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Changes in the Sugar, Amino Acid and Volatile Profiles of Black Soybean (*Glycine max* L.) on Cooking with Rice

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ABSTRACT

Background: Black soybean (*Glycine max* L.) is a nutritionally valuable legume widely consumed in Korea, particularly as a traditional dish co-cooked with rice. Its seeds contain sugars, free amino acids and volatile organic compounds that collectively contribute to flavor and nutritional quality.

Methods: To characterize taste-related properties of cooked black soybeans, twelve varieties of green-kernel black soybeans were co-cooked with rice and the following changes in free sugar, volatile organic compound and free amino acid profiles were evaluated.

Result: Four sugars-fructose, sucrose, raffinose and stachyose-were consistently detected and cooking decreased the total sugar content by an average of 21%, with fructose showing the greatest loss (59%). The total free amino acid content increased by an average of 42% as a result of cooking and the compositional proportions of leucine, aspartate and lysine remained high regardless of co-cooking with rice. A total of 51 volatile compounds were identified across all samples. Cooking substantially affected the volatile profiles, decreasing the level of alcohols, esters, ethers and bases, while increasing those of pyran derivatives such as maltol. Several compounds, including benzoic acid and maltol, were newly detected under our experimental conditions after cooking, whereas others disappeared or their levels changed in a variety-dependent manner. PLS-DA of volatiles clearly discriminated cooked from raw soybeans, with dibutyl phthalate, benzoic acid and nonanoic acid as marker volatiles. These results demonstrate that cooking significantly reshapes sugar, free amino acid and volatile compositions and influences the flavor characteristics of cooked black soybeans.

Key words: Amino acid, Black soybeans, SPME, Sugar, Volatile organic compounds.

INTRODUCTION

The soybean (*Glycine max* L.), originating from East Asia, is a staple crop of Korea, where it is divided into five types based on utilization: bean paste, bean curd or milk, bean sprouts, cooked-with-rice and a vegetable (Kim *et al.*, 2007). 'Seoritae' refers to soybean varieties characterized by a black seed coat, green kernel and late harvesting properties (Jang *et al.*, 2014). 'Seoritae' soybeans are generally cooked with rice and contain various phytonutrients including anthocyanins (Kim and Lee, 2006), isoflavones, vitamins and dietary fibers, in addition to proteins and carbohydrates (Lim *et al.*, 2003).

Carbohydrates constitute a substantial proportion of soybeans, with approximately 60% insoluble and 40% soluble sugars (Wang *et al.*, 2014). The primary soluble sugars include sucrose, raffinose and stachyose, with minor amounts of glucose and fructose (Chavez *et al.*, 2016; Tondé *et al.*, 2026). Sucrose enhances sweetness and flavor, whereas raffinose and stachyose are less desirable, being poorly digestible and often causing flatulence and diarrhea (Wang *et al.*, 2014). Therefore, detailed information on the sugar composition of soybeans is essential, especially for breeders who aim to develop cultivars with increased sucrose and reduced raffinose and stachyose levels that satisfy consumer preferences.

Proteins are one of the most nutritionally significant components of soybeans and their quality is largely determined by amino acid composition (Qin *et al.*, 2019). Soybeans provide a well-balanced profile of essential amino acids that supports human dietary requirements (Kannan *et al.*, 2001). Processing may substantially alter amino acid composition through thermal degradation of antinutritional factors and structural modification of storage proteins (Khattab *et al.*, 2009; Kim *et al.*, 2015).

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Flavor is also important for eating quality and customer preferences (Kim and Lee, 2006) imparted by a complex mixture of volatile compounds (Choi *et al.*, 2023; Kato *et al.*, 1981). Lee and Shibamoto (2000) found that the major aroma constituents of the soybean include hexanal, 1-octen-3-ol, γ-butyrolactone, maltol and phenylethyl alcohol. Processing methods such as frying, roasting and cooking play critical roles in flavor development, altering the volatile composition and inactivating lipolytic enzymes (Bi *et al.*, 2021). For example, Tsugita *et al.* (1980) showed that roasting soybeans at 200°C reduced the hexanal, 1-octen-3-ol and hexanol level, but increased those of furan compounds. In Korea, 'Seoritae' are usually consumed after cooking with rice. This involves soaking and boiling at high temperature and pressure, associated with substantial changes in nutritional composition, including degradation of organic compounds. Despite the importance of aroma, free sugar and free amino acid profiles to eating quality, the chemical profiles of black soybeans cooked with rice have rarely been investigated.

This study was conducted to define the changes in flavor-affecting volatile organic compounds, free amino acid and sugar compositions of 12 black soybean varieties before and after cooking.

MATERIALS AND METHODS

Materials

Twelve black soybean seoritae varieties-'Geomjeongkong 2-ho' (GJ2), 'Seonheuk' (SH), 'Socheongja' (SCJ), 'Socheong 2-ho' (SC2), 'Yeongjujaerae' (YJJ), 'Cheongdu 1-ho'(CD1), 'Cheongja 3-ho' (CJ3), 'Cheongja 4-ho' (CJ4), 'Cheongja 5-ho' (CJ5), 'Taecheong' (TC), 'Saebaram' (SBR) and 'Gwijok' (GJ)-were obtained from the National Institute of Crop Sciences, Rural Development Administration of the Republic of Korea and cultivated according to standard practices. Harvested soybeans were air-dried and stored at 4°C until experimental utilization. 'Samkwang' white rice was purchased from the market and cooked with soybeans. The mineral drinking water used for soybean washing and rice cooking was Jeju SamDaSoo (Kwangdong Pharmaceutical, South Korea) were commercially purchased and used for experiments. All experiments were carried out at the Department of Medical Biotechnology, Soonchunhyang University, Asan 31538, Republic of Korea, in 2024.

