DOCTORAL DISSERTATION

Effect of food processing on soyfood qualities relating nutraceutical and taste characteristics

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ABSTRACT

Soybean based foods or soyfoods are recommended to consumed as nutritious and health promoting food products which according to nutraceutical properties of soy active compounds such as preventing cardiovascular diseases properties of soy active compounds such as soyasaponin and soy isoflavones. Beside the nutraceutical property of the soy bioactive compounds, they also affect in taste characteristics of food products. The soyasaponin is reported that it is a major cause of undesirable taste in soyfoods such as bitter taste, green and beany flavor, and astringent flavor. However, both nutraceutical and taste characteristics of soyfoods can be altered by food process due to alter those chemical structure of soy active compounds.

This research aimed to evaluate nutraceutical property of various soyfood samples, and the food processing effect on soyactive compounds. Firstly, the antioxidant activity of various soyfood products were evaluated along with antioxidative capacity of soyactive compounds which were soyasaponin and isoflavone including their glycoside and aglycone forms. The antioxidative capacity of soy active compounds and soyfood samples were evaluated by hydrophilic-oxygen radical absorbance capacity (H-ORAC assay). The soyfood samples included thermal processed products and fermented products. The soaking treatment (pre-treatment) effect on soyactive compounds were evaluated. Moreover, the precise soyasaponin content was identified in nine different soybean varieties and 39 soyfood products by using LC-PDA/MS/MS analysis. The soyasaponin composition which including fully-acetylated soyasapogenol A glucoside (FSAGs), partially-acetylated SAGs (PSAGs), deacetylated SAGs (DSAGs), and DDMPs were clearly identified. The FSAGs was reported as a major cause of undesirable taste characteristics in soy products, while group B saponin, DDMPs derivative compounds, promoted health benefit. Lastly, the soymilk preparing process was used as food processing model to investigate the effect of soymilk process on nutaceutical properties and taste characteristic of soymilk products.

According to antioxidant capacity investigation of soy active compounds and soyfood products, the result revealled that isoflavone presented higher H-ORAC value than soyasaponin. In addition, their different chamical structure presented different antioxidant activity. The aglycone isoflavone showed stronger antioxidant activity than glycoside isoflavone and soyasaponin. Whilst glycoside soyasaponin presented higher H-ORAC value than aglycone soyasaponin. Beside of the different capacity in each soyfoods, general treatment as soaking also altered the antioxidant capacity and the active compound composition. The long-term soaking could change the chemical structure of isoflavone and soyasaponin which glycoside isoflavone and soyasaponin were degraded to aglycone forms. Moreover, amino acid composition and free sugar content also were changed after soaking for 48h. According to precise soyasaponin complexity investigation, minor components as PSAGs were newly detected including monoacetyl, diacetyl, and triacetyl in nine soybean varieties and various soyfood samples. Moreover, SAGs were newly detected in seed cotyledons by using LC-PDA/MS/MS analysis. Among 39 soyfood products, the fermented food products presented lowest ratio of FSAGs and DSAGs (including partially- and null- acetylated gr. A) which SAGs related the undesirable tastes. On the other hand, group B saponin in fermented samples showed highest content that presented health promoting effects. The effect of soymilk preparation on DDMPs and maltol production also was investigated. The experiment revealled that DDMPs saponin could be degraded by thernal and radical treatments, but maltol was produced only by heat degradation. The result revealed that DDMPs could be degraded to gr.B

when it was treated with AAPH radical which has one oxygen atom attached at DDMP-moiety. But the DDMPs would be degraded to gr.E when it was dehydrogenated by DPPH-radical.

The research could be cancluded that nutraceutical properties of soyfood products depended on the soy active compounds which also affects the taste characteristics. And the chemical structure of the soy active could be altered by food processing. The changing chemical structure of soy active compounds especially soyasaponin may affect both health promoting properties and taste characteristics of soyfood product. Therefore, understanding the effect of food processing on soy active components may be useful for the development of health soyfood production.

INTRODUCTION

Soybean (*Glycine max*) is an important grain legume and it has long been recognized for providing nutritious protein, essential fatty acid and health benefits such as soyasaponins, isoflavones, tocopherol, lecithin etc. (T. Yamamoto, 2006). These soy active compounds were reported as health promotion source which could be called "nutraceutical compounds". The term nutraceutical was first coined by Stepha DeFelice in 1989 as a combination of "nutrition" and "pharmaceutical" (DeFelice, 1989). Nutraceutical defines a food or a apart of food containing health-giving additives and having medical benefits, including the potential for prevention and/or treatment of disease (Santini & Novellino, 2014, 2018).

WHO's latest "global health estimates" revealed that non-communicable diseases (NCDs); heart disease, stroke, cancer, diabetes, etc. were responsible for 70 percent of all deaths. The WHO also noted that 85 percent of premature NCD-related deaths occur in low- and middle- income countries which had a correlation between income level and the prevalence of NCDs.

According to health functional properties of soybean, soybean is recommended to intake in many countries. The U.S. Food and Drug Administration established 25g of soy protein daily for cholesterol reduction (FDA, 1999) which health claim of soyfoods and coronary heart diseases was awards since 1999 (Anderson & Bush, 2011; Zhan & Ho, 2005). Similar claims were approved in many other countries (Xiao, 2008). However, only protein in soybean was approved which not including another functional compound especially soyasaponin and isoflavones. Even though soybean is recognized as health promoting food. But the consumption of soyfood still limit due to undesirable taste characteristics as bitterness, grassy flavor, and dry mouth feel. The bioactive compounds in soybeans were reported as the cause of undesirable taste characteristics especially soyasaponin (Okubo, Iijima, Kobayashi, Yoshikoshi, & Uchida, 1992). Soyasaponin is a general term of glycosides having an oleanane-triterpene aglycone and more than 50 structurally different compounds as shown in Figure I.1. Following the aglycone structure, soyasaponins have been classified into group A and DDMP (2,3-dihydro-2,5-dihydroxy-6-methyl-4*H*-pyran-4-one) saponins. Group A saponins (gr.A) composed of soyasapogenol A glycosides (SAGs) and usually two sugar chains attached at the C-3 and C-22 positions. DDMP saponins (DDMPs) are DDMP conjugated soyasapogenol B glycosides at the C-22 positions which degrades to group B (gr.B) and group E (gr.E) saponins. Gr.B and gr.E saponins composed soyasapogenol B and soyasapogenol E as the aglycone, respectively.

Gr.A saponins are a major cause of undesirable taste characteristics in soyfood products (Shiraiwa, Kudou, Shimoyamada, Harada, & Okubo, 1991). Thus gr.A saponins commonly were purposed to eliminate from soybean such as genetic modified breeding techniques (Sundaramoorthy et al., 2018) and food preparing process (Asano, Okubo, Igarashi, & Yamaguchi, 1987). However only the glycoside form of gr.A saponin, fully acetylated soyasapogenol A glycosides was reported as the major cause of undesirable taste and astringent flavor whilst null-acetylated soyasapogenol A glycosides did not present those characteristics (Okubo et al., 1992).

Beside undesirable tastes of gr.A saponin, its health functionalities also were reported. Gr.A saponin was revealed the effect of *in vitro* reducing inflammations of liver cell (Kuzuhara, Nishiyama, Minowa, Sasaki, & Omoto, 2000), and expressed anti-obesity abilities (Yang et al., 2015). On the other hand, DDMPs commonly were reported that they can promote free radical scavenging properties due to unpaired electron at C-6 of DDMP moiety which not be detected

in their aglycone forms as gr.B and gr.E saponins (Yoshiki & Okubo, 1995). However, gr.B saponin also are reported as nutraceutical bioactive compounds due to their low absorption in human intestine. The ingested gr.B saponins in human digestive tract, gr.B saponins promoted anti-hyperlipidemic activity by interrupting bile acid and lipid reaction (Hu, Reddy, Hendrich, & Murphy, 2004). The gr.B saponins can be metabolized to soyasapogenol B by human intestinal microorganisms *in vitro* (Hu, Reddy, et al., 2004), it might affect to suppression of human colon cancer cell proliferation (Ellington, Berhow, & Singletary, 2005, 2006; Hu, Zheng, Hyde, Hendrich, & Murphy, 2004), and hepato-protective activities through thyroid hormone receptor in small intestine (Wu and Kang, 2011). Furthermore, the absorption of soyasapogenol was reported that the aglycone saponin, soyasapogenol could be absorbed into blood and gr.B saponin is more easily absorbed than gr.A saponin in rats (Kamo, Suzuki, & Sato, 2014). It's seemed that chemical structure changes of soyasaponin can affect to both taste characteristics and health functionalities.

On the other hand, soybean is one of the greatest sources of isoflavones which are reported as a subgroup of phytoestrogen. Soy isoflavones are natural bioactive compounds with structure similar to 17 β-estradiol and capable to binding to estrogen receptors (Pilšáková, Riečanský, & Jagla, 2010). Soy isoflavones could be classified into glycoside and aglycone form. There are three major isoflavones in soybean, which are daidzein (4'7-dihydroxyisoflavone), genistein (4',5,7-trihydroxyisoflavone), and glycitein (4',7-dihydroxy-6-methoxyisoflavone), and their β-glycoside, daidzin, genistin, and glycitin, respectively (S. Yamamoto & Tsugane, 2006). In generally, soy isoflavones naturally exist as glycosides (Wang & Murphy, 1994). However the glycoside forms are too polar (hydrophilic) to cross cellular membranes by diffusion (Setchell et al., 2002). Thus the isoflavones glycosides require hydrolysis to release the biologically active form. The isoflavones in soybean based food also were evaluated, the report revealed that the profile of isoflavones of fermented foods and non-fermented foods were different. The fermented soy food products mainly composed of aglycone isoflavones while non-fermented soy food products composed of glycoside isoflavones (S. Yamamoto & Tsugane, 2006).

Beside the early mentioned health benefits from soyasaponin and soy isoflavones, soybean also promotes the health functional properties such antioxidant activities which play an important role on reducing risk of cardiovascular disease (CVDs). Generally, the free radicals in human body can be eliminated by two activities which are an indirect activity or enzymatic system such as superoxide dismutase, glutathione peroxidase, glutathione reductase, and catalase enzyme; and a direct activity or non-enzymatic system such as vitamins and antioxidant compounds. The antioxidative property of soybean relates to isoflavones, soyasaponins and other components which can defense free radicals. The isoflavones were reported that they could act as directly and indirectly antioxidants by promoting catalase activity, superoxide dismutase, glutathione peroxidase, and glutathione reductase (Kurzer & Xu, 1997). Phenolic compounds in soybean acts as antioxidants which helping to eliminate free radicals such as reactive oxygen species in cell (Heim, Tagliaferro, & Bobilya, 2002). According to those health promoting compounds and properties, soyfoods were recommended to consume as healthy foods. However, some scientific information of the bioactive compounds in soybean still be scant such as minor compounds of soyasaponins, the chemical structure of the compounds in different foods, and food processing effect on those compounds.

Thus, this research aimed to evaluate the effect of food process on soy bioactive compounds particularly soyasaponin and isoflavones in soyfood products. These soy bioactive compounds directly affect the quality of soybean based food products including health

promoting property and consumer acceptance od taste characteristics. Finally, the results will be useful for food product development as health promoting food products and contribute to supporting consumer health.

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CHAPTER I

Antioxidant capacities of soyfoods, and long-term soaking effect on soy active compounds

Introduction

A peroxyl radical was reported as a cause of non-communicable diseases (NCDs) such as cardiovascular disease, cancer, and neurological disease (Pizzino et al., 2017). As oxidative stress *in vivo*, the antioxidant promoting food products are strongly recommended to be consumed for these NCDs prevention. Soybean is a recommended plant-based protein source which additionally promotes an antioxidant effect due to its antioxidant components such as isoflavones (Han et al., 2009; M. S. J. Kim, Y.S. Jang, D. Cho, C.H. Lee, S.-H. Han, N.S. Kim, D-O., 2022), saponins (Yoshiki, Kahara, Okubo, Sakabe, & Yamasaki, 2001; Yoshiki & Okubo, 1995), and tocopherols (Carrera, 2016) including anthocyanins (Kähkönen & Heinonen, 2003; Zilic et al., 2019) and proanthocyanins (Xu, 2017) in black soybean.

