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Determination of saponin contents in raw soybean and fermented soybean foods of India

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Saponin composition and contents in seeds of raw dry soybean and fermented soybean foods of India (*kinema*, *bekang* and *tungrymbai*) were investigated by liquid chromatography-tandem mass spectrometry analysis. Raw dry seeds of soybeans (15 samples) were divided into 3 saponin types (Aa, Ab & AaAb). Saponin contents were found as follows: raw soybeans, 401.8±46.8 - 686.9±33.9; seed hypocotyls, 2118.5±160.7 - 9049.3±788.6; seed cotyledon, 226.5±5.0 - 508.3±19.2; and fermented soybean foods, 243.4±25.8 - 590.3±29.0 mg/100g. Due to large amount of Group B saponin in ethnic fermented soybeans of India, it is presumed that soybean foods have health promoting benefits.

Keywords: *Bekang*, Fermented soybeans, *Kinema*, Raw soybeans, Saponin, *Tungrymbai*

Introduction

Soybean (*Glycine max*) saponins, present in seeds[1,2] (0.2-0.9%, dry wt basis), are oleanane triterpenoid glycosides and divided into Group A and DDMP (2,3-dihydro-2,5-dihydroxy-6-metyl-4*H*-pyran-4-one)-conjugated saponins according to their type of aglycone[3]. Group A saponins, present only in seed hypocotyls[4], are bis-desmoside type saponins, which have two sugar chains at C-3 and C-22 position as hydroxyl groups of soyasapogenol A as aglycone[5]. DDMP and their derivatives, Group B and E saponins, show health beneficial effects in human colon for prevention of dietary hypercholesterolemia[6], suppression of colon cancer cell proliferation[7], and anti-peroxidation of lipids and liver-protecting action by acceleration of secretion of thyroid hormones[8]. DDMP saponin is bitterer than Group B saponin[9], and an aroma component maltol, which is generated by heating degradation of DDMP saponin, contributes to enrichment of quality in soybean foods[10]. *Kinema*, *bekang* and *tungrymbai* (*k*, *b* & *t*) are *Bacillus*-fermented, sticky soybean foods of India[11]. These ethnic fermented soybeans[12] are prepared and eaten mostly in Sikkim, Mizoram and Meghalaya states of India. Sticky fermented soybean foods (*k*, *b* & *t*) are similar to Japanese *natto*[13]. Saponin compositions and contents are reported[14,15] in Asian traditional soybean foods (soymilk, *tofu*, *miso*, *natto* and *tempeh*).

This study determines saponin compositions and contents in raw soybean and ethnic fermented soybean foods of India (*k*, *b* & *t*) by LC-PDA/MS/MS method.

Experimental Section

Sample Collection

Dry seeds of soybean [15 samples; yellow (Y) & brown (Br) varieties] were collected from different parts of Sikkim (Sm) [east (E), west (W) and south (S)] in India as follows: Sample 1 (Y), Tharpu, E Sm; Sample 2 (Y), Rautery Rumtek, E Sm; Sample 3 (Br), Daramdin, W Sm; Sample 4 (Y), Chisopani, S Sm; Sample 5 (Y), Damthang, S Sm; Sample 6 (Br), Assangthang, S Sm; Sample 7 (Br), Tarku, S Sm; Sample 8 (Y), Rateypani, S Sm; Sample 9 (Y), Mangar gaon, S Sm; Sample 10 (Br), Zwroh, S Sm; Sample 11 (Br), Ranka, E Sm; Sample 12 (Y), Dentam, W Sm; Sample 13 (Y), Pipaley, S Sm; Sample 14 (Br), Bhusuk Gaucharan, E Sm; and Sample 15 (Br), Aho, E Sm. Dried fermented soybeans (15 samples) were also collected from different regions of India (4 samples of *kinema* from Sikkim, 6 samples of *bekang* from Mizoram and 5 samples of *tungrymbai*