Cooking black soybeans with rice

To cook black soybeans with rice, approximately 24 g of raw soybeans of each variety were briefly washed three times with mineral drinking water and then soaked for 2 h. The soaked soybeans were mixed with 160 g of 'Samkwang' white rice that had been pre-washed three times with mineral drinking water. Mixture of soaked black soybeans and white rice were cooked in an automatic rice cooker (CR-0255 MW, Cuckoo, South Korea) at ~100°C for 35 min, in line with the manufacturer's protocol.

Sugar analysis

Two grams of cooked soybeans were carefully separated from rice to minimize any influence of rice sugars and transferred to 50 mL centrifuge tubes (BD Falcon™, NJ, USA). Distilled water (20 mL) was added, followed by homogenization (POLYTRON® PT 2500 E, Kinematica, USA) at 6,000 rpm for 10 s. Sugars were extracted in a water bath at 55°C for 30 min, vortexed for 30 s and cooled to room temperature for 20 min. After adding 20 mL of acetonitrile, each mixture was vortexed vigorously, centrifuged at 3,000 rpm for 10 min and the supernatant diluted fivefold with acetonitrile:distilled water (65:35, v/v) and filtered through a 0.45 µm PTFE syringe filter (ADVANTEC, Japan). For raw soybeans, samples were milled (A11 Basic, IKA, Germany) and 1 g of powder extracted as above. Sugars were separated on an HPLC system (Skyam S5300, GmbH, Germany) with an ELSD detector (Alltech-3300, BUCHI, USA) using a Kromasil 60-5-HILIC-D column (4.6 × 250 mm, Nouryon, Sweden), 20 µL injection. A distilled water-acetonitrile gradient was used, starting at 20% water, rising to 50% at 12 min and returning to 20% at 16 min, at 1.5 mL min^{-1} (20 min total). The ELSD was held at 55°C with nitrogen at 3.3 bar. Sugar contents were expressed on a dry-matter basis using moisture contents of pre- and post-cooking soybeans measured in triplicate (Mettler MJ33, Mettler-Toledo, Switzerland). Fructose, sucrose, raffinose and stachyose standards were from Sigma-Aldrich (St. Louis, MO, USA) and HPLC-grade acetonitrile from J.T. Baker (Avantor, PA, USA).

Free amino acid analysis

Soybeans (0.5 g) were extracted with 5 mL of methanol, sonicated at 35°C for 30 min and centrifuged at 4,000 rpm for 10 min. A 100 µL aliquot of the supernatant was evaporated to dryness under nitrogen, then derivatized with 150 µL of N-methyl-N-(trimethylsilyl)-trifluoroacetamide (MSTFA) at 60°C for 45 min. Quantification used a QP2010 Ultra GC-MS system (Shimadzu, Kyoto, Japan) with a DB-5 capillary column (30 m × 0.25 mm ID, Agilent, Santa Clara, CA, USA). The oven was held at 80°C for 2 min, ramped to 250°C at 5°C min^{-1}, then to 300°C at 25°C min^{-1} and held for 5 min. Helium (99.999%) was the carrier gas at 1 mL min^{-1}, with the inlet at 280°C in split mode (1:10). MS conditions were: ion source, 180°C; interface, 280°C; electron energy, 70 eV; scan range, 35-550 m/z. Free amino acids were quantified against a standard curve prepared from a free amino acid mixture (Supelco, Bellefonte, PA, USA) with five serial dilutions and expressed as mg g^{-1} dry weight (mg g^{-1} DW).

Volatile analysis

Three-grams of raw soybeans were placed in 20 mL headspace (HS) vials (Choongin Science, Suwon, South Korea), sealed with Teflon-lined magnetic caps and loaded onto an automatic HS sampler (Combi-PAL, East Petersburg, PA, USA). For cooked samples, 3 g were

carefully separated from rice before transferring to HS vials. Volatiles were released at 80°C for 10 min, adsorbed onto a divinylbenzene/carboxen/polydimethylsiloxane SPME fiber (Supelco, Bellefonte, PA, USA) for 10 min at 80°C and desorbed in the inlet of a GC-MS system (QP2010 Ultra, Shimadzu, Kyoto, Japan). The fiber was then thermally cleaned at 260°C for 10 min before the next sample. Cooked rice was analyzed separately as a blank to exclude rice-derived volatiles. Separation used an Rxi-5Sil MS capillary column (30 m × 0.25 mm ID, Restek, Bellefonte, PA, USA); the oven was held at 50°C for 1 min, ramped to 240°C at 2.8°C min^{-1} and held for 3 min. Helium (99.999%) was the carrier gas at 1.01 mL min^{-1}, with the inlet at 260°C in splitless mode. MS conditions were: ion source, 200°C; interface, 270°C; electron energy, 70 eV; scan range, 35-500 m/z. Volatiles were identified by comparing retention times and mass spectra with the NIST 14 library and the retention indices of C8-C20 n-alkane standards (Sigma-Aldrich, St. Louis, MO, USA). Each volatile was semi-quantified on a dry-weight basis from its TIC peak area, taken as its relative amount; peak areas were used only to compare the same compound between raw and cooked samples, not to compare absolute contents among different compounds. Odor descriptions were obtained from Volatile Compounds in Food (VCF) online (https://www.vcfonline.nl/VcfHome.cfm).

Statistical analysis

Descriptive statistics were derived, followed by analysis of variance (ANOVA) with the Duncan multiple range test and t-test (*p<0.05; **p<0.01; ***p<0.001), with using the SPSS software (IBM Corp., version 24). Multivariate analyses, including partial least squares-discriminant analysis (PLS-DA), employed MetaboAnalysis software (http://www.metaboanalyst.ca) after mean-centered scaling and division by the standard deviation of each volatile compound. All sugar, free amino acid and volatile analyses were performed in triplicates.