Isoflavones are naturally presented as glycoside forms particularly malonyglycoside (Wang & Murphy, 1994). Glycoside forms are too polar to cross cellular membranes by diffusion (Setchell et al., 2002). Thus, isoflavone glucosides require hydrolysis to release the biologically active form, aglycone. Soyasaponins are reported to inhibit colon cancer cell proliferation (Ellington, Berhow, & Singletary, 2005) and to eliminate oxidative compounds (Yoshiki et al., 2001; Yoshiki & Okubo, 1995). Moreover, black soybean highly contains antioxidative components such as anthocyanins in the seed coat (Kanamoto et al., 2011; Koh, Youn, & Kim, 2014). The black soybean also was evaluated and reported that can reduce plasma triglyceride, total cholesterol, low-density lipoprotein (Byun, Han, & Lee, 2010; Kanamoto et al., 2011; S. Y. Kim et al., 2015; Y. K. Kim et al., 2012). However, there is no report about an evaluation of the antioxidant capacity of individual isoflavones, soyasaponins, and their derived compounds by the same methodology including several processed soybeans.

Beside the antioxidant compounds, soybean also composes of prebiotic oligosaccharides such as stachyose and raffinose which can promote growth of intestinal bacteria in human tract (Yamamoto, 2006). These oligosaccharides were reported as promoting bifidobacteria and lactic acid bacteria growths which improving the immune function (Ma, Wu, Giovanni, & Meng, 2017). On the other hand, these health beneficial compounds also affect taste characteristics. For example, sugars are related to sweetness, free amino acids are related to savory taste (Kudou et al., 1993; Shallenberger, 1993; Solms, 1969; Zhao, Schieber, & Ganzle, 2016), and isoflavones and soyasaponins show undesirable taste characteristics (Okubo, Iijima, Kobayashi, Yoshikoshi, & Uchida, 1992).

Those components in soybeans can be altered during food processing treatment. The processing, including non-fermented and fermented process can induce degradation and/or conversion of soy bioactive compounds and affect their functionalities (Chitisankul et al., 2021; Chitisankul, Omizu, Uemoto, Varanyanond, & Tsukamoto, 2015; Khosravi & Razavi, 2021; Ping, Shih, Rong, & King, 2012). Since soaking process is a common pretreatment process which is generally required for soyfood production. And previous research revealed that some bioactive components changed in quantity and composition during soaking treatment which generated new components (deLima, Kurozawa, & Ida, 2014; Guo, Chen, Song, & Gu, 2011; Toda, Sakamoto, Takayanagi, & Yokotsuka, 2001). As a result, soaking treatment might affect bioactive compounds which contribute to health functionalities. This experiment revealed the effect of soaking treatment and so on the long-term soaking effect.

This research explained the antioxidant activity of soy active compounds and several processed soybeans. The isoflavone, soyasaponins and their derived compounds were evaluated. Moreover, the effect of long-term soaking on the active compounds in soybean also was investigated.

Objective

- To obtain the antioxidant activity of isoflavone, soyasaponins, and their derivative compounds by hydrophilic-oxygen radical absorbance capacity (H-ORAC) assay, which regarding health promoting effect.
- To investigate the antioxidant activities of several soyfood products.
- To evaluate the effect of soaking treatment on antioxidative capacities and changes of soy active compounds regarding health promoting properties.

Materials and methods

1. Samples and reagents

Soyfood samples : soyfoods were purchased from food supermarkets in Morioka (Iwate, Japan). The samples included non-fermented soyfoods (NFS) and fermented soyfoods (FS) as shown in Table I.1. The 11 samples of six kinds of NFS included steamed soybean, young soybean, soymilk, soy beverage, tofu, and fried tofu. The nine samples of four kinds of FS included natto, tempeh, miso, and soy sauce.

Soybean samples : Thai yellow soybean; *Glycine max* (L.) Merr. cv Chaing-Mai 60 (CM60) cultivated in Chaing-Rai Province of Thailand. Thai black soybean; *Glycine max* (L.) Merr. cv Sukhothai 3 (SK3). Soybean seeds were kept at below 10°C until analysis (about 2 months).

Reagents : all standard reagents were of high performance liquid chromatography (HPLC) grade. They included six isoflavone standard reagents; malonyldaidzin, malonylgenistin, daidzin, genistin, daidzein, and genistein, and soyasaponin standard reagents; soyasapogenol A and soyasapogenol B were purchased from Wako Pure Chemical Industries, Ltd., Osaka, Japan. Amino acid analysis reagents and amino acid standard reagents were purchased from Waters following PICO-Tag method. Kidney acetone powder porcine type II was purchased from Sigma-Aldrich. Actinase E and VitaFast® Folate Kit (P 1001) were purchased from Kaken Pharmaceutical Co., Ltd. and Azumax corporation, respectively. Solvents and other reagents for HPLC analysis were purchased from Wako Pure Chemical Industries, Ltd. as HPLC grade reagent. 2,2'-Azobis(2-amidinopropane) dihydrochloride (AAPH) and 6-hydroxy-2,5,7,8-tetramethylchroman-2-carboxylic acid (Trolox) was purchased from Sigma-Aldrich, Japan.

2. Sample preparations

Soyfood samples : Liquid soyfoods were directly extracted by 10-fold volume (*w/v*) of a 70% (*v/v*) aqueous ethanol solution. The sample solution was homogenized (15,500 rpm, 1 min, Polytron homogenizer, Jakarta, Indonesia) and left to stand for 1 h with every 10 min vortex mixing. The supernatant was obtained by centrifugation at 27,000×*g* and 15°C for 15 min (Kubota 7780 centrifuge, Tokyo, Japan) as a crude extract, and then filtered with a 0.45 µm membrane filter. For all tofu samples, the outer part was cut off with a ceramic knife, then the samples were fined in a mortar. Steamed soybean, edamame, natto, tempeh, and miso were crushed with a mallet and then fined in a mortar. Each solid soyfood sample was

extracted in the same manner as liquid samples. The crude extracts were kept in the opaque bottles at -20°C until analysis.

Table I.1 : Code, name, and description of soyfoods including non-fermented (NF) and fermented (F) products

Code	Sample Name	Sample description
NF1	Steamed soybean	Steamed whole seeds
NF2	Edamame	Heated and frozen green (young) soybean
NF3	Soymilk	Regular type soymilk
NF4	Soymilk w/o LOX	Soymilk from lipoxygenase enzyme deficient soybean
NF5	Modified soymilk	Blended soymilk
NF6	Soybean beverage	Beverage from whole soybean (including okara)
NF7	Black soybean beverage	Beverage from whole black soybean (including okara)
NF8	Momen tofu	Regular type tofu
NF9	Silken tofu	Very soft tofu
NF10	Abura-age	Twice-fried tofu, deep fried thin sliced tofu
NF11	Nama-age	Deep fried tofu
F1	Natto	Regular type natto
F2	Black soybean natto	Black soybean natto
F3	Tempe	Fermented soybean with *Rhizopus* spp.
F4	Rice miso, sweet- white	Rice-koji miso: sweet-white type
F5	Rice miso, light yellow	Rice-koji miso: light yellow type
F6	Barley miso	Barley-koji miso
F7	Soy miso	Soybean-koji miso
F8	Dark soy sauce	Dark soy sauce
F9	Light soy sauce	Light soy sauce

Soybean samples : Soybean sample was washed several times with running tab water then added distilled water for 5 times weight (w/w) of soybean. Samples were kept at 35°C in the dark incubator. Soaking time were 0, 12, 24, 48 and 72 h (at 72 h, measurement of free amino acids only). During soaking, water was replaced with new distilled water every 6 hours to prevent microorganism growth and spoilage. Soaked soybean samples were washed with distilled water before freeze drying treatment. Each soaked soybean sample was frozen in liquid nitrogen and then dried in a vacuum freeze dryer for 72 h. The moisture content of each sample was measured. The lyophilized sample was pulverized by multi-beads shocker (Yasui Kikai, Osaka, Japan) at 1,500 rpm for 30 sec. Dried powder samples were kept in airtight opaque plastic bottle and stored at -20°C until analysis. The samples were prepared in triplicate.

3. Antioxidative capacity determination

Hydrophilic-oxygen radical absorbance capacity (H-ORAC) assay was used to measure peroxyl radical scavenging capability (Ou et al., 2001). The standard and sample solution were diluted with phosphate buffer (75 mM, pH 7.0), then the Trolox standard solution of 6.25 µM to 100 µM was used as a standard curve. The H-ORAC values of isoflavone and soyasaponin were reported as mol Trolox equivalent (TE)/mol and those of the processed soybean food as

mmol Trolox equivalent (TE)/100 g sample. A fluorescence plate reader (Bio-Tek FL600, Bio-Tek Instruments, Inc., Winooski, VT, USA) was used for measurements.

4. Isoflavone determination

Soy powder sample was precisely weighted for 0.1 g then added 5 ml of 700 mL/L acidic aqueous ethanol (including 1 mL acetic acid) solvent. The sample solution was extracted at room temperature for 1 h while stirring every 10 min. Centrifugation (KUBOTA 5310) was performed at 1200 *xg* for 15 min, and about 2 ml of the supernatant was collected and passed through a membrane filter (0.45 µm) to prepare a sample solution. The sample solution was stored at -30 °C until HPLC analysis. Each sample was extracted in triplicate.

Isoflavone content in each sample was determined by HPLC analysis; Shimadzu high-performance liquid chromatography LC-10AD (Shimadzu Corporation) was operated with UV detector (Shimadzu UV - VIS detector SPD - 10A (Shimadzu Corporation)) at 260 nm and column of LiChrosorb RP-18 (7 µm, 4 × 250 mm, Kanto Kagaku Co., Ltd.) at 40 °C. Solvent A is 20 mM phosphate buffer (pH 3.2) which prepared by dissolving 3.12 g of sodium dihydrogen phosphate dihydrate with distilled water to make 1 L then the pH of solution was adjusted to 3.2 using hydrochloric acid solution. Solvent B was acetonitrile. Gradual elution was performed with flow rate 1.0 ml/min 9:1, solvent A:B as at 0 min with then shifted to 5:5, solvent A: solvent B at 30 min. The injection volume was 5 µl.

The isoflavone content was calculated from a calibration curve prepared using a standard reagent and reported in three group which were malonyl glycoside, glucosyl glucoside and aglycone. Malonyl glycoside included malonyl daidzin, malonyl genistin and malonyl glycitin. Glucosyl glycoside included daidzin, genistin and glycitin. Aglycone included daidzein, genistein and glycitein. Malonyl glycoside, glucosyl glycoside and aglycone were included as the total amount of isoflavone.

5. Soyasaponin determination

Soy powder sample was precisely weighted for 0.1 g then extracted with 19-fold volume of 800 mL/L aqueous methanol, and the sample solution was stirred at room temperature for 1 h. Thereafter, centrifugation (at 1200 *xg* for 15 min) was performed, and the supernatant was collected and passed through a membrane filter (0.45 µm) to prepare a sample solution. The sample solution was stored at -30°C until HPLC analysis. Each sample was extracted was in triplicate.

HPLC analysis for soyasaponin content determination was carried out under the following conditions with partial modification of the previous research. Shimadzu high-performance liquid chromatography LC-10AD (Shimadzu Corporation) was operated with UV detector (Shimadzu UV - VIS detector SPD - 10A (Shimadzu Corporation)) at 205 nm and column of Shiseido CAPCELL PAK (C18, UG 120, S-5 µm, 4.6 mm i.d. × 150 mm). The mobile phase was acetonitrile: 2-propanol: ultrapure water: acetic acid in the ratio of 360: 60: 580: 1 (v / v) including 0.1 mL EDTA·2Na with flow rate 1.0 mL/min. The injection volume is 10 µl. The DDMP saponin and group B saponin content in the sample were calculated by equation (1) and (2), respectively.

$$\text{DDMP saponin content (nmol / 10 µl)} = ([Bb] \times A_{DDMP} \times \varepsilon) / A_{std} \quad (1)$$

$$\text{Group B saponin content (nmol / 10 µl)} = ([Bb] \times A_{B}) / A_{std} \quad (2)$$

Whereas:

[Bb] = Bb concentration in the saponin standard solution (1.99 nmol/10 µl)

A_{std} = Peak area of saponin standard solution (Bb saponin)

A_{DDMP} = Peak area of DDMP saponin (αg, βg, βa, γg, γa) of the sample

A_B = Peak area of group B saponin (Ba, Bb, Bc, Bb') of the sample

ε = Conversion factor based on the molecular extinction coefficient of DDMP saponin which are αg: 0.854, βg: 0.810, βa: 0.900, γg: 0.810, γa: 0.810

The total saponin content composed of DDMP saponin and group B saponin. DDMP saponin included αg, βg, βa, γg and γa saponin. Group B saponin included Ba, Bb, Bc and Bb' saponin.