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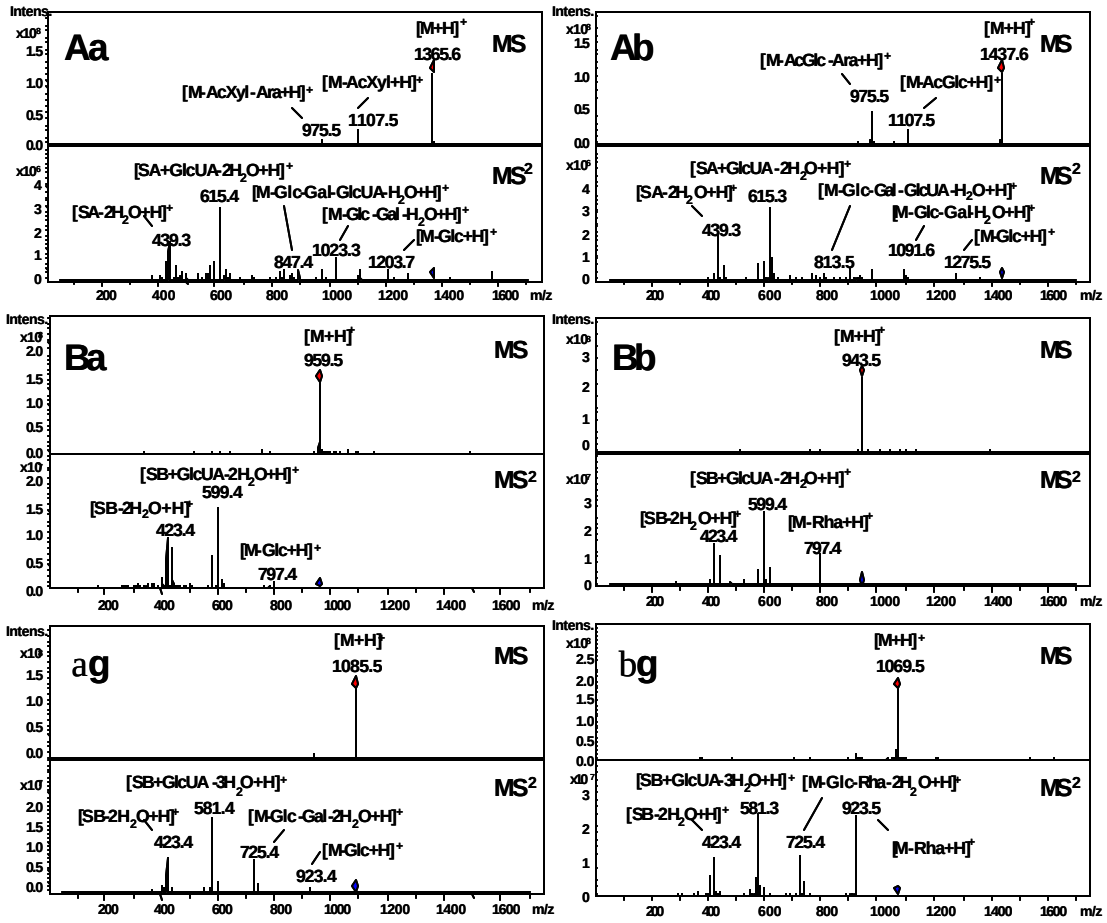

Fig.1—MS and MS^2 spectra of purified saponins, Aa, Ab, Ba, Bb, αg, βg at ESI (+) mode (AcXyl: 2,3,4-tri-*O*-acetyl- β-D-xylopyranosyl,AcGlc:2,3,4,6-tetra-*O*-acetyl-β-D-glucopyranosyl, Ara: α-D-arabinopyranosyl, Glc: β-D-glucosyl, Gal: β-D-galactopyranosyl, GlcUA: β-D-glucoronopyranosyl, Rha: α-D-rhamnopyranosyl, H_2O: water, SA: soyasapogenol A, SB: soyasapogenol B)

from Meghalaya). HPLC grade acetonitrile, formic acid, and analytical grade methanol were purchased from Kanto Chemical Co. Ltd (Tokyo, Japan). Standard saponins (Aa, Au, Ae, Ab, Ac, Af, Ba, Bb, Bb', Bc, Bd, Be, αg, βg, γg, βa) were prepared by reported method[16].

Extraction of Saponins

Soybean seeds were cut with a hand cutter, and hypocotyls and cotyledons were separated. Cotyledons of raw soybean seeds and dried samples of (*k*, *b* & *t*) were milled using Multi Beads Shocker (Yasui Kikai Co. Osaka, Japan) at 2,500 rpm for 15 s. Saponins of hypocotyl parts were extracted with a 10-fold volume (v/w) of aqueous 80% methanol for 12 h at 25°C. Cotyledons and samples of (*k*, *b* & *t*) were extracted with a 5-fold volume (v/w) of aqueous 80% methanol for 12 h at 25°C. Crude extracts were used directly for LC-MS/MS analysis.