RESULTS AND DISCUSSION

Sugar contents in raw and cooked soybeans

Soybeans contain a range of mono-, di-and oligo-saccharides. In our present study, fructose, sucrose, raffinose and stachyose were consistently detected in raw and cooked soybeans. The sugar components, including glucose, have been reported in many previous studies (Hou *et al*., 2009a; Hou *et al*., 2009b; Oboh *et al*., 2000). However, glucose could not be detected in either raw or cooked black soybeans in our study, which may due to differences in experimental conditions compared to previous studies (Choi *et al*., 2023).

In raw soybeans, sucrose was the dominant sugar across all tested varieties (Table 1), averaging 55% of total sugar content and ranging from 43.16 to 115.74 mg g^{-1} DM, with a mean of 81.51 mg g^{-1} DM, consistent with previously reported ranges of 11.10-105.48 mg g^{-1} DM and an average of 50.13 mg g^{-1} DM (Hou *et al*., 2009b). The average stachyose content in raw soybeans was 51.92 mg g^{-1} DM, approximately 66% higher than those of previous studies (Hou *et al*., 2009a; Silva *et al*., 2009), a discrepancy likely attributable to genotypic differences among varieties, as sugar content in soybean is known to vary considerably with genetic background (Hou *et al*., 2009b). The average raffinose content in raw soybeans was 7.43 mg g^{-1} DM, within the previously reported ranges of 0.85-19.73 mg g^{-1} DM (Hou *et al*., 2009b; Silva *et al*., 2009). Fructose was the least abundant sugar, contributing only 3.4% of total sugar content in raw soybeans, with an average of 4.97 mg g^{-1} DM (Table 1), consistent with reports of rare monosaccharides in raw soybeans (Hou *et al*., 2009a; Silva *et al*., 2009). Among individual sugars, fructose exhibited the highest genetic variation across varieties (RSD = 39%), while stachyose showed the lowest (RSD = 8%), reflecting the genotypic diversity of the tested materials.

Following cooking, total sugar content decreased across all varieties, with an average reduction of 21% relative to raw soybeans (maximum: 31% in 'Yeongjujaerae'; minimum: 7% in 'Gwijok'). Sucrose remained the dominant sugar after cooking, averaging 58% of total sugars and 66.72 mg g^{-1} DM, with an average reduction of 17%, though no cooking-induced reduction was apparent in the 'Socheong 2-ho' and 'Taecheong' varieties. Stachyose and raffinose declined by an average of 24% and 9%, respectively, with post-cooking levels ranging from 30.57 to 47.25 mg g^{-1} DM for stachyose and averaging 6.76 mg g^{-1} DM for raffinose; notably, the raffinose levels of 'Geomjeongkong 2-ho' and 'Cheongja 3-ho' were largely unchanged after cooking. Fructose showed the greatest sensitivity to heat, with an average reduction of 59% after cooking and in the 'Cheongja 3-ho' variety, fructose was undetectable in the cooked form despite a raw level of 1.18 mg g^{-1} DM (Table 1). Cooked black soybeans overall exhibited fructose levels ranging from 1.41 to 3.11 mg g^{-1} DM, with a mean of 1.86 mg g^{-1} DM. These cooking-induced reductions in sucrose, raffinose, stachyose and fructose are consistent with those of previous reports (Oboh *et al*., 2000; Silva *et al*., 2009; Vidal-Valverde *et al*., 1993).

The variety-dependent variation in sugar composition observed in raw soybeans was consistently maintained after cooking, suggesting that cooking with rice does not induce sugar-specific compositional changes beyond the general thermal reduction. The decreases in sugar content following cooking can be attributed to hydrolysis during water soaking and thermal degradation at high cooking temperatures (Oboh *et al*., 2000; Silva *et al*., 2009). Fructose, as the most heat-sensitive sugar, is particularly susceptible to breakdown or conversion into other compounds during cooking (Vidal-Valverde *et al*., 1993), consistent with the near-complete loss observed in some varieties. The results of this study are in good agreement with Vidal-Valverde *et al*. (1993), who reported only unquantifiable trace levels of fructose in water-boiled lentils.

Table 1: Sugar contents of raw and cooked black soybeans (mg g^{-1} DM).