6. Free sugar determination

Soy powder sample was accurately weighted for 0.25 g and defatted with 2.5 ml of hexane then stirred for 10 min at room temperature. Thereafter, the mixture was centrifuged at 1200 *xg* for 15 min. The supernatant was discarded, and sample pellet would be defatted for three times. Then 0.25 ml of aqueous xylitol solution (30 mg/mL) was added as an internal standard solution and further 2.25 ml of 900 mL/L aqueous ethanol adding. The mixture was stirred and heated at 100 °C for 1 h then cooled to room temperature, and the sample was centrifuged at 1200 *xg* for 15 min then supernatant was collected. The sample residue was added 2.5 ml of 800 mL/L aqueous ethanol, followed by stirring and heating at 100 °C for 1 h. The collecting supernatant were totally repeated for three times. Approximately 2 ml of the total of three extracted supernatants was passed through a Sep-Pak Plus C-18 cartridge which was treated with 5 ml of methanol and washed with 5 ml of deionized water, and then passed through a membrane filter (0.45 μm) to prepare a sample solution. The sample solution was stored at -30°C until HPLC analysis. Each sample was extracted in triplicate.

Free sugar content was analyzed by HPLC (Shimadzu high-performance liquid chromatography LC-10AD VP (Shimadzu Corporation)) with differential refractometer detector CTO - 10A (Shimadzu Corporation). The extracted sample was analyzed by Polyspher CH PB column (7.8 × 300 mm, Kanto Kagaku Co., Ltd.) at 80 °C with 0.4 mL/min flow rate by using water as mobile phase. The injection volume was 10 μL. The content of stachyose, raffinose, sucrose, glucose and xylitol in the sample were calculated from the calibration curve which obtained from the standard compound. The total amount of free sugar included those free sugar contents.

7. Free amino acid determination

Sample preparation and HPLC analysis were performed according to Waters' PICO-Tag method (White, Hart, & Fry, 1986). The soy powder sample was precisely weighted at 0.1 g and extracted with 1.5 ml of 700 mL/L aqueous ethanol solvent. The mixture was stirred and refluxed for 10 min in hot water at 90 °C. This sample solution was stirred with a tube mixer for 30 min, then centrifuged at 10000 *xg* (CFM-100 micro- centrifuge; Iwaki, Tokyo, Japan) for 20 min, and the obtained supernatant was recovered. The residue pellet was repeatedly extracted with 100 μl of 700 mL/L aqueous ethanol and stirred for 10 min with a tube mixer. Then the sample mixture was centrifuged at 10000 *xg* for 20 min, and the obtained supernatant was recovered. The recovered supernatant was concentrated to dryness by a vacuum centrifuge dryer. 800 μl of 1 mL/L aqueous trifluoroacetic acid (TFA) solution was added to the crude extract and stirred for 30 min with a tube mixer, then centrifuged at 10000 *xg* for 20 min. The supernatant was passed through a Sep-Pak C-18 cartridge (treated with 1 mL/L TFA) and washed with 1 ml of 1 mL/L aqueous TFA solution. The sample was freeze dried at -80 °C. The 20 μl of an amino acid washing solution (ethanol:H_2O:TEA = 2:1:1) was added to crude extract and stirred for 20 min and concentrated to dryness by a vacuum centrifuge dryer.

Further, phenylthiocarbamyl reagent (ethanol:H_2O:TEA:phenylisothiocyanate = 7:1:1:1, mL/mL) was added the dried samples and allowed to react at room temperature for 20 min, after stirring for 30 min, then concentrated to dryness. The dried sample was added 100 µl of Pico-Tag diluted solution (0.05M Na_2HPO:CH_3CN = 95:5) and the mixture was stirred and centrifuged, and the supernatant was used as a sample solution for HPLC.

Free amino acid content was determined by HPLC (Waters 600E system) with PICO-TAG™ column (3.9 mm × 150 mm) at 46°C. Injection volume was 10 µl. The solvents were: (A) 140 mM $CH_3COONa{\cdot}3H_2O$ + 0.05% TEA (pH 6.5): CH_3CN = 940:60, and (B) CH_3CN: H_2O = 60:40. The sample was eluted and monitored at 254 nm. Free amino acid amount was calculated by following equation:

Amount of amino acid (mg/100g sample) = A x B x 10000

Whereas:

A = (amino acid MW) × (amino acid content in STD solution (0.00625 µmol)) x 10^{-3}

B = (sample peak area) / (standard amino acid peak area)

8. Folate determination

Folate content was determined by AOAC method (AOAC, 2000a, 2000b; Bassett & Sammán, 2010). The soy powder sample was precisely weighted for 1.0 g and added 10 ml of HEPES/CHES buffer solution (containing 20 g/L AsA, 0.8 mL/L 2ME, pH 7.85; hereinafter referred to as buffer solution) and mixed well for 1 minute. The sample was extracted about 20 h. The extract was washed with 15 mL buffer solution and transferred to a 100 ml heat-resistant screw-tip bottle, and subjected to autoclave (121 °C, 15 min) as pressure extraction. After cooling, 10 mL of Actinase E solution was added to the crude extract, and allowed to react at 37°C for 3 h, then heated in a boiling water bath for 10 min. After cooling, 5 mL of a conjugase solution (prepared from Kidney acetone powder (Porcine, Type II)), 25 mg of cysteine hydrochloride, 3 drops of toluene were added then the mixture was reacted at 37°C for 20 h. The mixture was heated in a boiling water bath for 10 min and let it cool, then centrifuged at 10000 *xg* at 4°C for 10 min in a high-speed cooling centrifuge. The supernatant was filtered and adjusted to 100 ml with a buffer solution.

Determination of folic acid was carried out as VitaFast® folic acid kit protocol. The sample was filtered through a 0.2 µm filter and appropriately diluted with deionized water. 150 µl of folic acid test component was added dropwise to all wells, and folic acid standard solutions (0.16, 0.32, 0.64, 0.96 and 1.28 µg/100 ml) or diluted sample was dropped in a predetermined well. After mixing, the mixture was incubated at 37°C for 48 h. The solution absorbance was monitored at a wavelength of 630 nm using a microplate reader (Model No. IMark, BIO-RAD). Folic acid content was expressed as µg / 100 g of powder dried sample.

Folic acid content (µg / 100 g) = A × F × W / 100 × 100

Whereas:

A = Folic acid concentration (µg / 100 g) in the sample solution obtained from the calibration curve

F = dilution factor

W = sample volume (g)

9. Color measurement

For miso and soy sauce, CIE-L* (lightness), a* (green to red color), b* (blue to yellow color) were measured using a colorimeter (color meter Z-300A, Nippon Denshoku Industries

Co., Ltd., Tokyo, Japan). Miso was packed in a Petri dish, the lid was put on, and the reflectance was measured. In addition, soy sauce was placed in a glass cell and permeation was measured. Miso was triplicate measured per sample, and soy sauce was measured once per sample.

10. Statistical analysis

Analysis of variance (ANOVA) of the experimental data was performed and the least significant difference was evaluated by Tukey's test at a 95% confidence interval. The correlation coefficient (r) of the experimental data was analyzed. All analyses were repeated in triplicate.

Result and Discussion

1. Antioxidative capacity of isoflavones and soyasaponins

The peroxyl radical scavenging capacity of isoflavones and soyasaponins had been shown as H-ORAC values (mol TE/mol) in Figure I.1. Isoflavones presented higher antioxidative capacity than soyasaponins. The chemical structure of active ingredients promoted different activities. For isoflavones, high H-ORAC values presented in the order of aglycone form then glycoside form, and malonyl glycoside form. Daidzein had the highest antioxidant ability, with the H-ORAC value reaching 9.94±0.45 mol TE/mol, followed by genistein, genistin, daidzin, and malonyl glycoside isoflavone, respectively. The result was supported by a previous report which revealed that the antioxidative activity of aglycones (daidzein, genistein) was higher than that of glycosides (daidzin, genistin) depending on the method of measuring the lag time of LDL oxidation (lag time assay) (Lee *et al*., 2005). Recently, Kim *et al.* (2022) also found that the $ABTS^{\bullet+}$ scavenging activity and ferric-reducing antioxidant power (FRAP) of soy isoflavones as aglycones were higher than those of their glycosides, and malonyl glycoside derivatives had the lowest activity. Isoflavones are generally found as glycoside forms in natural sources, but their bioavailability is lower compared to aglycones (Kim *et al*., 2022; Setchell *et al*., 2002). However, the glycoside isoflavone would be converted to aglycone by gut microflora (Bultosa, 2016).

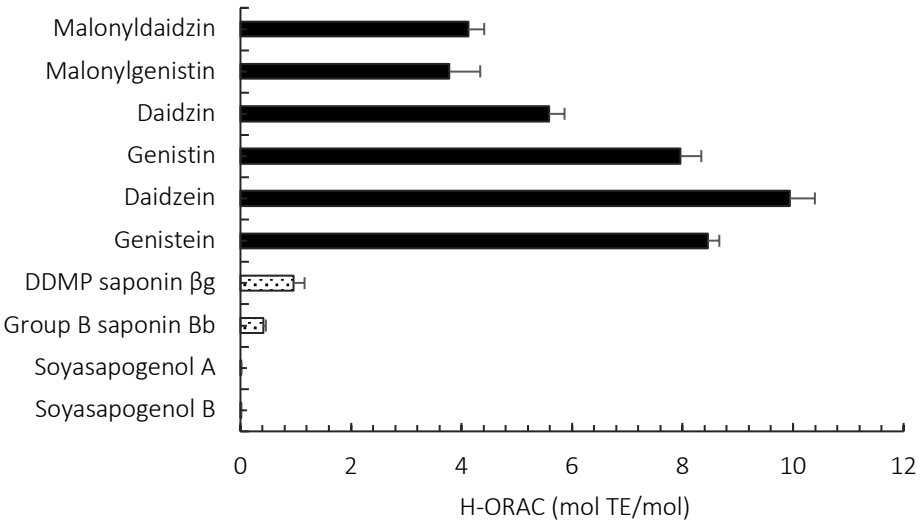

Figure I.1 : Hydrophilic-oxygen radical absorbance capacity (H-ORAC) of isoflavones and soyasaponins.

On the other hand, high H-ORAC values of soyasaponins are presented in the order of DDMP glycoside (DDMP saponin βg) then glycoside (group B saponin Bb), and aglycone

(soyasapogenol A, soyasapogenol B) (Figure I.1). According to this statement, it clearly shows that the changing chemical structure could affect the antioxidant activity of the compound. The degradation of DDMP saponin resulted in antioxidant activity reduction, which was agreeable with other reports (Yoshiki *et al*., 2001; Yoshiki & Okubo, 1995). The reports also revealed that the DDMP site was involved in superoxide scavenging ability (Yoshiki *et al*., 2001; Yoshiki & Okubo, 1995), thus group B saponin Bb which lacked the DDMP site would not present scavenging activity. Following the evaluation of soyasaponin peroxyl radical scavenging activity in this research, DDMP saponin βg presented strong peroxyl radical scavenging activity while group B saponin Bb presented low peroxyl radical scavenging activity. These results suggested that DDMP saponin βg could scavenge both peroxyl radicals and superoxide. Although, group B saponin Bb could not scavenge superoxide (Yoshiki *et al*., 2001; Yoshiki & Okubo, 1995) but it can eliminate peroxyl radical. On the other hand, the aglycone saponins; soyasapogenol A and soyasapogenol B, had no scavenging activity against peroxyl radicals (Figure I.1). From the above findings, it was speculated that the peroxyl radical scavenging ability was related to the DDMP site and sugar chain portion of soyasaponin. Nevertheless, DDMP saponin could be naturally found in fresh soybean or low processed soybean products which might vary depending on soybean variety and processing treatment (Chitisankul *et al*., 2019, 2021).