LC-PDA/MS/MS analysis and HPLC analysis

Liquid chromatography with a photo diode array detector and a tandem mass spectrometry (LC-PDA/MS/MS-analysis) was performed by Agilent HP 1200 series (Agilent Technol Inc., Santa Clara, CA, USA) equipped with Brukar Daltonics HCTultra series (Brukar Daltonics, Inc., MA, USA). Separation was performed using a Develosil C30-UG-3 column (2.0 mm i.d. x 150 mm, Nomura Chemical, Aichi, Japan), and column temperature was kept at 40°C. Solvents used were 0.1% formic acid (v/v) in water (A) and 0.1% formic acid (v/v) in acetonitrile (B). Gradient condition was performed as follows: 0-38 min, 20-77% B (linear gradient); 38-43 min, 100% B (isocratic); and 43-63 min, 20% B (isocratic). Flow rate was 0.15 ml/min, and 10 µl of sample solution were injected. Elution was monitored at 205 and 292 nm with a photodiode array. MS analysis was performed by positive mode of electrospray ionization

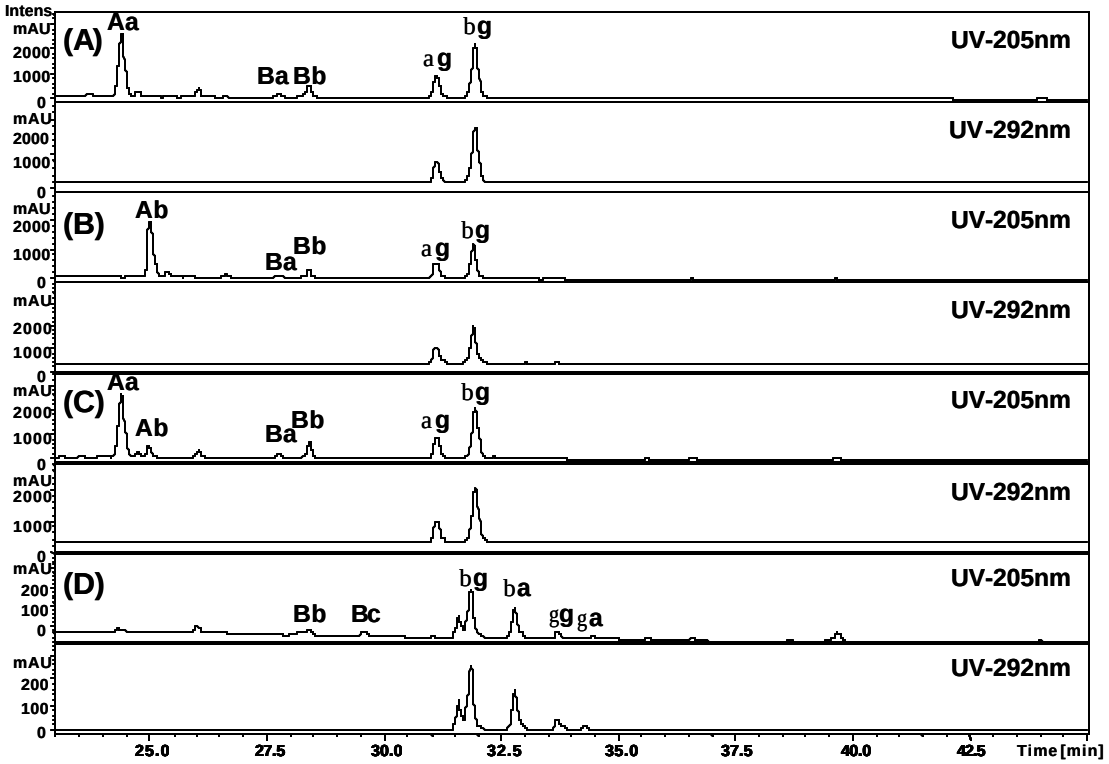

Fig. 2—Representative HPLC chromatograms (UV 205 and 292 nm) of aqueous 80% methanol extracts of seed hypocotyls and cotyledons of 15 soybean varieties in India [(A-C0 are extracts of seed hypocotyls; (A): Aa type, sample (soybean) No. 1, (B): Ab type, sample (soybean) No. 3, (C): AaAb type, sample (soybean) No. 6, (D): Seed cotyledon of sample (soybean) No. 1]

(ESI) method with: capillary voltage, 4.0 kV; capillary exit voltage, 121V; dry temp., 250°C; N_2 dry gas, 10.0 l/min; and N_2 nebulizer pressure, 50.0 psi. Full scan mass spectra ranged 50-1700 m/z.