Variety	Fructose		Sucrose		Raffinose		Stachyose		Total sugar	
	Raw	Cooked	Raw	Cooked	Raw	Cooked	Raw	Cooked	Raw	Cooked
'Geomjeongkong 2-ho'	4.77±0.15^{d†}	2.25±0.07^{f}	101.06±1.64^{h}	82.80±0.85^{h}	9.43±0.22^{f}	9.83±0.23^{g}	50.28±1.07^{bcd}	41.97±0.38^{gh}	165.54±2.62^{f}	136.86±0.92^{i}
'Seonheuk'	4.36±0.10^{c}	1.81±0.04^{e}	74.95±0.78^{cd}	58.10±1.53^{cd}	5.53±0.09^{a}	3.22±0.02^{a}	59.78±1.40^{g}	42.81±1.04^{h}	144.62±1.13^{d}	105.95±1.73^{c}
'Socheongja'	7.06±0.32^{f}	2.17±0.05^{f}	73.56±2.20^{c}	53.91±0.87^{b}	7.60±0.19^{c}	5.98±0.10^{c}	55.09±1.29^{f}	37.15±0.57^{d}	143.30±0.97^{cd}	99.22±1.10^{b}
'Socheong 2-ho'	1.89±0.11^{b}	3.11±0.05^{h}	43.16±0.76^{a}	43.03±1.15^{a}	5.14±0.31^{a}	4.32±0.05^{b}	59.44±1.17^{g}	45.11±1.04^{i}	109.63±0.85^{a}	95.57±1.12^{a}
'Yeongjujaerae'	7.16±0.23^{f}	1.59±0.07^{c}	115.74±1.79^{i}	85.02±1.19^{i}	9.96±0.26^{g}	6.45±0.14^{d}	51.73±1.17^{d}	33.51±0.89^{c}	184.59±1.01^{g}	126.58±0.33^{h}
'Cheongdu 1-ho'	6.59±0.13^{e}	1.59±0.05^{c}	67.21±3.24^{b}	61.08±1.65^{de}	6.64±0.21^{b}	6.02±0.21^{c}	53.41±0.35^{e}	41.30±0.74^{fg}	133.85±2.79^{b}	109.99±2.28^{d}
'Cheongja 3-ho'	1.18±0.10^{a}	ND‡	82.46±3.08^{f}	63.91±0.80^{ef}	6.74±0.14^{b}	7.02±0.28^{e}	50.43±0.35^{bcd}	41.70±0.90^{fgh}	140.80±2.66^{c}	112.62±1.49^{e}
'Cheongja 4-ho'	7.31±0.28^{f}	0.86±0.07^{a}	81.13±1.74^{ef}	66.44±0.72^{f}	6.40±0.04^{b}	7.79±0.15^{f}	51.16±0.57^{cd}	47.25±0.69^{j}	145.99±2.27^{d}	122.33±0.49^{g}
'Cheongja 5-ho'	5.07±0.13^{d}	1.52±0.06^{c}	86.32±1.91^{g}	68.01±1.60^{fg}	6.68±0.24^{b}	6.11±0.14^{c}	46.91±0.84^{a}	30.57±0.37^{a}	144.99±1.93^{d}	106.22±1.39^{c}
'Taecheong'	4.83±0.21^{d}	1.72±0.04^{d}	86.83±0.95^{g}	86.85±0.79^{i}	8.62±0.35^{e}	9.61±0.34^{g}	49.38±0.70^{b}	40.42±1.22^{f}	149.66±0.99^{e}	138.61±1.60^{i}
'Saebaram'	4.44±0.22^{c}	1.41±0.07^{b}	78.06±1.68^{de}	68.98±0.67^{g}	8.11±0.24^{d}	7.56±0.20^{f}	49.95±0.56^{bc}	38.59±0.91^{e}	140.56±2.21^{c}	116.54±1.19f
'Gwijok'	5.04±0.14^{d}	2.41±0.01^{g}	87.69±2.10^{g}	62.50±1.98^{de}	8.37±0.37^{de}	7.22±0.23^{e}	45.53±0.44^{a}	31.99±0.39^{b}	146.63±1.67^{de}	104.13±2.29^{c}
Average	4.97±1.95	1.86±0.60	81.51±17.68	66.72±13.01	7.43±1.50	6.76±1.90	51.92±4.39	39.37±5.18	145.85±17.64	114.55±14.00
Maximum	7.31±0.28	3.11±0.05	115.74±1.79	86.85±0.79	9.43±0.22	9.83±0.23	59.78±1.40	47.25±0.69	184.59±1.01	138.61±1.60
Minimum	1.18±0.10	1.41±0.07	43.16±0.76	43.03±1.15	5.14±0.31	3.22±0.02	45.53±0.44	30.57±0.37	109.63±0.85	95.57±1.12
% RSD	39%	32%	22%	20%	20%	28%	8%	13%	12%	12%

† Values represent means of triplicate experiments., Results in the same column of sample groups with different letters (a-j) are significantly different by Duncan's multiple range test (p<0.05).

‡ ND: Not detected.

Free amino acid content in raw and cooked soybeans

Free amino acid profiles of 12 soybean varieties were determined in both raw and cooked form, measuring 14 individual free amino acid (Ala, Asp, Glu, Gly, Ile, Leu, Lys, Met, Phe, Pro, Ser, Thr, Tyr and Val) and expressed as mg g^{-1} dry weight (DW).

In raw soybeans, the total free amino acid content (14 free amino acids) ranged from 0.69 to 1.27 mg g^{-1} DW across 12 varieties, with the three highest values (mg g^{-1} DW) observed in 'Gwijok' (1.27), 'Cheongja 3-ho' (1.25) and 'Taecheong' (1.07) (Fig 1). Leucine (Leu) was most abundant free amino acid, accounting for an average of 34.8% of total free amino acid contents, followed by asparagine (Asp; 12.4%), lysine (Lys; 10.8%) and alanine (Ala; 9.1%). Glutamate (Glu), phenylalanine (Phe) and methionine (Met) contributed moderate levels at 7.3%, 6.3% and 4.7%, respectively, while proline (Pro), serine (Ser) and threonine (Thr) were consistently minor components, each below 1% across all 12 varieties.

Following cooking, the total free amino acid content increased substantially across all varieties, ranging from 0.97 to 1.80 mg g^{-1} DW, with a mean of 1.32 mg g^{-1} DW, representing an average increase of approximately 41.7% over the raw form (Fig 1). The three varieties with the highest total free amino acid content remained 'Gwijok' (1.80 mg g^{-1} DW), 'Cheongja 3-ho' (1.76 mg g^{-1} DW) and 'Taecheong' (1.52 mg g^{-1} DW), mirroring the ranking observed in raw soybeans. Notably, the relative free amino acid composition was conserved after cooking: leucine remained dominant, followed by aspartate, lysine and alanine which proportions nearly identical to those in the raw form. The increase across individual free amino acids was also uniform, ranging from 28.6% (Pro) to 52.4% (Ser), indicating that cooking elevated all free amino acids proportionally rather than selectively.