2. Antioxidative capacity of soyfoods

2.1 Antioxidative capacity of non-fermented soyfoods

The nutraceutical property due to the antioxidative capacity of 11 non-fermented soyfoods (NF) was different and depended on type of product (Figure I.2), relating their processing treatments. The NFS products could be categorized into three major groups: (1) steamed soybean (NF1) and frozen boiled edamame (NF2) as low-processed products, (2) soymilk or soy beverages (NF3-NF7), and (3) tofu (NF8-NF11). Only heat processes were applied to obtain the products of the first group whilst several treatments such soaking, extracting, and heating were required to produce other NF. Among all NF products, the steamed soybean showed the highest H-ORAC value (2.35±0.31 mmol TE/100 g). However, H-ORAC of the second low-processed product – edamame (young soybean seed), was much lower. It could be assumed that the difference was due to the content of the main antioxidative compounds which were, isoflavones and soyasaponins. Indeed, it was reported that contents of daidzein and genistein in soybean were at 0.25–1.23 mg/g and 0.33–1.17 mg/g, respectively (Wu et al., 2004). On the other hand, edamame contained daidzein and genistein in the amounts of 0.11–0.55 mg/g and 0.16–0.62 mg/g, respectively (Wu et al., 2004). Moreover, as reported in the previous research, edamame contained much less soyasaponins than mature soybeans, and more active DDMP saponins were degraded to group B saponin during heating (Chitisankul et al., 2021). Thus, the different antioxidant capacities of these low-processed samples might not be related to the food process but varied depending on the maturity of soybean.

For soymilk and soy beverages, four of five samples showed non-significant differences in H-ORAC values and only H-ORAC of modified soymilk (NF5) was significantly ($p<0.05$) lower (Figure I.2). Soy solid content of soymilk and soy beverages might play a significant role in their functional properties. On the report of product labeling, solid contents of regular (non-modified) soymilk (NF3), lipoxygenase-deficient soymilk (prepared from a 'Kinusayaka' soybean variety which is deficient in all of 3 lipoxygenase isozymes) (NF 4) and soy beverage (NF6) were 9 to 14 g/100 g or more, whereas the modified soymilk (NF5) contained about 7

g/100 g. Therefore, it could be considered that soy solid content might play an important role in antioxidative capacity in soymilk and soy beverages. In turn, black soybean beverage (NF7) was expected to be a rich antioxidative compound source. The previous research revealed that black soybean, in addition to isoflavones and soyasaponins, contained significant amounts of anthocyanins and proanthocyanidins (Xu et al., 2017; Zilic et al., 2019) and showed significantly higher antioxidative capacity than regular soybean (Chitisankul et al., 2019). However, anthocyanins are unstable water-soluble components that could be degraded by enzymatic reaction and thermal treatment (Slavu(Ursu) et al., 2020). The thermal treatment is generally required to preserve and process food for safety purposes. The traditional soymilk process might require thermal treatment at 99˚C while steam-infusion treatment might facilitate a higher temperature of about 99–154˚C (Johnson et al., 1981). In the last group of NFS samples, tofu products showed the lowest peroxyl radical scavenging capacity, especially abura-age, twice-fried tofu (NF10) (Figure I.2). There was no significant difference in H-ORAC between momen tofu (NF8) and silken tofu (NF9), with their H-ORAC values being lower than that of nama-age, fried tofu (NF11).

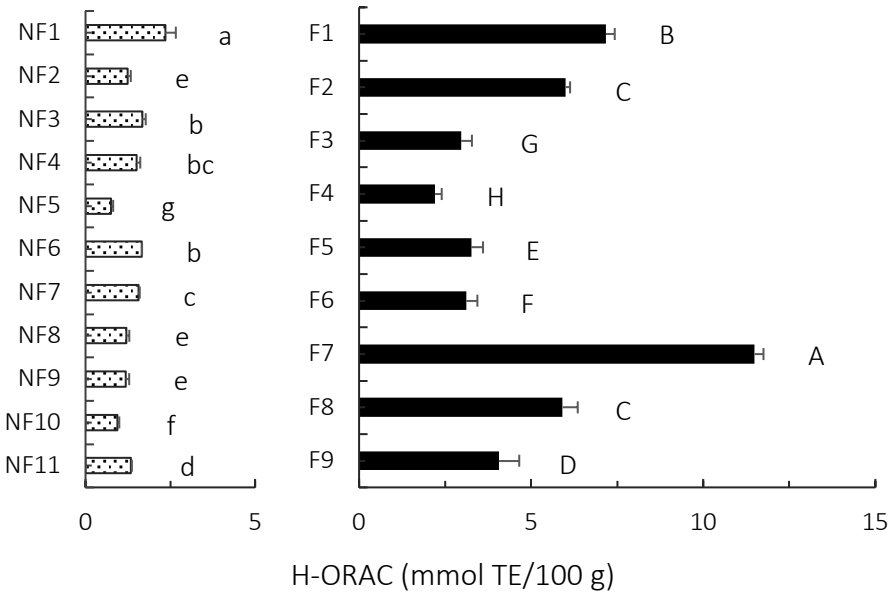

Figure I.2 : Hydrophilic-oxygen radical absorbance capacity (H-ORAC) of non-fermented (NF) and fermented (F) soyfoods. Details on the samples NF1–NF11 and F1–F9 are provided in Table I.1. Different letters presented significant differences between samples (p<0.05), lowercase letter for non-fermented soyfoods and uppercase letter for fermented soyfoods.

In summary, there was a significant difference in H-ORAC values among each group of NF soyfood products. It was considered that the slight difference found was mainly due to the difference in the isoflavone and soyasaponin contents and the respective compositions caused by the difference in the food processing treatment such as thermal process, extraction, and coagulation which following process requirement in the manufacturing production. The chemical structures of active compounds such as isoflavones and soyasaponins have an important role in nutraceutical property in soyfood as mentioned above. Malonyl glycoside, which is the main component of soybean isoflavone, is unstable to heat and derived from malonyl glycoside to glycoside and further to aglycone upon thermal treatment (Kasuga et al., 2006; Toda et al., 2000). DDMP saponin can be degraded to group B saponin by thermal

treatment and group E saponins by lipoxygenase-induced radical reaction during grinding (Chitisankul et al., 2015). It was considered that the non-fermented soybean foods had different antioxidative capacities due to their isoflavone and DDMP saponin compositions, depending on the processing treatment during the manufacturing process.

2.2 Antioxidative capacity of fermented soyfoods

Fermented soyfood (F) products could be categorized into four groups; natto (F1 and F2), tempeh (F3), miso (F4-F7), and soy sauce (F8 and F9). Among all F samples, soy miso showed the highest H-ORAC value followed by natto, soy sauce, other types of miso (F5 and F6), and tempeh, respectively (Figure I.2). In the comparison of NF and F samples, all F products had higher H-ORAC values than NF samples. The H-ORAC value of F samples ranged from 2.21±0.19 to 11.53±0.41 mmol TE/100 g while whole soybean seed (NF1) had 2.35 mmol TE/100 g.

High antioxidative capacity of black soybean natto was expected as mentioned above, because the seed coat of black soybean is rich in anthocyanins. Moreover, the previous research revealed that the H-ORAC value of black soybean was 5,870±115 μmol TE/100 g while that of soybean was 4,369±418 μmol TE/100 g (Chitisankul *et al*., 2019). However, the previous research also reported that isoflavone content of soybean was higher than that of black soybean (Chitisankul *et al*., 2019). Thus, it could be considered that isoflavones make a significant contribution to the peroxyl radical scavenging capacity instead of anthocyanins in natto. In addition, the H-ORAC value of natto was 3-fold higher compared to steamed soybean. Therefore, it clearly showed that the fermented process of natto could induce nutraceutical properties as enhancing antioxidant capacity. The biotransformation of isoflavones plays an important role to enhance those functionalities. The consistent reports explained the glycoside conjugates of isoflavones could be converted to isoflavone aglycones by β-glucosidase of *Bacillus subtilis* during fermentation (Dajanta *et al*., 2009; Khosravi & Razavi, 2021; Ping *et al*., 2012). Hence, the enhanced antioxidative capacity of regular soybean natto compared to black soybean natto might be due to three reasons which were 1) regular soybean had a higher isoflavone content, 2) aglycone isoflavones presented stronger antioxidative capacity than anthocyanins, and 3) degradation of water-soluble anthocyanins during natto production resulted in functionality losses. Moreover, it was found that natto had a higher antioxidant capacity than steamed soybeans (Figure I.2). The browning substances derived from the amino-carbonyl reaction (Ando *et al*., 2003) and antioxidant peptides released from proteins during fermentation (Sanjukta & Rai, 2016; Tonolo *et al*., 2020) could additionally contribute to the antioxidant potential of natto.

Although soy-miso (F7) showed the highest antioxidant activity, other kinds of miso (F4-F6) presented much lower peroxyl radical scavenging capacity especially the sweet-white type of rice miso (F4) with H-ORAC value of 2.21±0.19 mmol TE/100 g (Figure I.2). In the consent report, the antioxidant properties of different kinds of miso were evaluated by determining 2,2-diphenyl-1-picrylhydrazyl (DPPH) radical and superoxide scavenging activities (Matsuo & Hitomi, 2007). The soy-miso presented the highest antioxidative capacity following dark-yellow rice miso, light yellow rice miso, and sweet-white rice miso. And barley miso showed similar property with dark-yellow rice miso. Moreover, it was reported that the DPPH radical scavenging ability and the superoxide scavenging activity have a strong positive correlation with the total isoflavone content and aglycone content, respectively (Matsuo & Hitomi, 2007). During *Aspergillus* spp. fermentation in miso, the β-glucosidase was produced and activated to hydrolyze glycoside isoflavone to aglycone type isoflavones (Yamabe *et al*., 2007; Yan *et al*., 2016). Furthermore, during the malting process in soy miso, aglycones (daidzein and genistein) could be converted to *o*-dihydroxyisoflavones (ODI) while rice miso and barley miso do not

contain ODI (Esaki *et al*., 2001b). It was reported that ODI also contributes to the antioxidant properties of miso, since the higher the amount of ODI, the higher the antioxidant capacity (Esaki *et al*., 2001a). Additionally, the lower lightness (*L**) of the miso sample was revealed as related to higher antioxidative capacity. It was speculated that the browning substance derived from the amino-carbonyl reaction during fermentation and aging could contribute to the peroxyl radical scavenging activity of miso. For soy sauce, the H-ORAC value of dark-colored soy sauce (F8) was higher than that of light-yellow soy sauce (F9) (Figure I.2). Similarly to miso, soy sauce undergoes fermentation and aging which aglycone isoflavone producing but there was low total isoflavone content in soy sauce (Toda *et al*., 2000). Although the aglycone isoflavones were formed during the soy sauce production, they were detected as remaining in soy sauce cake, a by-product (Esaki *et al*., 2004). However, a positive correlation was found between the ODI content of soy sauce and its antioxidative capacity, and it was reported that ODI also contributes to the antioxidative capacity of soy sauce (Esaki *et al*., 2002). According to this evaluation, it is considered that the peroxyl radical scavenging activity of soy sauce, which was recognized by the ORAC value, is related to the browning substance derived from the amino-carbonyl reaction and ODI. Tempeh (F3) had a similar H-ORAC value as rice miso and barley miso, but lower than that of natto (Figure I.2). β-Glucosidase produced by *Rhizopus filamentous* fungi, which is used for tempeh fermentation, hydrolyzes isoflavone glycosides into aglycones [Kameda *et al*., 2018]. It was suggested that the isoflavone aglycone produced during fermentation might contribute to the antioxidant properties of tempeh (Murakami *et al*., 1984).

2.3 Color of fermented soyfood relating antioxidative capacity

The color parameters of long-term fermented soyfood samples, miso, and soy sauce were measured and *L**, *a**, and *b** values are shown in Table I.2. The relationship of color parameters with antioxidative capacity was also evaluated. The *L** value of miso decreased in order of sweet-white rice miso, light yellow rice miso, barley miso, and soy miso, the darkest miso. The *a** value of light-yellow rice miso was slightly higher than that of the other four miso samples. There was no significant difference in the *b** value among miso samples, except soy-miso which showed the lowest yellowness. On the other hand, dark soy sauce showed lower *L**, *a**, and *b** values than light soy sauce. After *L**, *a**, *b** value measurement, the correlations between these values and peroxyl radical scavenging capacity were found for miso and soy sauce. The results revealed that there were no significant correlations of H-ORAC values with *a** and *b** values.