Quantification of Saponin Components

Purified saponin Bb was used as a standard. Total saponins (sum of 16 components: Aa, Au, Ae, Ab, Ac, Af, Ba, Bb, Bb', Bc, Bd, Be, αg, βg, γg, βa) were calculated from standard curve based on peak area of Bb monitored at λ = 205 nm. Molecular absorbance coefficient at 205 nm for saponin Bb was used to quantify 16 saponins[17].

Microsoft excel 2000 was used for statistical analysis of data, including average and standard deviation. Samples were analyzed for saponins in triplicate.

Results and Discussion

Saponins were identified by LC-PDA/MS/MS to determine saponin contents in raw soybean and (*k*, *b* & *t*). Molecular ion peaks $[M+H]^+$ of Aa, Ab, Ba, Bb, αg and βg were detected with a m/z = 1365.6, 1437.6, 959.5, 943.5, 1085.5, and 1069.5, respectively (Fig. 1). Fragment ion peaks, derived from chemical structures of their aglycones and loss of sugar moieties (2,3,4-tri-*O*-acetyl-β-D-xylopyranosyl,2,3,4,6-tetra-*O*-acetyl-β-D-glucopyranosyl, α-D-arabinopyranosyl, β-D-galactopyranosyl, β-D-glucopyranosyl, α-D-rhamnopyranosyl), and water were observed. These results are consistent with reported[18,19] results. MS/MS fragment patterns of saponins reflect chemical structures of their aglycones and sugar chains. Using relation between chemical structure of saponins and MS/MS fragment patterns, saponin composition and contents in raw soybean and ethnic fermented soybean foods of India were determined.

HPLC chromatograms of aqueous 80% methanol extracts were obtained from hypocotyls (Fig. 2A-C) and cotyledons (Fig. 2D) of 15 varieties of raw types. Samples (15) of soybeans were divided into three saponin phenotypes as follows: Aa type varieties (Fig. 2A), Sample numbers 1, 8, and 12; Ab type varieties (Fig. 2B), Sample 2, 3, 5, 7, 9, 10, 11 and 14; and AaAb type varieties (Fig. 2C), Sample 4, 6, 13 and 15. Saponin contents [2118.5±160.7 - 9049.3±788.6 mg/100 g (2.3 - 9.1% dry wt basis)] were found in seed hypocotyls (Table 1). In seed cotyledons (Table 1), saponin contents were 226.5±5.0–508.3±19.2 mg/100 g (0.2-0.5% dry wtbasis).

Table 1—Saponin composition and contents in raw soybean seeds collected from Sikkim in India.