The cooking-induced increase in free amino acid content is primarily attributable to the thermal inactivation of heat-labile protease inhibitors in raw legumes (Khattab *et al*., 2009), which upon denaturation allow more complete protein hydrolysis and release of free amino acids from storage proteins; thermal unfolding of protein structures further enhances susceptibility to enzymatic breakdown (Chauhan *et al*., 2022; Khattab *et al*., 2009). These results are consistent with previous literature resulting that thermal processing methods; including soaking (Chauhan *et al*., 2022), boiling (Kim *et al*., 2015) and microwave cooking (Rodríguez-Bernaldo De Quirós *et al*., 2000), reliably increase total essential free amino acid content in legume seeds (Khattab *et al*., 2009; Kim *et al*., 2015). From a nutritional perspective, soybeans are rich in lysine yet deficient in sulfur-containing free amino acids such as methionine and cysteine (Kannan *et al*., 2001), while rice, a global staple presents the complementary lysine deficiency (Kim *et al*., 2015). The cooking-induced lysine increase observed in this study (0.095 to 0.135 mg g^{-1} DW; +41.6%) reinforces the value of cereal-legume complementation and supports the incorporation of cooked soybean into rice-based diets to achieve a more balanced free amino acid intake. Although soybeans were carefully separated from rice prior to extraction, no cooked-rice control was analyzed for sugars and free amino acids; therefore, a minor contribution of rice-derived solutes to the observed compositional changes cannot be excluded.

Volatiles in raw and cooked black soybeans

A total of 51 volatiles were identified from all tested samples, including 25 odor-active volatiles (Table 2). By the chemical groups, the identified volatiles were categorized as follows: 3 acids, 10 alcohols, 8 aldehydes, 1 base, 7 esters, 3 ethers, 1 furan, 12 hydrocarbons, 3 ketones, 1 lactone, 1 phenol and 1 pyran. For raw soybeans, the three principal volatiles by their peak area were 2-(2-methoxyethoxy)ethanol, caprolactam and octanal, in all varieties. Cooking with rice significantly altered the qualitative profiles. The major volatiles of cooked soybeans were maltol, 2-butoxyethanol and nonanal. The total peak area of identified volatiles in cooked soybeans were about half those in raw soybeans, suggesting significant loss of volatiles during cooking (Fig 2). The chemical groups principally affected by cooking were

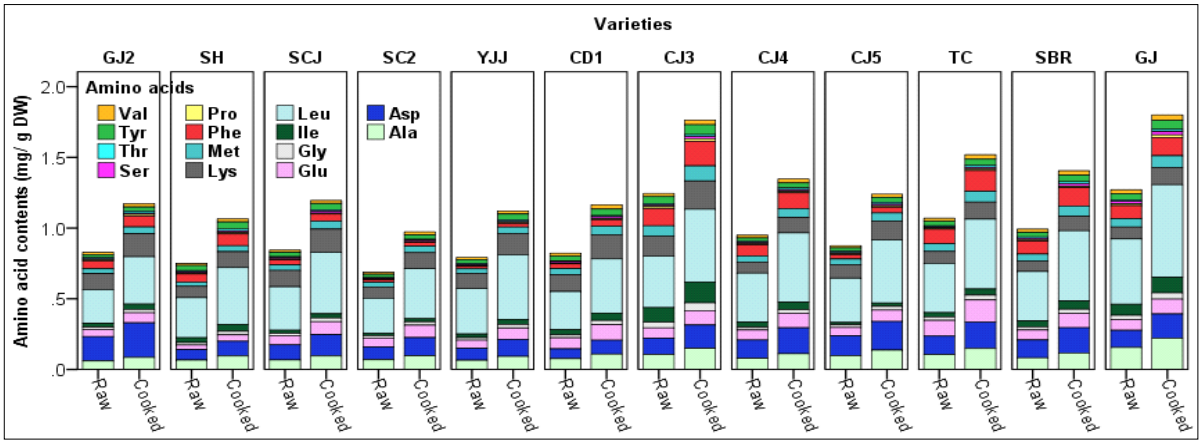


Fig 1: Free amino acid content (mg g^{-1} DW) in raw and cooked soybean black soybean.

Table 2: List of volatiles identified in raw and cooked black soybeans.