Table I.2 : Color parameters of miso and soy sauces

Soybean foods	*L**	*a**	*b**
Rice miso: sweet-white type	65.33 ± 0.03^{a}	4.35 ± 0.05^{d}	35.37 ± 0.03^{c}
Rice miso: light yellow type	48.82 ± 0.04^{b}	9.33 ± 0.08^{a}	41.96 ± 0.02^{a}
Barley miso	44.86 ± 0.08^{c}	6.86 ± 0.01^{b}	35.68 ± 0.08^{b}
Soy miso	10.90 ± 0.09^{d}	5.73 ± 0.26^{c}	8.24 ± 0.26^{d}
Dark soy sauce	14.16	29.92	23.38
Light soy sauce	43.64	36.73	72.23

Note : Values for miso are expressed as mean ± standard deviation (*n*=3); Different letters in column presented significant differences ($p<0.05$); for soy sauce value *n*=1; *L**, lightness; *a**, redness; *b**, yellowness.

On the other hand, a strong negative correlation (r=-0.9747, *p*=0.0048) was found between the *L** and H-ORAC values of miso samples and soy sauce, excluding soy miso (Figure I.3). Therefore, the low *L** value, darkness of soy sauce or miso, might be an index of browning reagent contents, the amino-carbonyl reaction product which induced a higher H-ORAC value. The result was supported by other consistent reports. The correlations of *L** value with both DPPH radical and superoxide scavenging activities of various miso paste samples were negative (Matsuo & Hitomi, 2007). In our study, soybean miso presented a significantly higher H-ORAC value than the expected H-ORAC value based on the *L** value (Figure I.3). It could be explained that soybean miso had a higher ratio of soybean content than other kinds of miso, and the soybean miso production might be different too. Therefore, the strong peroxyl radical scavenging capacity of soybean miso might be due to the high content of isoflavones, soyasaponins, ODI (Esaki *et al*., 2001a,b), and antioxidative peptides released from soy proteins (Sanjukta & Rai, 2016).

3. Effect of long-term soaking on soy active compounds

Both soybean varieties absorbed water at 35°C within 12 h soaking, thereafter moisture content of the soaked soybeans did not change significantly. Moisture content of the samples increased from 13.5±0.2% to 53.5±0.7% after soaking. Soybean morphology changed during soaking; the seed hypocotyl elongated and germinated about 0.5-2.0 mm depending on soaking time. Weight of the seeds did not change significantly during long-term soaking. Nutraceutical compound contents of CM60 and SK3 were evaluated during long-term soaking as follows.

3.1 Isoflavone content

Malonyl isoflavones naturally exist in soybean (Toda et al., 2001; Toda, Sakamoto, Takayanagi, & Yokotsuka, 2000) and were detected in raw CM60 and SK3 seeds (Figure I.3). In CM60, malonyl isoflavones and total isoflavone contents did not change after 24h soaking but decreased thereafter. The isoflavone glycosides (daidzin and genistin) decreased whilst aglycones (daidzein and genistein) increased during soaking. Total glycoside forms degraded from 263.22±2.35 to 121.55±6.08 (mg/100g DM) during 48 h soaking, while the aglycone forms increased from 11.43±0.49 to 122.72±3.23 mg/100 g DM.

Malonyl isoflavones hydrolyse into glycoside and aglycone by thermal and enzymatic degradation via processing treatment (Toda et al., 2000; Y. Zhang, Chang, & Liu, 2015). Reduction of malonyl isoflavone in CM60 during soaking occurred through decomposition to glycoside or other substances. Soybeans contain β-glucosidase which hydrolyzes glycoside to produce aglycone (deLima et al., 2014; Wardhani et al., 2008); therefore, reduction of glycosides and increasing aglycones in CM60 resulted from β-glucosidase activity. Reduction of glycoside in SK3 was lower than in CM60. Reduction is activated by α-glucosidase and β-glucosidase, with the former inhibited by anthocyanin. Black soybean seed coat contains polyphenols such as flavonoids and catechins which inhibit digestive reactions by binding with various enzymes (J.-S. Kim, Kwon, & Son, 2000). Thus, lower reduction of malonyl isoflavones into aglycones in SK3 may be caused by the presence of anthocyanin and other polyphenols in the seed coat of SK3 which inhibit the enzymatic reaction (Matsui et al., 2001; McDougall & Stewart, 2005).

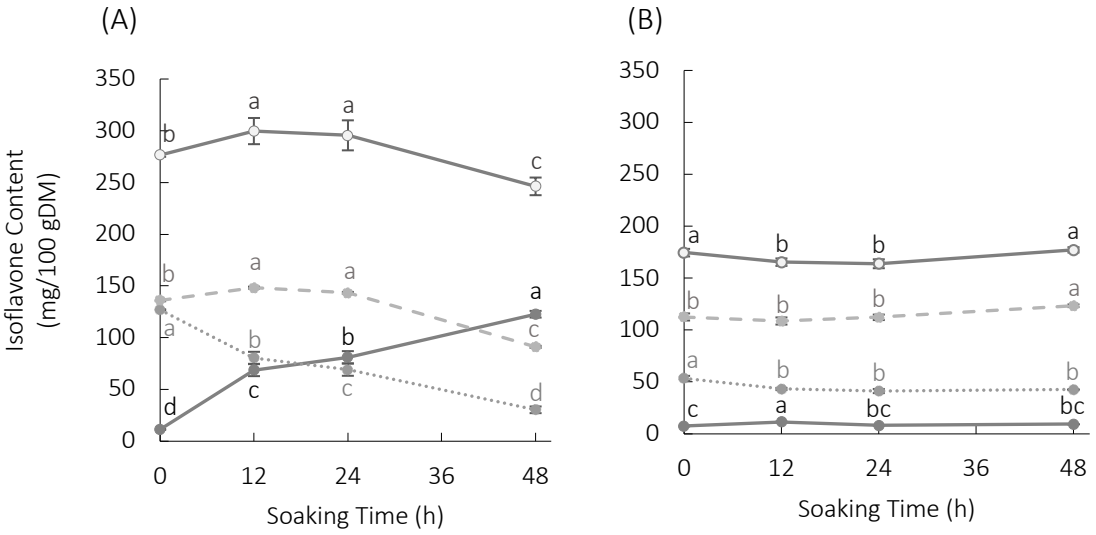

Figure I.3 : Isoflavone content (mg/100g DM) in CM60 (A) and SK3 (B) samples at different soaking times (h); value: mean ± SD (n=3); —●— : aglycone; ···●··· : glycoside; - - ●- - : malonyl-glycoside; —○— : total amount of isoflavones; Different letters (a-d) within the same sample show significant difference ($p<0.05$).

Previous research revealed that aglycone could be absorbed faster and in higher amounts than glycoside (Izumi et al., 2000). Thus, isoflavone aglycone-rich products might offer greater health benefits than glycoside-rich products (Izumi et al., 2000). Genistein inhibits cancer cell proliferation (Mostrom & Evans, 2011) and long-term soaking induced conversion of malonyl and glycoside forms into the aglycone, genistein, in CM60. Genistein in CM60 increased from 7.65±0.35 to 85.45±3.30 mg/100 g DM after 48h soaking while genistin and malonylgenistin degraded from 78.07±0.63 to 17.32±0.60 and 91.09±1.02 to 67.60±0.85 (mg/100 g DM), respectively. Thus, increasing genistein in long-term soaked CM60 could promote higher nutraceutical functions which contribute to phytoestrogen action. The nutraceutical properties of phytoestrogen in soybean were reported to manage diabetes, reduce low-density lipoprotein and triglycerides, and reduce the risk of coronary heart disease (Meghwal & Sahu, 2015).

3.2 Soyasaponin content

DDMP-saponins contain DDMP (2,4-dihydro-2,5-dihydroxy-4-methyl-*4H*-pyran-4-one) moiety at the C-22 hydroxyl residue of soyasapogenol B and decompose to group B and/or group E saponins by heat, acid, alkali, and enzymatic treatments (Heng et al., 2006; Serventi et al., 2013). Both CM60 and SK3 showed no significant change in total soyasaponin contents during long-term soaking but the ratio of DDMP-saponins and group B saponins were altered (Figure I.4).

The DDMP-saponins decreased while group B saponins increased after 24h soaking in both seed varieties. The DDMP moiety contains an unpaired electron at C-6 which promotes radical scavenging activity and is not observed in group B and group E (Yoshiki & Okubo, 1995). Long-term soaking treatment of 48h degraded DDMP-saponins and affected soybean antioxidant capacity. However, increasing group B saponins might promote different health benefits (Bau, Villanme, Nicolos, & Mejean, 1997). Group B health functionality was reported to inhibit and prevent colon cancer (Ellington et al., 2005), and also offer a possible treatment against Alzheimer's disease (Hong, Yoo, Jeong, Yang, & Kim, 2014). After 48h soaking, group B saponins increased from 75.64 ± 2.94 to 153.25 ± 3.73, and 57.59 ± 3.83 to 135.77 ± 43.89

(μmol/100 g DM) in CM60 and SK3, respectively. Thus, the 48h soaking treatment enhanced nutraceutical effects in both soybean varieties.

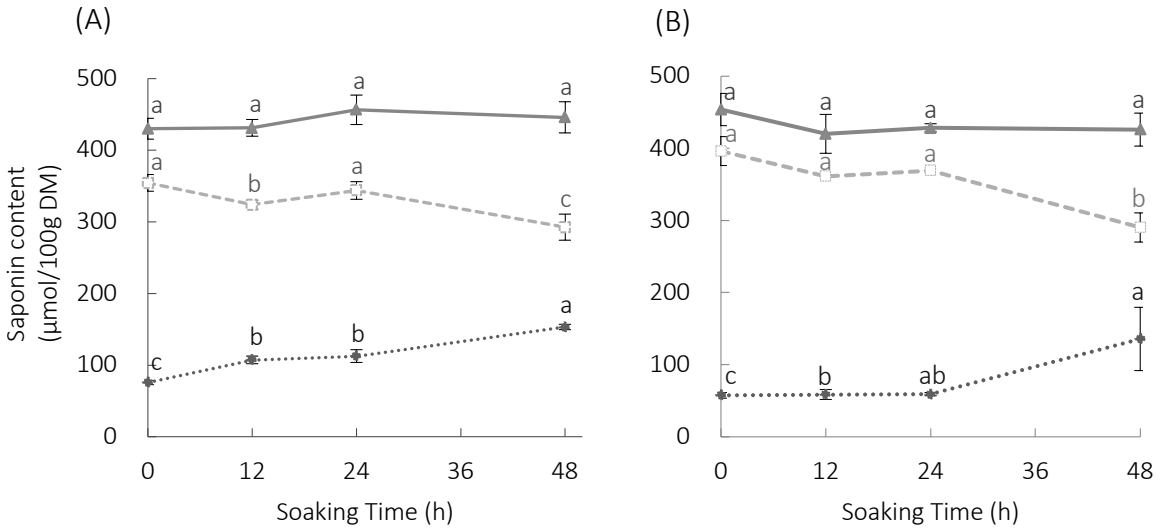

Figure I.4 : Soyasaponin content (μmol/100g DM) in CM60 (A) and SK3 (B) samples at different soaking times (h); value: mean ± SD (n=3); : group B saponin; : DDMP-saponin; : total amount of saponins; Different letters (a-c) within the same sample show significant difference ($p<0.05$).

For taste characteristics, a previous study indicated that degradation of DDMP-saponins affected the sweet flavor and taste characteristics of soy products (Chitisankul et al., 2015). During thermal degradation of DDMP-saponins, group B saponins and maltol (natural sweet components) are produced; however, this phenomenon occurs only by thermal degradation, not enzymatic reaction. Thus, long-term soaking, which degrades DDMP-saponins by enzymatic degradation, could reduce the naturally sweet taste by maltol production. DDMP-saponins in CM60 and SK3 degraded by 17% and 27%, respectively during long-term soaking. Therefore, maltol could be produced by the cooking process later.

3.3 Free sugar content

Stachyose, raffinose, sucrose, and glucose were determined in long-term soaked soybean samples. The main saccharide in soybean samples was sucrose, which presents as a sweet taste, followed by stachyose, raffinose, and glucose respectively. Sucrose and stachyose content in CM60 continuously decreased with longer soaking time, while raffinose and glucose were less affected (Figure I.5A). The α-1,6 bonds of stachyose and raffinose can be hydrolyzed by α-galactosidase to galactose and sucrose in soybeans (Guimarães, Rezende, Moreira, Barros, & Felix, 2001; Porter, Herrmann, & Ladisch, 1990; Viana et al., 2005). Thus, stachyose and raffinose in CM60 were hydrolyzed under the action of α-galactosidase during soaking. Sucrose has higher solubility in water and smaller molecules than stachyose and raffinose; therefore, it was eluted from the soybean seeds into the soaking liquid without accumulating in the seeds and the sweet taste of soaked soybean reduced due to decreasing amounts of total sugar. In SK3, only sucrose content decreased during soaking from elution, while stachyose and raffinose showed non-significant differences (Figure I.5B). Degradation of free sugars was activated by enzymatic reaction such as the α-galactosidase reaction which

occurred during soaking as seed germination. On the other hand, stachyose and raffinose in SK3 did not degrade following enzymatic reaction. Isoflavone degradation was inhibited by polyphenol compounds in the seed coats. Active compounds such as anthocyanin inhibited α-galactosidase, then enzymatic hydrolysis did not occur.