Soybean No.	Saponin Type	Hypocotyl, mg/100 g				Cotyldon, mg/100 g			Soybean seed, mg/100 g			
		Group A	Group B, E	DDMP	Total	Group B, E	DDMP	Total	Group A	Group B, E	DDMP	Total
1	Aa	4601.4±441.1	618.5±73.9	3112.9±256.7	8332.8±605.1	27.9±3.4	255.0±35.5	282.9±36.2	118.3±11.3	43.1±4.1	328.4±39.8	489.8±49.5
2	Ab	3418.4±477.1	1477.8±126.9	1391.9±173.6	6288.1±614.2	307.8±11.7	200.5±11.2	508.3±19.2	105.6±14.7	344.0±13.6	237.3±14.8	686.9±33.9
3	Ab	3560.1±306.2	415.0±11.4	1959.3±95.2	5934.4±265.7	30.3±0.9	253.4±13.8	283.7±14.7	90.1±7.7	40.0±1.2	296.6±15.0	426.7±19.4
4	AaAb	3686.1±304.6	813.7±77.3	2610.5±292.9	7110.3±575.1	25.2±0.9	276.1±20.2	301.3±20.6	87.7±7.2	44.0±2.7	331.7±25.8	463.4±28.8
5	Ab	4084.6±187.4	448.3±31.6	2068.3±193.5	6601.2±195.6	40.3±3.9	348.6±23.6	388.9±27.4	110.7±5.1	51.4±4.0	395.2±23.3	557.3±31.5
6	AaAb	5106.0±661.5	934.6±54.9	3008.7±223.7	9049.3±788.6	129.4±3.7	316.9±26.1	446.3±29.4	140.9±18.3	151.6±3.4	391.2±28.2	683.7±49.0
7	Ab	2815.8±361.2	327.1±16.6	1343.6±69.0	4486.5±446.7	37.6±2.5	317.8±22.4	355.4±24.2	84.2±10.8	46.3±2.8	348.5±23.8	478.9±36.8
8	Aa	3465.5±296.5	700.5±62.2	2863.7±167.7	7029.7±113.1	21.7±0.9	204.8±4.4	226.5±5.0	80.1±6.8	37.4±1.7	266.2±1.4	383.7±5.3
9	Ab	3089.6±308.9	1674.7±138.8	1215.8±78.6	5980.1±379.9	225.8±13.2	141.6±2.7	367.4±15.7	81.9±8.2	264.2±12.7	170.1±4.7	516.1±23.4
10	Ab	998.0±47.1	807.9±89.4	312.6±50.0	2118.5±160.7	273.8±36.9	90.1±9.2	363.9±44.3	21.6±1.0	285.3±38.0	94.9±10.1	401.8±46.8
11	Ab	3928.8±439.6	611.5±51.3	2171.9±115.0	6712.2±587.4	51.5±2.8	360.9±40.5	412.4±43.3	98.2±11.0	65.5±4.0	406.2±42.1	569.9±56.9
12	Aa	2467.1±213.0	2626.2±155.5	366.1±19.7	5459.4±372.4	379.5±36.1	40.9±2.8	420.4±36.8	63.4±5.5	437.2±39.1	49.3±2.5	549.9±45.5
13	AaAb	3284.6±181.2	1011.5±84.9	2680.8±243.2	6976.9±508.4	38.0±1.6	383.4±16.2	421.4±17.8	84.7±4.7	63.1±3.7	442.7±22.1	590.5±30.4
14	Ab	2948.0±178.2	2210.3±86.8	867.7±43.4	6026.0±276.0	204.3±14.5	67.2±6.2	271.5±16.6	78.4±4.7	257.7±16.4	88.5±7.1	424.6±22.6
15	AaAb	3137.0±307.3	2271.6±125.8	985.0±124.4	6393.6±530.1	258.5±25.2	83.8±3.8	342.3±28.0	61.2±6.0	297.8±26.9	101.4±5.9	460.3±37.7

Data represents the means (± SD) of triplicate of each sample.

Table 2— Saponin contents of ethnic fermented soybean foods of India

Fermented food	Sample code	Group B, mg/100 g
Kinema	K-1	405.5±13.2
	K-2	350.1±33.1
	K-3	285.0±10.2
	K-4	253.3±24.0
Bekang	B-1	428.6±48.8
	B-2	248.8±14.5
	B-3	340.7±23.8
	B-4	345.2±8.6
	B-5	229.5±11.1
	B-6	394.9±25.1
Tungrymbai	T-1	518.4±28.1
	T-2	447.1±28.4
	T-3	590.3±29.0
	T-4	440.2±22.2
	T-5	243.4±25.8

Data represents e means (± SD) of triplicate of each sample

Total saponin contents of soybean seeds (Table 1) were found as 401.8±46.8 mg/100 g - 686.9±33.9 mg/100 g (0.4-0.7% dry wt basis). Saponin contents of fermented soybean foods (*k*, *b* & *t*) were 243.4±25.8 mg/100 g - 590.3±29.0 mg/100g (0.2-0.6% dry wt basis) (Table 2). Only Group B and E saponins were detected in (*k*, *b* & *t*); Group A and DDMP saponins were not detected due to complete degradation duÿ:ring fermentation of soybeans (Fig. 3).

In this study, a large amount of Group B saponins were detected in fermented soybean foods of India, however, Group A saponins and DDMP saponins were not detected. Group B saponin is expected to show health beneficial effects[20]. Kudou *et al*[21] reported that soybean saponins were hydrolyzed at th glucuronide bond of saponins by hydrolyzing enzyme produced by microorganisms. Group B saponin (Bb) was degraded into soyasapogenol B by human gut microorganisms, and sugar moieties of saponins were hydrolyzed sequentially to yield smaller and more hydrophobic metabolites[20]. Group A saponins and DDMP saponins might have degraded into unknown components by fermentation. Some unknown peaks were detected at 33.5, 34.5, 38.5, and 39.5 min in fermented soybean foods of India (Fig. 3), suggesting that these peak components have more hydrophobic properties than saponin components. Fermented soybean foods are expected to contain functional saponin-like components.