Chemical group†	Volatiles name	RT (min)	CAS number†	RI (exp)‡	RI (ref)†	Odor description†
Acids	Benzoic acid	21.81	65-85-0	1,164	1,171	Floral, fruit, pungent, sour, urine
Acids	Hexanoic acid	12.27	142-62-1	976	1,014	Acid, cheese, fermented, goat, pungent
Acids	Nonanoic acid	26.83	112-05-0	1,267	1,264	Fat, green, sour, sweat
Alcohols	1-Dodecanol	36.00	112-53-8	1,472	1,473	Fat, wax
Alcohols	1-Heptanol	11.91	111-70-6	968	969	Chemical, green, putrid, unpleasant, wood
Alcohols	1-Hexanol	7.72	111-27-3	867	870	Bread, flower, fruit, grass, green, herb, wood
Alcohols	1,1-Oxydi-2-Propanol	14.18	110-98-5	1,021	1,017	
Alcohols	2-Ethyl-1-hexanol	14.71	104-76-7	1,028	1,032	Citrus, green, oil, rose
Alcohols	2-Methyl-1-octanol	21.97	818-81-5	1,171		
Alcohols	2-Methyl-1-undecanol	28.00	10522-26-6	1,291		
Alcohols	2-Octyl-1-decanol	34.00	45235-48-1	1,413		
Alcohols	Benzyl alcohol	14.97	100-51-6	1,032	1,039	Almond, boiled cherries, floral, fruit, moss
Alcohols	2-Butoxyethanol	9.00	111-76-2	903	909	
Aldehydes	(E)-2-Nonenal	21.28	18829-56-6	1,159	1,160	Beany, cucumber, tallow, watermelon
Aldehydes	(E)-2-Octen-1-al	16.15	2548-87-0	1,056	1,059	Nut, peanut, seaweed, sweet
Aldehydes	Benzaldehyde	11.58	100-52-7	961	966	Almond, berry, caramel, cherry
Aldehydes	Benzeneacetaldehyde	15.45	122-78-1	1,042	1,056	Berry, honey, lavender, nut, rose, sweet
Aldehydes	Decanal	23.69	112-31-2	1,205	1,205	Citrus, fat, floral, fried, orange peel
Aldehydes	Hexanal	5.00	66-25-1	799	802	Apple, cut grass, fresh, fruit, grass, green, oil
Aldehydes	Nonanal	18.51	124-19-6	1,103	1,106	Citrus, cucumber, rubber, soap, sweet
Aldehydes	Octanal	13.21	124-13-0	1,002	1,006	Citrus, honey, nut, orange peel, pungent
Bases	Caprolactam	26.02	105-60-2	1,253	1,255	
Esters	2-Ethylhexyl salicylate	50.00	118-60-5	1,799	1,807	
Esters	Butyl nonanoate	34.00	50623-57-9	1,422		
Esters	Dibutyl phthalate	55.00	84-74-2	1,949	1,967	
Esters	Diisopropyl adipate	35.00	6938-94-9	1,446	1,464	
Esters	Diisobutyl adipate	45.00	141-04-8	1,677	1,680	
Esters	Methyl 2-hydroxyhexanoate	13.92	68756-64-9	1,011	1,011	
Esters	Nonanoic acid, ethyl ester	28.06	123-29-5	1,293	1,294	Banana, fruit, grape
Ethers	2-(2-Methoxyethoxy)ethanol	10.15	111-77-3	930	932	
Ethers	2-Dodecoxyethanol	46.00	4536-30-5	1,711		
Ethers	Isopropyl tetradecyl ether	40.00		1,561	1,630	
Furan	2-Pentylfuran	12.92	3777-69-3	989	994	Butter, caramel, floral, fruit, green
Hydrocarbons	1-Tetradecene	32.76	1120-36-1	1,390	1,390	
Hydrocarbons	2,2-Dimethylbutane	23.09	75-83-2	1,194	1,189	

Table 2: Continue...

Table 2: Continue...

Hydrocarbons	2,6,10-Trimethyltridecane	36.00	3891-99-4	1,459			
Hydrocarbons	2,6,11-Trimethyl dodecane	23.90	31295-56-4	1,209	1,215		
Hydrocarbons	Azulene	22.66	275-51-4	1,184	1,190		
Hydrocarbons	cis-Calamenene	39.00	72937-55-4	1,520	1,523		
Hydrocarbons	Dodecane	23.44	112-40-3	1,200	1,200	Alkane, undesirable	
Hydrocarbons	Hexadecane	42.00	544-76-3	1,597	1,598	Alkane, root	
Hydrocarbons	Hexylcyclopentane	25.54	4457-00-5	1,242	1,212		
Hydrocarbons	3-Methylpentadecane	41.00	2882-96-4	1,568	1,570		
Hydrocarbons	2,5-Dimethylundecane	15.07	17301-22-3	1,036	1,030		
Hydrocarbons	3-Methylideneundecane	27.83	71138-64-2	1,285			
Ketones	2-Decanone	23.02	693-54-9	1,191	1,192	Fat, fruit	
Ketones	6,10,14-trimethyl-2-pentadecanone	51.00	502-69-2	1,837	1,842	Fat	
Ketones	Acetophenone	16.44	98-86-2	1,062	1,068	Almond, animal, floral, must, plastic	
Lactones	4-Nonanolide	31.00	104-61-0	1,355	1,366	Apricot, cocoa, coconut, peach	
Phenols	2,4-Di-tert-butylphenol	38.00	96-76-4	1,504	1,514		
Pyrans	Maltol	18.85	118-71-8	1,107	1,107	Butterscotch, caramel, cotton candy	

†Information was used from references (https://www.vcf-online.nl), ‡Retention index, which was experimentally determined in this report.

ethers, bases, alcohols and esters, whose relative peak areas decreased by 95, 98, 42 and 78%, respectively (Fig 2). These results are consistent with Rodríguez-Bernaldo De Quirós *et al.* (2000) who reported that the levels of esters and alcohols in raw green beans decreased significantly during cooking, to the extent that they could barely be detected. Esters are generally derived from short-chain acids and are thermally unstable. They may therefore be completely degraded during cooking (Chyau *et al.*, 1992). The reductions in alcohol levels during cooking may reflect their high solubility in water (Maarse, 1991). This facilitates transfer to the cooking medium, followed by loss attributable to heat-induced degradation, evaporation, or leaching. In contrast, the levels of pyran volatiles such as maltol increased significantly (by up to 95%) after cooking (Fig 2), perhaps attributable to the high cooking temperature which thermally degrades and rearranges pyran precursors such as sugars, amino acids and fatty acids, resulting in formation of smaller volatile compounds, including pyran derivatives, as suggested by Maarse (1991).

Classification of volatiles according to changing patterns during cooking

Based upon quantitative changing patterns during cooking, volatiles were classified into five groups; (I) disappeared, (II) decreased, (III) increased, (IV) newly detected and (V) variety-dependent irregular changing patterns (Fig 3).

