}^{n} aibi}{\sqrt{\sum_{i-1}^{n} ai^2}\sqrt{\sum_{i-1}^{n} bi^2}}.$$

Dendrograms of the relationship among the samples based on total and free amino acid compositions were prepared using θ of the pattern similarities by the complete linkage cluster analysis method using SAS software (ver. 6.09) at the Computer Center of the Tsukuba office, Agriculture, Forestry and Fisheries Research Council Secretariat, Ministry of Agriculture, Forestry and Fisheries (Tsukuba, Japan).

Results and Discussion

Proximate components Average values of various chemical and physical components of the Kinema samples, the soybean sample and the Natto sample are shown in Table 2. The sun-dried Kinema samples contained about 15% moisture. This value is slightly higher than 8.9% which is shown by Karki (1986) as an average moisture content of sun-dried Kinema. Sarkar *et al.* (1994) also have shown that the average moisture content of fresh Kinema samples prepared in Sikkim and the Darjeering Hills is 60–63%. This value is similar to the result for the Natto sample.

TCA-N and EtOH-N contents in the Kinema samples were high. Higher TCA-N and EtOH-N contents were observed in the Natto sample. The reason for the higher contents can be presumed to be that Natto is produced by a controlled fermentation process using a pure culture starter along with hydrolysis of soybean proteins while both Kinema and Thua-nao are allowed to undergo natural fermentation without adding a starter.

Minerals In the traditional method of Kinema preparation, a small amount of ash is often added to the cooked and lightly crushed soybeans. Table 3 shows the contents of

Table 2. Proximate compositions of the Kinema, soybean, Thua-nao and Natto samples.

	Moisture (%)	Ash (%)	Lipid (%)	TN (%)	TCA-N (%)	TCA-N/TN (%)	EtOH-N (%)	EtOH-N/TN (%)
Kinema (n=5)	15.2±1.6	6.02±0.31	22.7±2.3	7.62±0.31	2.68±0.45	35.3±6.6	2.31±0.54	30.4±7.1
Soybean (n=1)	11.9	5.47	17.0	7.07	0.28	4.0	0.79	11.1
Thua-nao (n=1)	16.5	5.19	23.0	7.48	2.21	29.6	1.40	18.8
Natto (n=1)	60.0	5.35	21.7	7.01	3.05	43.5	3.23	46.1

Data are expressed in mean±s.d. and dry weight percentages.
TN, total nitrogen; TCA-N, trichloroacetic acid-soluble nitrogen; EtOH-N, ethanol-soluble nitrogen.

Table 3. Mineral contents of the Kinema, soybean, Thua-nao and Natto samples.

	Ash (%)	Mineral (mg/100 g)									
		K	Mg	Cu	Fe	Mn	Zn	Na	P	Ca	Total
Kinema (n=5)	6.02±0.31	1768±130	252±19	1.71±0.18	17.7±3.7	5.41±1.87	4.52±0.71	27.7±37.1	729±84	432±98	3238±137
Soybean (n=1)	5.47	1936	240	1.31	8.7	2.70	3.76	1.7	721	186	3102
Thua-Nao (n=1)	5.19	1478	240	1.48	11.8	3.41	6.13	10.4	778	386	2915
Natto (n=1)	5.35	1697	221	1.46	7.2	3.63	4.55	14.4	731	281	2961

Data are expressed in mean±s.d. on dry matter basis.

Table 4. Total amino acid compositions of the Kinema, soybean, Thua-nao and Natto samples.

	Amino acid (mg/100 g)			
	Kinema (n=5)	Soybean (n=1)	Thua-nao (n=1)	Natto (n=1)
Asp	4823±382	4842	5006	4335
Thr	1761±110	1742	1849	1593
Ser	2081±226	2304	2353	1967
Glu	9289±769	8525	8740	9363
Gly	2025±130	1923	2134	1804
Ala	2156± 80	1920	2220	1668
Val	2166±192	2054	2136	1971
Cys	501± 84	353	542	492
Met	393± 90	282	512	420
Ile	2157±171	2099	2186	1879
Leu	3697±222	3564	3680	3300
Tyr	1978±157	1441	1894	1903
Phe	2670±121	2455	2384	2213
NH_3	930± 58	810	984	963
Orn	335± 69	0	107	91
Lys	2951±151	2751	2628	2699
His	1016± 41	1012	1026	1055
Arg	2894±273	3533	3297	2601
Pro	2394±145	2047	2297	1990
Total	46218±2411	43654	45976	42309

Data are expressed in mean±s.d. on dry matter basis.

Table 5. Free amino acid compositions of the Kinema, soybean, Thua-nao and Natto samples.