Free sugar degradation in CM60 concurred with previous research by Luo et al. (2009) who reported that sugar content in soaking water increased after 5h soaking. Moreover, the bran layer also resisted ingress of water and reduced leaching. Diverse bran layers of different soybeans affected soluble solid loss which was reported in the range of 0.2% to 0.7%. Previous research noted that the major sugars in soaking water were raffinose and stachyose, including soluble protein (Luo et al., 2009), while long-term soaking resulted in excessive loss of nutrients, especially carbohydrates. Free sugar degradation impacted on taste characteristics and health functionality, especially for stachyose and raffinose which are major health functional oligosaccharides in soybean as prebiotic sources (Yamamoto, 2006).

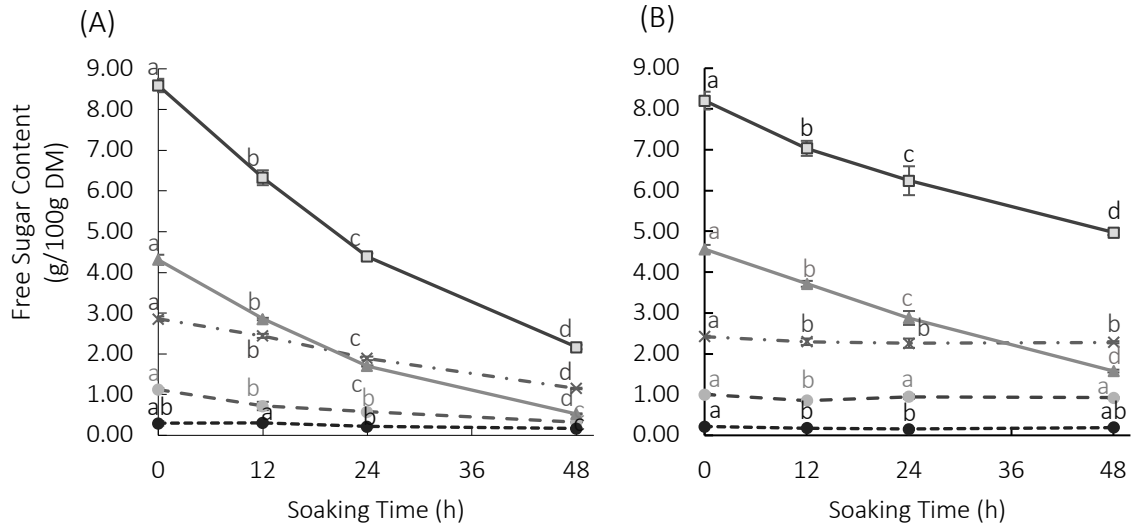

Figure I.5 : Free sugar content (g/100g DM) in CM60 (A) and SK3 (B) samples at different soaking times (h); value: mean ± SD (n=3); : stachyose; : raffinose; : sucrose; : glucose; : total amount of free sugars; Different letters (a-d) within the same sample show significant difference ($p<0.05$).

3.4 γ -aminobutyric acid (GABA) and free amino acid content

The health functionalities of GABA, especially as an inhibitory neurotransmitter in the sympathetic nervous system (T. H. F. Wang, Tsai, Lin, & Ou, 2006), have been researched for the past decade. Results revealed that germination enhanced GABA content in soybeans (Matsuyama et al., 2009; Xu & Hu, 2014), legumes (Bau et al., 1997) and cereals such as wheat (Moongngarm & Saetung, 2010), barley (Chung, Jang, Cho, & Lim, 2009) and rice (M. Y. Kim et al., 2015; Komatsuzaki et al., 2007; Thuwapanichayanan, Yoosabai, Jaisut, Soponronnarit, & Prachayawarakorn, 2015; H. Zhang, Yao, Chen, & Wang, 2007a). Moreover, amino acids play an important role in plant metabolism as preventive and development actions. Various abiotic stress conditions can induce amino acids, especially GABA and proline accumulations in plant tissues (Ashraf & Foolad, 2007; Aurisano, Bertani, & Reggiani, 1995; Hayat et al., 2012).

Long-term soaking induced stress as oxygen lacking condition in soybean seeds and affected GABA accumulation in both CM60 and SK3 seeds (Tables I.3 and I.4). GABA is produced from the decarboxylation of glutamic acid by glutamic acid decarboxylase (H. Zhang, Yao, Chen, & Wang, 2007b). Considering the amount of GABA and the amount of glutamic acid in CM60

and SK3 at each soaking period, glutamate sharply decreased after 12h soaking while GABA showed a marked increase. After 12h soaking, GABA content continuously decreased but remained higher than raw samples. Thus, the soaking process activated enzymatic decarboxylation of glutamic acid and GABA was produced. Highest GABA contents were detected at 12h soaking time for both soybean varieties. The highest GABA accumulation after 12h soaking promoted the highest nutraceutical nervous system functions. Total amino acids in CM60 greatly reduced after soaking for 48h while amino acid contents in SK3 changed slightly after long-term soaking.

Amino acids can impact taste characteristics in soy products such as savory, sweet, sweet to bitter, bitter to sweet and bitter (Shallenberger, 1993; Solms, 1969; Zhao et al., 2016). Taste characteristics of amino acids are listed in Tables I.3 and I.4. For raw CM60, about 60% of total amino acid (954.78 mg) presented undesirable bitter-sweet and bitter taste characteristics (Table I.2). These undesirable taste amino acids continuously increased during soaking treatment but decreased from 562.1 to 254.0 mg/100 g DM after soaking for 48h. Thus, the undesirable taste of CM60 degraded after soaking for 48h or longer. Moreover, the total amount of amino acids in CM60 was almost 50% degraded after soaking in water for 48h. Nevertheless, GABA reported health functional compounds greatly increased after soaking for 12h and then slightly decreased with longer soaking in water. This result suggested that 12h soaking was suitable for enhancing GABA accumulation in CM60, while longer soaking (48-72h) reduced undesirable taste characteristics. For SK3, GABA content was highest in 12h soaked samples; however, the ratio of undesirable and desirable taste amino acids did not vary greatly in soaked SK3 samples.

The ratio of desirable and undesirable taste amino acids in soybean samples can be used to predict taste characteristics. As examples, Japanese soybean was composed of 65.4% desirable taste amino acids (Tamura, 1970) including umami savory taste and sweet taste, US soybean comprised 64.3% desirable taste amino acids (Kuiken & Lyman, 1949), while CM60 and SK3 contained 38.4% and 44.7% desirable umami, sweet and sweet-bitter taste amino acids, respectively. According to these ratios, Japanese soybean may provide the most delicious taste and CM60 may present the most bitterness. Furthermore, the most bitter amino acid is tryptophan and which was reported to be about 0.133% bitterness strength of caffeine (Solms, 1969). CM60 was composed of 7.21% tryptophan followed by 1.49 and 1.36% for Japanese soybean and US soybean, respectively. Accordingly, CM60 contained higher bitter amino acids than both Japanese and US soybean.

Table I.3 : Amino acid content (mg/100 g DM) in CM60 samples at different soaking times (h)

Amino acid	0 h	12 h	24 h	48 h	72 h
Glutamic acid*	87.45 ± 0.68 [a]	29.44 ± 1.14 [b]	18.30 ± 1.85 [c]	33.93 ± 3.38 [b]	27.21 ± 1.35 [b]
Aspartic acid*	9.90 ± 0.02 [a]	6.33 ± 0.36 [b]	nd	9.08 ± 0.02 [a]	nd
Total umami	**97.35 ± 0.69 [a]**	**35.77 ± 1.10 [c]**	**18.30 ± 1.85 [e]**	**43.00 ± 3.71 [b]**	**27.21 ± 1.35 [d]**
Glycine†	9.85 ± 0.26 [c]	11.46 ± 0.16 [c]	15.29 ± 1.64 [b]	11.84 ± 0.44 [c]	19.43 ± 0.26 [a]
Alanine*	79.24 ± 0.15 [a]	54.35 ± 2.26 [c]	52.13 ± 1.87 [c]	53.49 ± 3.63 [c]	60.31 ± 2.10 [b]
Threonine†	21.33 ± 0.41 [b]	24.12 ± 0.72 [a]	25.45 ± 1.58 [a]	10.58 ± 0.20 [d]	13.95 ± 0.53 [c]
AABA†	47.36 ± 0.27 [b]	50.86 ± 0.41 [a]	46.13 ± 1.50 [b]	6.34 ± 0.03 [d]	8.21 ± 0.14 [c]
Total sweet	**158.78 ± 0.63 [a]**	**140.79 ± 0.81 [b]**	**139.00 ± 5.11 [b]**	**82.25 ± 4.30 [d]**	**101.91 ± 2.46 [c]**
Serine†	14.39 ± 0.04 [a]	11.52 ± 0.08 [b]	10.57 ± 1.01 [b]	8.61 ± 0.59 [c]	9.57 ± 0.48 [b]
Valine†	38.93 ± 0.55 [c]	47.14 ± 1.38 [b]	50.37 ± 2.06 [a]	16.81 ± 0.33 [d]	51.27 ± 0.69 [a]
Proline†	44.33 ± 2.31 [c]	59.95 ± 1.95 [b]	66.12 ± 3.80 [a]	19.16 ± 0.65 [e]	40.67 ± 0.14 [d]
Lysine†	3.56 ± 0.02 [b]	4.12 ± 0.09 [a]	3.49 ± 0.16 [b]	nd	nd
Total sweet-bitter	**101.21 ± 2.70 [b]**	**122.73 ± 2.26 [a]**	**130.54 ± 5.97 [a]**	**44.58 ± 1.57 [c]**	**100.87 ± 0.86 [b]**
Methionine†	201.81 ± 0.73 [a]	177.76 ± 3.70 [b]	150.56 ± 5.96 [c]	31.89 ± 0.38 [e]	36.10 ± 0.40 [d]
Asparagine†	71.06 ± 0.69 [a]	38.49 ± 1.22 [d]	44.68 ± 3.79 [c]	55.02 ± 3.09 [b]	58.17 ± 3.43 [b]
Cysteine†	4.50 ± 0.08 [a]	3.90 ± 0.30 [b]	4.52 ± 0.16 [a]	nd	nd
Total bitter-sweet	**277.37 ± 0.26 [a]**	**220.15 ± 1.38 [b]**	**199.76 ± 8.10 [c]**	**86.90 ± 3.47 [d]**	**94.11 ± 3.83 [d]**
Arginine†	51.52 ± 0.45 [b]	37.96 ± 2.37 [c]	39.93 ± 0.64 [c]	49.52 ± 1.73 [a]	38.52 ± 1.46 [c]
Isoleucine†	25.18 ± 0.51 [b]	26.02 ± 0.39 [b]	29.70 ± 1.17 [a]	8.87 ± 0.21 [d]	15.28 ± 0.33 [c]
Histidine†	13.98 ± 0.26 [b]	15.34 ± 0.02 [a]	14.76 ± 1.11 [ab]	12.77 ± 0.93 [b]	10.73 ± 0.93 [c]
Glutamine†	6.54 ± 0.12 [e]	10.91 ± 0.54 [b]	12.25 ± 0.77 [a]	7.23 ± 0.16 [d]	8.13 ± 0.25 [c]
Tyrosine*	57.31 ± 2.12 [b]	87.09 ± 4.26 [a]	91.05 ± 3.55 [a]	25.31 ± 0.40 [d]	49.61 ± 0.63 [c]
Leucine*	32.81 ± 0.96 [c]	39.59 ± 0.23 [b]	54.73 ± 2.19 [a]	20.81 ± 0.08 [d]	33.00 ± 9.18 [bc]
Tryptophan*	68.82 ± 1.14 [b]	85.28 ± 4.85 [a]	72.82 ± 3.30 [b]	23.82 ± 0.07 [d]	39.30 ± 0.14 [c]
Phenylalanine*	28.56 ± 0.83 [d]	63.04 ± 2.29 [b]	71.11 ± 4.20 [a]	18.76 ± 0.14 [e]	35.57 ± 0.17 [c]
Total bitter	**284.72 ± 5.12 [b]**	**365.23 ± 7.25 [a]**	**386.34 ± 13.36[a]**	**167.08 ± 3.56 [d]**	**235.30 ± 5.01 [c]**
GABA (tasteless)	36.04 ± 0.52 [e]	86.73 ± 1.69 [a]	77.26 ± 1.45 [b]	54.28 ± 1.98 [d]	57.86 ± 0.58 [c]
Total amino acid	**954.78 ± 9.92 [b]**	**981.91 ± 10.67 [a]**	**951.20 ± 1.44 [b]**	**478.10 ± 18.59 [d]**	**617.25 ± 4.57 [c]**