Volatiles in group I completely disappeared or decreased to below the detection limits after cooking (Fig 3A), regardless of the variety. Hydrocarbons, alcohols and aldehydes including 2,2-dimethylbutane, benzyl alcohol, cis-calamene and octanol were in group I. Cooking-induced decreases in the levels of hydrocarbons, alcohols and aldehydes have been previously reported for a soymilk product (Yuan and Chang, 2007). Similarly, Mishra *et al.* (2017) also reported octanal decreases in three varieties of cooked red kidney beans compared to raw beans. Such reductions are attributable to the roles played by alcohols and aldehydes as reactants involved in production of sugars and amino acids during cooking (Bi *et al.*, 2021). Volatiles of group II, which exhibited decreases during cooking, included 1-dodecanol, benzeneacetaldehyde, decanal, hexanoic acid and nonanoic acid. Specifically, the fall in the level of benzene acetaldehyde (Fig 3B) averaged 69% after cooking, with a maximum of 76% ('Taecheong') and a minimum of 58% ('Seonheuk'). A previous study found that the soybean benzeneacetaldehyde level decreased after roasting at 200°C (Kato *et al.*, 1981).

In contrast, cooking increased the levels of certain volatiles (Fig 3C) including 2-pentylfuran, 1-heptanol, 1-hexanol, hexanal and benzaldehyde. For 2-pentylfuran, the relative peak areas rose by an average of 551% after cooking, with a maximum of 1,752% ('Taecheong') and a minimum of 59% ('Seonheuk'). Kato *et al.* (1981) also reported that furan volatiles were not detectable in raw soybean flour but were in roasted flour. Furan volatiles are

produced *via* reactions between amino acids and reducing sugars during heating (Fiddler *et al.*, 1967). Benzaldehyde increased by an average of 159% after cooking, with a maximum increase of 327% ('Gwijok') and a minimum increase of 25% ('Socheongja'). These findings are consistent with those of previous reports on benzaldehyde increases in cooked green beans (Rodríguez-Bernaldo De Quirós *et al.*, 2000), cooked adzuki bean (Bi *et al.*, 2021), roasted soybean (Kato *et al.*, 1981) and cooked red kidney bean (Mishra *et al.*, 2017). Benzaldehyde is thermally generated *via* Strecker degradation as beans cook (Kato *et al.*, 1981).

Two volatiles were newly detected after cooking (group IV): *e.g.*, benzoic acid and maltol (Fig 3D). Maltol had been reported as new volatiles found in cooked soybean (Lee and Shibamoto, 2000) and cooked adzuki bean (Bi *et al.*, 2021; Tokitomo and Kobayashi, 1988). Maltol could be produced by the interactions between amino acids and carbohydrates, such as glucose and fructose reacting with lysine and alanine, under high temperature conditions (Lee and Shibamoto, 2000). Benzoic acid may arise from phenylalanine degradation or benzaldehyde oxidation (Selvarani *et al.*, 2024), which remains to be verified. Both benzoic acid and maltol are categorized as odor-active volatiles that may contribute to floral and fruity notes, while maltol imparts roasted nut and caramel flavors (Bi *et al.*, 2021; Mishra *et al.*, 2017). Tokitomo and Kobayashi (1988) reported maltol as the characteristic aroma of cooked