	Amino acid (mg/100 g)			
	Kinema (n=5)	Soybean (n=1)	Thua-nao (n=1)	Natto (n=1)
Asp	213± 89	48	135	84
Thr	124± 57	4	17	62
Ser	33± 11	7	18	44
Glu	860±378	66	182	412
Gly	164± 90	26	59	72
Ala	365±160	25	198	66
Val	344±127	10	71	219
Cys	10± 15	0	34	0
Met	73± 16	7	0	124
Ile	286± 60	5	60	210
Leu	588±157	6	113	456
Tyr	328±226	4	46	377
Phe	570±205	10	96	526
NH_3	203± 54	35	166	153
Orn	207± 74	0	46	51
Lys	417±161	11	59	324
His	113± 52	9	36	170
Arg	30± 26	200	96	17
Pro	200± 62	0	21	30
Total	5129±1700	472	1453	3397

Data are expressed in mean±s.d. on dry matter basis.

ash and minerals in the samples. The average value of the ash content of the Kinema samples was 6%. This value was higher than those of the other samples. This may be due to the firewood ash addition process in Kinema preparation.

Because the higher ash content of the Kinema samples was recognized, the minerals were determined. Potassium comprised about 30% of the ash prepared from the Kinema sample. However, this value was not so different from those of the soybean sample and the other fermented soybean samples. The average values of the iron, manganese, sodium and calcium contents of the Kinema samples were higher than those of the other samples. Although it can be presumed that the higher value may be due to the water and/or pans used in the soybean cooking process, further investigations should be carried out.

Amino acid compositions Total amino acid compositions of the Kinema, soybean, Thua-nao and Natto samples are shown in Table 4. The correlation coefficients between samples using the data for Asp, Thr, Ser, Glu, Gly, Ala, Val, Met, Ile, Leu, Tyr, Phe, Lys, His, Arg, and Pro ranged from 0.9822 (soybean, Natto) to 0.9990 (Kinema D2, Kinema D3). The pattern similarities ($\cos\theta$) between samples using the same data ranged from 0.9926 (soybean, Natto) to 0.9996 (Kinema D1, Kinema D2). Therefore, it can be generalized that there were no significant differences in the total amino acid compositions of the Kinema, soybean, Thua-nao and Natto samples.

Free amino acid compositions of the samples are shown in Table 5. Higher amino acid contents were observed in the fermented soybean products. The total of the free amino acid

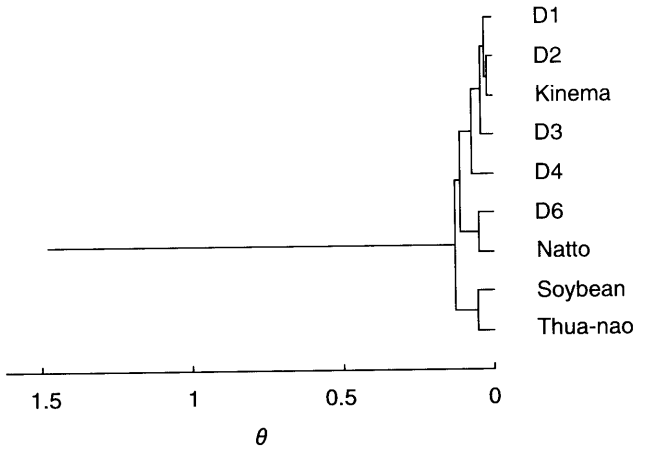

Fig. 1. Dendrogram showing the relationship of the Kinema samples to the soybean, Thua-nao and Natto samples as determined by the pattern similarity ($\cos\theta$) of total amino acid compositions, using the complete linkage cluster analysis. Kinema, average values of total amino acid contents of 5 Kinema samples shown in Table 4; D1, D2, D3, D4, D6, see Table 1.

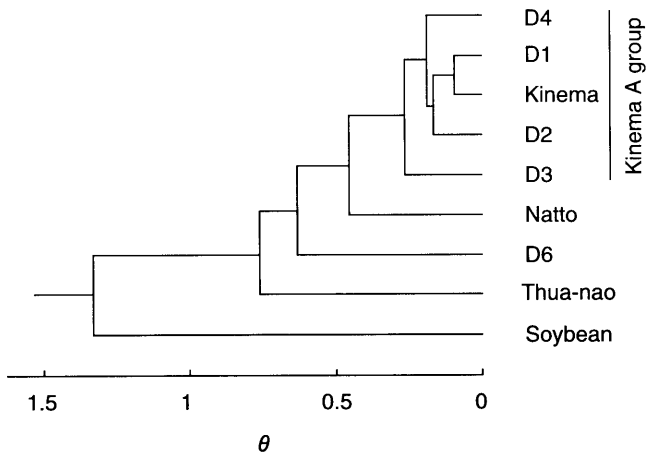

Fig. 2. Dendrogram showing the relationship of the Kinema samples to the soybean, Thua-nao and Natto samples as determined by the pattern similarity ($\cos\theta$) of free amino acid compositions, using the complete linkage cluster analysis. Kinema, average values of free amino acid contents of 5 Kinema samples shown in Table 5; D1, D2, D3, D4, D6, see Table 1.