Note : value: mean ± SD; GABA: γ-aminobutyric acid; AABA: α-aminobutyric acid; nd: not detected; Taste characteristics of amino acids followed *Solms (1969) and †Shallenberger (1993); Different superscripts (a-e) within the same row show significant difference ($p<0.05$)

Table I.4 : Amino acid content (mg/100 g DM) in SK3 samples at different soaking times (h)

Amino acid	0 h	12 h	24 h	48 h	72 h
Glutamic acid*	103.92 ± 0.45 [a]	58.54 ± 0.40 [b]	52.60 ± 1.14 [c]	44.81 ± 2.13 [d]	40.63 ± 0.78 [e]
Aspartic acid*	17.59 ± 0.08 [a]	8.28 ± 0.19 [b]	8.43 ± 0.28 [b]	7.78 ± 0.46 [c]	7.21 ± 0.15 [c]
Total umami	**121.51 ± 0.37 [a]**	**66.82 ± 0.57 [b]**	**61.03 ± 1.00 [c]**	**52.58 ± 2.58 [d]**	**47.84 ± 0.70 [e]**
Glycine†	6.80 ± 0.02 [e]	11.20 ± 0.27 [d]	13.53 ± 0.21 [c]	17.54 ± 0.37 [b]	20.21 ± 0.22 [a]
Alanine*	81.61 ± 0.68 [a]	69.38 ± 0.48 [b]	70.22 ± 2.22 [b]	70.98 ± 2.44 [b]	71.19 ± 1.59 [b]
Threonine†	23.86 ± 1.74 [d]	27.61 ± 0.29 [c]	32.84 ± 0.93 [a]	34.63 ± 1.88 [a]	29.46 ± 0.30 [b]
AABA†	49.54 ± 1.35 [a]	46.59 ± 0.36 [b]	49.10 ± 1.30 [a]	49.49 ± 1.37 [a]	42.70 ± 1.31 [c]
Total sweet	**161.81 ± 2.41 [b]**	**154.77 ± 0.34 [c]**	**165.70 ± 1.05 [b]**	**172.64 ± 5.66 [a]**	**164.11 ± 1.09 [b]**
Serine†	12.68 ± 0.11 [a]	12.75 ± 0.22 [a]	11.00 ± 0.28 [b]	9.55 ± 0.38 [c]	7.76 ± 0.16 [d]
Valine†	41.46 ± 0.90 [d]	45.65 ± 0.35 [c]	51.98 ± 0.32 [b]	54.96 ± 2.43 [a]	53.65 ± 0.97 [a]
Proline†	38.58 ± 0.83 [e]	54.35 ± 1.30 [d]	69.82 ± 1.42 [c]	85.89 ± 2.85 [b]	92.14 ± 1.15 [a]
Lysine†	2.27 ± 0.02 [c]	3.11 ± 0.11 [a]	2.99 ± 0.13 [ab]	2.73 ± 0.19 [b]	3.86 ± 1.11 [a]
Total sweet-bitter	**94.99 ± 1.61 [d]**	**115.87 ± 1.87 [c]**	**135.79 ± 1.09 [b]**	**153.13 ± 5.78 [a]**	**157.42 ± 0.87 [a]**
Methionine†	104.67 ± 6.19 [a]	82.29 ± 0.85 [b]	84.47 ± 3.34 [b]	78.48 ± 3.00 [c]	61.45 ± 2.30 [d]
Asparagine†	55.39 ± 1.18 [a]	47.17 ± 0.05 [b]	41.81 ± 1.41 [c]	41.22 ± 2.11 [c]	34.05 ± 0.82 [d]
Cysteine†	5.31 ± 0.20 [a]	nd	nd	nd	nd
Total bitter-sweet	**165.37 ± 2.31 [a]**	**129.46 ± 0.87 [b]**	**126.28 ± 2.10 [c]**	**121.11 ± 7.12 [d]**	**95.50 ± 1.54 [e]**
Arginine†	45.36 ± 1.48 [a]	40.69 ± 0.93 [b]	36.56 ± 1.32 [c]	32.53 ± 2.14 [d]	31.40 ± 0.82 [d]
Isoleucine†	27.20 ± 0.67 [d]	31.22 ± 0.28 [c]	37.82 ± 0.26 [b]	40.59 ± 1.92 [a]	40.92 ± 5.31 [ab]
Histidine†	13.06 ± 0.10 [c]	14.47 ± 0.13 [b]	15.09 ± 0.45 [b]	15.65 ± 0.73 [b]	17.08 ± 0.41 [a]
Glutamine†	6.78 ± 0.34 [c]	7.17 ± 0.05 [b]	7.26 ± 0.18 [a]	7.29 ± 0.56 [a]	7.36 ± 0.03 [a]
Tyrosine*	53.50 ± 3.38 [d]	67.10 ± 0.26 [c]	76.07 ± 2.11 [b]	88.26 ± 4.01 [a]	92.61 ± 1.84 [a]
Leucine*	36.51 ± 0.84 [d]	43.51 ± 0.38 [c]	57.14 ± 0.64 [b]	65.41 ± 3.27 [a]	64.91 ± 0.05 [a]
Tryptophan*	57.12 ± 1.89 [b]	55.69 ± 0.63 [b]	63.65 ± 1.40 [a]	59.73 ± 4.99 [ab]	49.41 ± 0.80 [c]
Phenylalanine*	28.72 ± 0.94 [d]	45.77 ± 0.63 [c]	57.14 ± 0.88 [b]	68.74 ± 3.80 [a]	66.90 ± 0.78 [a]
Total bitter	**268.25 ± 6.74 [d]**	**305.62 ± 2.94 [c]**	**350.72 ± 3.24 [b]**	**378.21 ± 20.50 [ab]**	**361.97 ± 7.63 [a]**
GABA (Tasteless)	33.86 ± 0.69 [e]	81.39 ± 2.37 [a]	62.28 ± 1.17 [b]	50.48 ± 2.11 [d]	58.03 ± 2.61 [c]
Total Amino acid	**845.79 ± 13.40 [c]**	**853.93 ± 5.72 [c]**	**901.79 ± 3.91 [a]**	**928.16 ± 43.56 [a]**	**879.76 ± 3.19 [b]**

Note : value: mean ± SD; GABA: γ-aminobutyric acid; AABA: α-aminobutyric acid; nd: not detected; Taste characteristics of amino acid followed *Solms (1969) and †Shallenberger (1993); Different superscripts (a-e) within the same row show significant difference ($p<0.05$)

3.5 Folate content

Extreme folate deficiency is a serious cause of megaloblastic anemia (Green & Miller, 1999). Folate is very important for pregnancy and adequate folate intake during early pregnancy is recommended to prevent the development of fetal neural tube closure disorder (DeWals et al., 2007). Soybean is a good source of folate. Raw SK3 contained higher folate content than CM60 at 187.7 and 151.0 μg/100 g DM, respectively (Table I.5).

The soaking process increased folate contents of CM60 and SK3 which were highest after 12h soaking at 166.3 and 216.5 μg/100 g DM, respectively. Previous research suggested that the germinating enzymatic reaction could be activated as regular germination during soaking, while cell division increased folate synthesis by accelerating seed development (Spronk & Cossins, 1972). This could explain the increased folate of 12h soaked samples. The germinating reaction was reported to increase folic acid content 1.7-4.3 times compared with non-germinated seeds (Hefni & Witthoft, 2011). Soybean research revealed that germination led to a maximum of 3.5 and 3.7 times increase in total folate content on the fourth day of germination as soybean sprouts, then strongly declined thereafter (Shohag, Wei, & Yang, 2012). Folate content of soaked CM60 significantly decreased after 48h soaking and at 24h soaking for SK3 samples, suggesting that folate was dissolved during long-term soaking as a water-soluble vitamin. Furthermore, folate has also been reported to exhibit antioxidant properties by free radical scavenging (Joshi, Adhikari, Patro, Chattopadhyay, & Mukherjee, 2001). Thus, decrease in folate after long-term soaking may result in lower antioxidative capacity of soaked soybean samples.

Table I.5 : Folate content and antioxidative capacity (H- ORAC) in CM60 and SK3 samples at different soaking times (h)

Sample (soaking time (h))	Folate content (μg/100g DM)	Antioxidative capacity (μmol TE/100g DM)
CM60 (0)	151.0 ± 16.7 ^{d}	4,368.75 ± 418.04 ^{b}
CM60 (12)	166.3 ± 9.5 ^{c}	4,342.57 ± 431.56 ^{b}
CM60 (24)	156.7 ± 14.0 ^{d}	3,894.19 ± 268.63 ^{c}
CM60 (48)	139.0 ± 6.1 ^{e}	3,770.99 ± 142.29 ^{c}
SK3 (0)	187.7 ± 6.5 ^{b}	5,870.48 ± 115.21 ^{a}
SK3 (12)	216.5 ± 11.4 ^{a}	3,682.12 ± 114.70 ^{c}
SK3 (24)	177.7 ± 14.6 ^{b}	3,253.86 ± 146.32 ^{d}
SK3 (48)	171.0 ± 11.5 ^{b}	3,670.24 ± 204.16 ^{c}

Note : value: mean ± SD; Different superscripts (a-e) within the same column show significant difference ($p<0.05$)

3.6 Antioxidant capacity

Antioxidative capacities of soaked soybean samples were evaluated by the H-ORAC method which determine hydrophilic antioxidative activity by measuring peroxy radical eliminating capacity (Ou et al., 2001). Total antioxidant capacity was evaluated using H-ORAC because this contributes more than 90% of most food total antioxidant capacity (X. Wu et al., 2004). The extractable compounds of crude soybean samples were estimated for antioxidant capacity (Durazzo & Lucarini, 2018). Raw black soybean, SK3 had higher antioxidative capacity than CM60 as their seed coats contained high anthocyanins and polyphenols. However, the soaking process critically reduced antioxidative properties in 12h soaked SK3 and 24h soaked

CM60 samples as shown in Table I.5. This decreasing activity can be explained because hydrophilic antioxidative components were eluted through soaking in water and degraded during soaking, especially anthocyanin in SK3.

Anthocyanin pigments in black soybean seed coats were identified in previous research to contain three major anthocyanins as cyanidin-3-glucoside, petunidin-3-glucoside and delphinidin-3-glucoside (Y. K. Kim et al., 2012; Koh et al., 2014). Although soaking or germinating resulted in decreasing hydrophilic antioxidant capacity which agreed with previous research (Pérez-Balibrea, Moreno, & García-Viguera, 2011; Shohag et al., 2012), antioxidant capacities of soaked SK3 slightly increased thereafter. Variation of the H-ORAC value was caused by chemical structural changes of other bioactive compounds during soaking such as isoflavone (Rüfer & Kulling, 2006), saponin (Yoshiki et al., 2001; Yoshiki & Okubo, 1995) and free amino acid (Marcuse, 1960) which promoted antioxidative properties. A report concerning the relative antioxidative activity of soybean isoflavones noted that the antioxidant potency of aglycones was much stronger than glycosides in LDL oxidation assay (Lee et al., 2005). Moreover, increased phenolic compounds (antioxidative components) in beans and legumes naturally occurred during germination (Xu et al., 2009); metabolism was initiated in the presence of water and generated new compounds including phenolics (López-Amorós, Hernández, & Estrella, 2006).