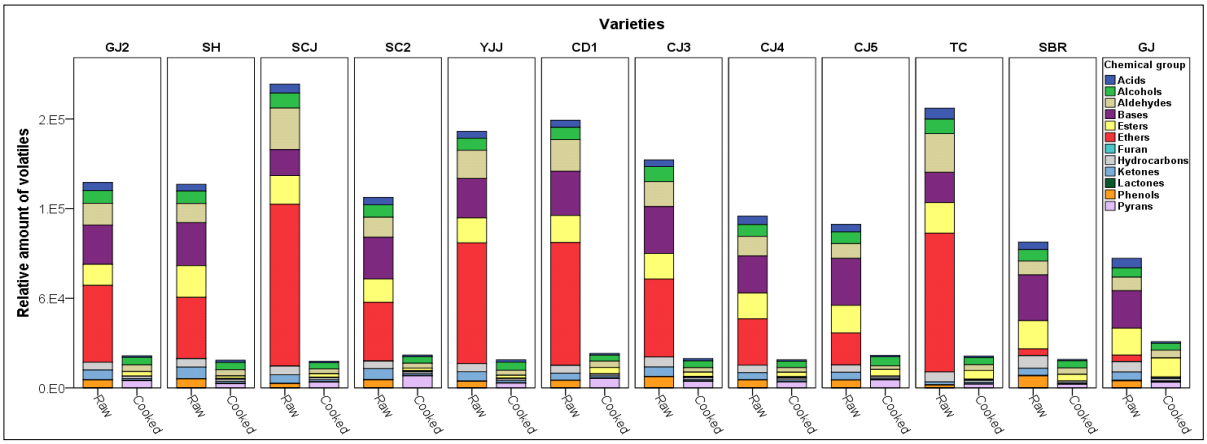
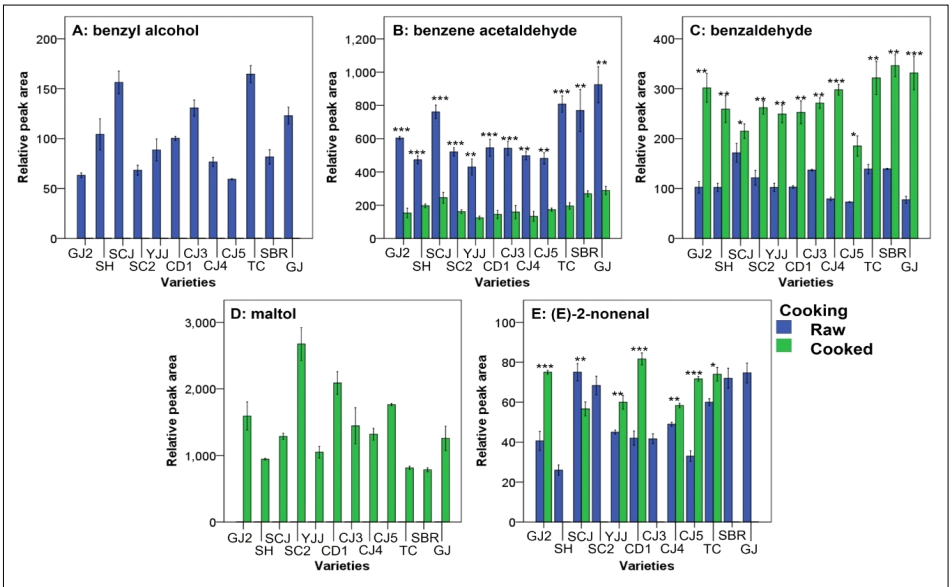
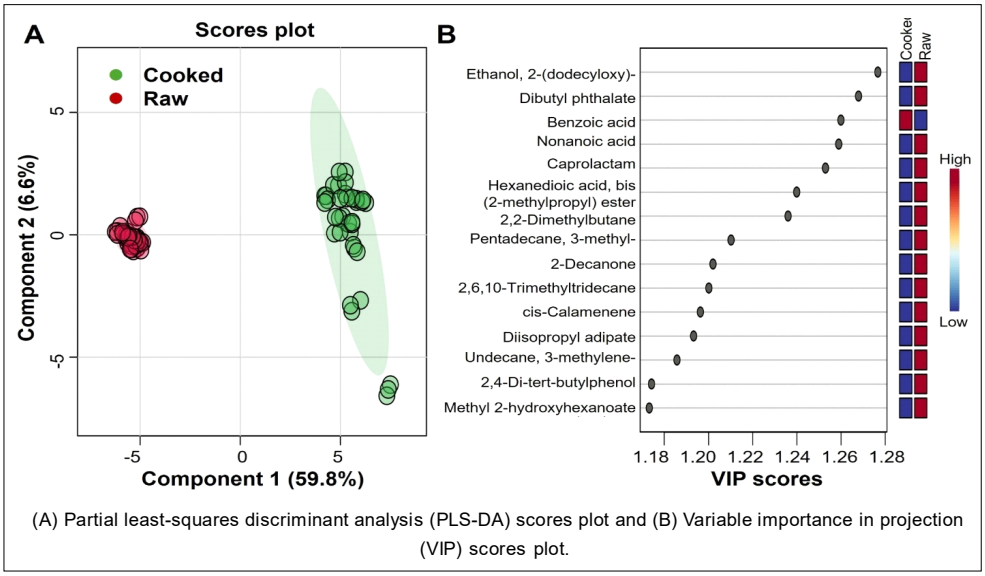


Fig 2: Relative amounts of volatiles by chemical groups in raw and cooked black soybean.



(A) Disappeared, (B) Decreased, (C) Increased, (D) Newly detected and (E) variety-dependent irregular changing patterns. Data represent mean±SD (n = 3). Asterisks indicate significant differences between raw and cooked soybeans by t-test: *p<0.05; **p<0.01; ***p<0.001.

Fig 3: Typical cases of five groups of volatiles classified by quantitative changes in their patterns during cooking.



(A) Partial least-squares discriminant analysis (PLS-DA) scores plot and (B) Variable importance in projection (VIP) scores plot.

Fig 4: Multivariate analysis of 51 volatiles observed of raw and cooked black soybeans.

adzuki bean, which produces a sugary flavor. All these results suggest that benzoic acid and maltol, newly detected under our experimental conditions after cooking, may significantly contribute to the flavor of cooked soybeans.

Six volatiles categorized into Group V (Fig 3E) exhibited inconsistent cooking-induced changing patterns depending upon variety. For example, (E)-2-nonenal completely disappeared after cooking in five varieties (SH, SC2, CJ3, SBR and GJ) but increased in six varieties (GJ2, YJJ, CD1, CJ4, CJ5 and TC), while decreased in SCJ (Fig 3E). These variety-dependent irregular changing pattern of volatiles are similar to Kato *et al.* (1981) who reported complicated and different behavior of volatiles during roasting.

Volatile profile-based discrimination of raw and cooked black soybeans

Partial least squares-discriminant analysis (PLS-DA) demonstrated that volatile profiles can effectively differentiate cooked soybeans from raw ones, regardless of variety (Fig 4A). Dibutyl phthalate, 2-dodecoxyethanol, benzoic acid and nonanoic acid showed the highest Variable Importance in Projection (VIP) scores (Fig 4B), suggesting these compounds could serve as markers differentiating raw and cooked soybeans.

Conclusion

This study characterized sugar, free amino acid and volatile profiles in raw and cooked black soybean to understand their changes during cooking with rice. The results showed that cooking reduces the total sugar content, significantly lowering fructose, while sucrose may remain stable or even increase. Cooking consistently increased the total free amino acid content across all tested varieties, while the proportional composition of individual free amino acids was preserved, with leucine, aspartate and lysine remaining the dominant free amino acids before and after cooking. Additionally, volatile compounds such as benzoic acid and maltol were newly detected after cooking, while other volatiles were decreased or completely lost under our experimental conditions. These changes are impacting both the nutritional content and sensory characteristics of soybeans. All these findings may provide important insights into how cooking soybeans with rice influences their chemical composition, with implications for culinary and nutritional contexts. However, this study did not assess the direct relationship between sensory properties and changes in sugar and volatiles; panel tests relating these components to consumer preference are warranted. Moreover, the study did not measure markers of Maillard thermal sugar-amino acid reactions and future studies should target their analysis.

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Conflict of interest

On behalf of all authors, the corresponding author states that there is no conflict of interest.

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