contents of the Kinema samples varied from 2.9% to 7.0% and was higher than that of Thua-nao sample (1.5%). The percent liberation of amino acids (ratio of the sum of the free amino acid contents to the sum of the total amino acid contents) of the Kinema samples ranged from 6.0% to 16.5% with an average value of 11.4% (s.d.=4.3) and the Natto, Thua-nao and Soybean samples were 8.0, 3.2 and 1.1%, respectively. Taira *et al.* (1964) presented 11% as that of Natto. Based on the results of TCA-N, EtOH-N and liberation percents of amino acids, it is suggested that Kinema bears a closer similarity to Natto than to Thua-nao.

The free amino acid pattern similarities ($\cos\theta$) between Kinema samples using the data for Asp, Thr, Ser, Glu, Gly, Ala, Val, Met, Ile, Leu, Tyr, Phe, Lys, His, Arg, and Pro (the same amino acids as in the total amino acid pattern similarities) ranged from 0.8064 (D3, D6) to 0.9865 (D1, D2). However, when the data for the D6 sample was eliminated from the calculations, the values ranged from 0.9658 (D1, D3) to 0.9865 (D1, D2). These results suggest that the Kinema D6 sample is unique among the collected Kinema samples. The pattern similarity between the Natto sample in this paper and that prepared by Nagai *et al.* (1994) was calculated using the data for Thr, Ser, Glu, Gly, Ala, Val, Met, Ile, Leu, Tyr, Phe, Lys and His. The result was 0.9771 and this value was almost the same as those among the Kinema samples except for the D6 sample. The free amino acid pattern similarities ($\cos\theta$) among all the samples ranged from 0.2375 (soybean, Kinema D6) to 0.9865 (Kinema D1, Kinema D2). These values were smaller than those of the total amino acid pattern similarities.

Because the value of θ of the pattern similarity ($\cos\theta$) shows the distance between samples (Tamura, 1978), dendrograms of the relationship among the samples based on the total and free amino acid compositions were prepared using the values of θ. The same cluster dendrograms in the free amino acid pattern were obtained from the cluster analysis of the complete linkage cluster analysis, the single linkage cluster analysis and the average linkage cluster analysis. Therefore, the results of the complete linkage cluster analysis are shown in Figs. 1 and 2. Because the total amino acid pattern similarities among samples ranged from 0.9926 (θ=0.1219) to 0.9996 (θ=0.0270) as described above, the values of θ were not as big in comparison with those of the free amino acid patterns which ranged from 0.2375 (θ=1.3310) to 0.9865 (θ=0.1648). The free amino acid patterns were classified into 2 groups, the fermented soybean group and the unfermented soybean sample. The fermented soybean group was also further classified into four sub-groups, the Kinema A group (D1, D2, D3 and D4), the Natto sample, the Kinema D6 sample and the Thua-nao sample. All the samples belonging to the Kinema A group were collected from the same area, Hille, while the D6 sample was collected at Shirdhuwa about 20 km away from Hille. The Kinema samples collected at Hille were closer to the Natto sample rather than the Thua-nao sample. This result agrees with those obtained by the analyses of TCA-N, EtOH-N and the liberation percents of amino acids.

The plasmids, pTNH14 (Hara *et al.*, 1986) and pNKH (Hara, 1990; Hara *et al.*, 1995) of γ-polyglutamate-producing *Bacillus* strains isolated from Thua-nao of Thailand and Kinema of Nepal, respectively, have been isolated and compared to pUH1, the plasmid of *B. subtilis* (*natto*), which is responsible for polyglutamate production (Hara *et al.*, 1983, 1992). Hara (1990) has revealed that pUH1 resembles pNKH in partial sequences more than pTNH4. However, although the number of samples in this paper is very limited, our results show that the Kinema of Nepal is similar to Japanese Natto rather than the Thua-nao of Thailand. It is suggested that the hydrolysis products of the soybean proteins of Kinema, Thua-nao and Natto are affected by their fermentation process rather than the characteristics of the *Bacillus* strains.

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