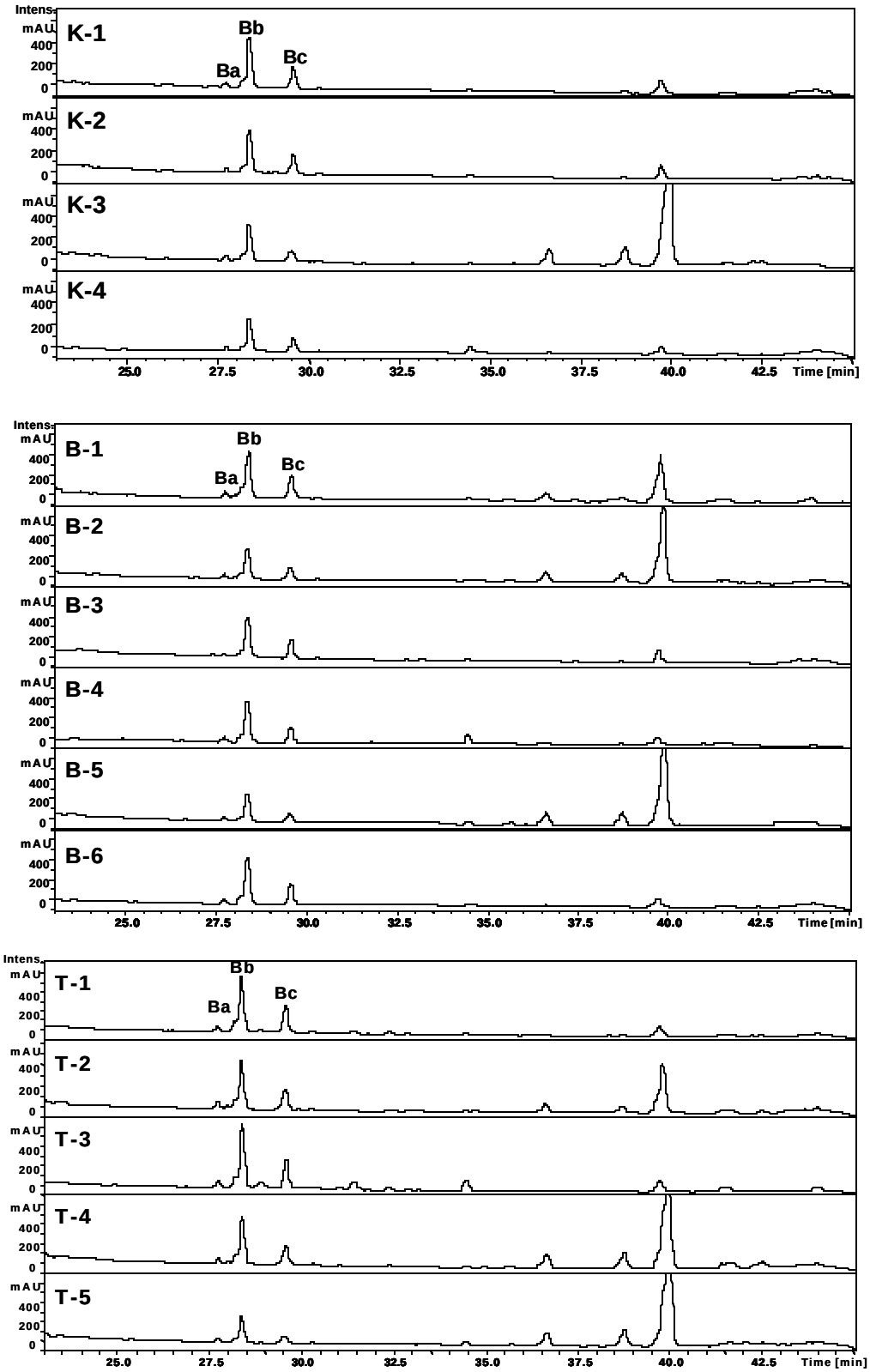

Fig. 3—HPLC chromatograms of aqueous 80% methanol extracts of ethnic fermented soybean foods of India (B1 - B6 = *Bekang*; K1 - K4 = *Kinema*; T1 - T5 = *Tungrymbai*)

Conclusions

Chemical structures and contents of saponins affect taste and health benefits. Due to large amount of Group B saponins in *kinema, bekang* and *tungrymbai,* it is presumed that these soybean foods of India have health promoting benefits. Group A saponins were degraded into unknown components (not deacetyl Group A saponins), which may also show health benefits.

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