Health problems including macronutrient malnutrition, lack of protein, carbohydrates and lipids, micronutrient malnutrition, and hidden hunger from lack of vitamins and minerals are now a global concern. Therapeutic food has been developed and formulated for therapy of acute malnutrition (Santini et al., 2013). If the appropriate condition of soaking treatment could be defined, it may enhance nutraceutical ingredients in soybean and may be useful to develop therapeutic food from natural sources instead of using chemical ingredients.

Conclusion

The food processing play an important role in health functional properties especially an antioxidant capacity of soybean-based food products, due to the changing chemical structure of soybean bioactive compounds. The derivative reaction could enhance or diminish the antioxidative capacity of those health functional compounds. The experiment revealed that derived isoflavone as aglycone could promote higher antioxidative capacity while derived soyasaponins such group A and group B saponin showed lower activity than DDMP-saponin. According to the H-ORAC value of soyfood sample, fermented soyfood presented highest antioxidant capacity which due to high aglycone isoflavone content. Furthermore, it was also considered that peptides and free amino acids produced by aging also contribute to the antioxidant capacity of fermented soybean foods. However, the soaking treatment also affected on the antioxidant capacity. The capacity decreased after soaking for 48 hours. On the other hand, the active isoflavones; daidzein and genistein increased together with health promoting group B saponins after 48 hour soaking. Regarding undesirable taste characteristics, long-term soaking might promote more undesirable tastes by increasing aglycone isoflavones and undesirable taste amino acid. Thus, it could be concluded that health-promoting compounds of soybean were altered by food processes such as soaking, heating, and fermenting treatment.

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CHAPTER II

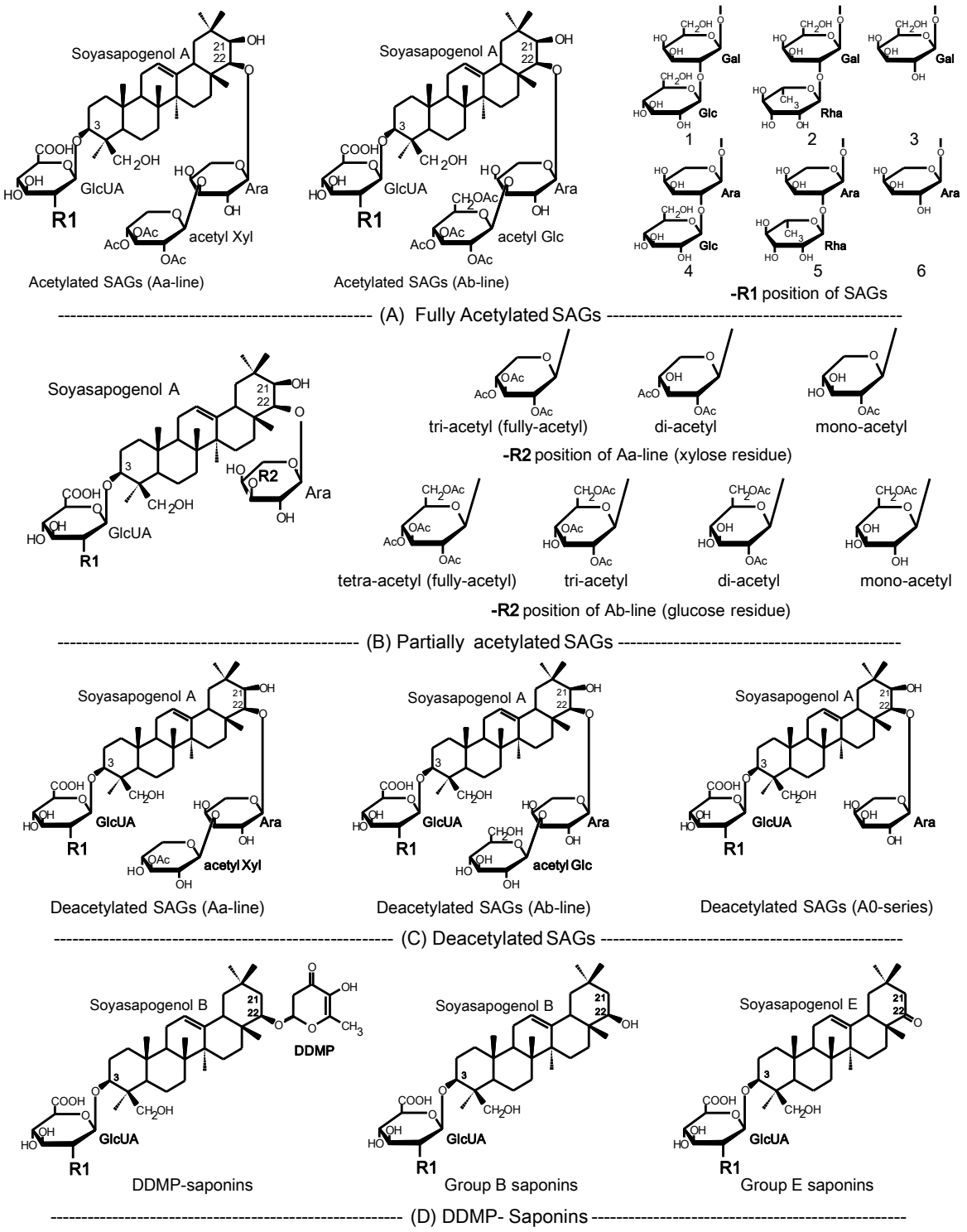
The effect of soybean variety and food processing on soyasaponin composition complexities relating health beneficial properties and undesirable taste characteristics

Introduction

Soyasaponins are a general term for glycosides having an oleanane-triterpene aglycone with more than 50 structurally different soyasaponin components (Tsukamoto & Yoshiki, 2006). Based on the aglycone structure, they have been classified into group A saponins (gr.A) and DDMP saponins (DDMPs). Group A saponins are soyasapogenol A glycosides (SAGs) with two sugar chains attached at the C-3 and C-22 positions of the aglycone. All hydroxyl groups of the terminal sugar of the sugar chain attached to the C-22 position are fully acetylated; while DDMP saponins are 2,3-dihydro-2,5-dihydroxy-6-methyl-4H-pyran-4-one (DDMP)-conjugated soyasapogenol B glycosides at the C-22 position. DDMPs degrade to group B saponins (gr.B) and group E saponins (gr.E), which have soyasapogenol B and soyasapogenol E as the aglycone, respectively. The nomenclature of all soyasaponin components is derived from the combination of these three aglycone structures and the sugar moiety composition of each aglycone (Figure II.1). The different saponin chemical structures present different health promoting properties and taste characteristic. Soyasaponins are reported as major cause of these characteristics, especially group A saponin (Okubo, Iijimi, Kobayashi, Yoshikoshi, & Uchida, 1992; Yumilko Yoshiki, Kodou, & Okubo, 1998).

Among gr.A saponin, fully acetylated SAGs (FSAGs) are the major cause of undesirable bitter tastes and astringent flavors (Okubo et al., 1992). FSAGs are generally located in seed hypocotyls (Shiraiwa, Harada, & Okubo, 1991; Shiraiwa, Kudou, Shimoyamada, Harada, & Okubo, 1991). Thus, seed hypocotyls are commonly removed in soymilk or soy food production (Asano, Okubo, Igarashi, & Yamauchi, 1987). Although seed hypocotyls are removed but soymilk products still present those undesirable characteristics. Besides FSAGs, other forms of SAGs also present these undesirable effects. The complexity of soyasaponin composition in each part of the soybean is not completely understood. The previous research reported that FSAGs presented undesirable taste characteristics which were not detected in null acetylated SAGs (NSAGs) (Okubo et al., 1992). Partially acetylated SAGs (PSAGs) might be responsible. However, the existence of PSAGs has never been reported.

On the other hand, DDMPs including DDMP, gr. B and gr. E saponins have reported health benefits. Free radical scavenging activity of DDMP saponin was investigated *in vitro*. The DDMP moiety probably contains of an unpaired electron at C-6 which promotes radical scavenging activity, which is not observed in gr.B and gr.E. Thus, only DDMP saponins can act as antioxidative compounds (Yoshiki & Okubo, 1995) which related to reduce risk of cardiovascular disease. Gr.B saponins have low absorbability in human intestinal cells and are metabolized to soyasapogenol B by human intestinal microorganisms *in vivo* and excreted in the feces. Therefore, daily intake of processed soybean foods can be expected to have health beneficial effects in the human digestive tract, e.g. anti-hyperlipidemic activity relating to bile acid binding to lipids, suppression of human colon cancer cell proliferation, and hepato-protective activities through thyroid hormone receptors in the small intestine (Hu, Lee, Hendrich, & Murphy, 2002; Hu, Reddy, Hendrich, & Murphy, 2004; Hu, Zheng, Hyde, Hendrich, & Murphy, 2004).



C-3 position sugar chain (**-R1**)		Soyasapogenol A glycoside (SAGs)					DDMP-saponins (DDMPs)		
		Acetylated SAGs		Deacetylated SAGs			DDMP	Group B*	Group E
		Aa-line	Ab-line	Aa-line	Ab-line	A0-series			
1	β-D-glucosyl-(1→2)-*O*-β-D-galactose	Aa	Ab	A_4	A_1	A0-αg	αg	Ba (IV)	Bd
2	β-L-rhamnosyl-(1→2)-*O*-β-D-galactose	Au	Ac	deacetyl-Au	deacetyl-Ac	A0-βg	βg	Bb (I)	Be
3	β-D-galactose	Ae	Af	A_5	A_2	A0-γg	γg	Bb' (III)	Be'
4	β-D-glucosyl-(1→2)-*O*-α-L-arabinose	Ax	Ad	deacetyl-Ax	deacetyl-Ad	A0-αa	αa	Bx	Bf
5	β-L-rhamnosyl-(1→2)-*O*-α-L-arabinose	Ay	Az	deacetyl-Ay	deacetyl-Az	A0-βa	βa	Bc (II)	Bg
6	α-L-arabinose	Ag	Ah	A_6	A_3	A0-γa	γa	Bc' (V)	Bg'

*group B saponins have two names for the same compound. Kitagawa's names are put in parenthesis.

Figure II.1 : Classification of soyasaponins according to basic chemical structures and table of soyasaponin nomenclature.

However, some consumer rejects soyfood as its undesirable characteristics such as bitter taste, astringent flavor, and green beany flavor. As early statement, group A saponins were reported as a cause of undesirable taste characteristics while DDMPs saponins were health promoting compounds. Thus, the variation of soyasaponin composition might present the different taste characteristic and nutraceutical property in soyfood products.

According to the previous chapter, a nutraceutical property such antioxidant activity was various in different soyfood products. Moreover, the soaking process which is a pretreatment process of soyfood production also interpreted the effect on antioxidant activity and soyasaponin composition.

In this chapter, the precise soyasaponin composition is disclosed from individual parts of nine different variety soybean to soyfood products. The conducted result might lead to a development of health promoting soyfood products with an acceptable taste characteristic.

Objective

- To obtain precise soyasaponin content in each soybean seed organs such as hypocotyls and cotyledons of nine soybean varieties.
- To evaluate soyasaponin composition in different kind of soyfoods and understand the processing effects due to the variable chemical structures relating taste characteristics of group A saponins and health beneficial properties of DDMPs saponins.

Materials and methods

1. Samples and reagents

Soybean seeds : nine soybean varieties were grown and harvested in Japan including two Japanese wide soybean varieties, *Glycine soja* and seven Japanese domestic soybean varieties, *Glycine max*. Sample No. 1 was 'GD50326-2' (a near inbred line, a progeny of the F8 line obtained from the cross between 'Shirosennari' and a wild soybean), it was grown and harvested in the field at Iwate University (Morioka, Japan). Sample No. 2 was 'GD50029-2', wide soybean (*Glycine soja*). Sample No.3 was 'Norin No.3', followed by 'Shirosennari', 'Ibarakimame No. 7', 'Suzuyutaka', 'Mikuriya-ao', 'Tohoku No. 152', and 'Kinusayaka', respectively. Sample No. 3 to 9 were *Glycine max*. Their soyasaponin phenotypes are listed in Table II.1. Soybean sample No. 2 to 9 were obtained from the National Agricultural Research Center for Tohoku Region, Kariwano, Japan. Hypocotyls and cotyledons were separated from dried whole seeds. All samples were kept in airtight opaque plastic bottles until required for extraction. Each part of seeds was milled separately with a multi-beads shocker (Yasui Kikai, Osaka, Japan) at 1,500 rpm for 30 sec.

Soyfood samples : All samples were collected from Thai and Japanese markets. Raw material of soyfood products in Thailand and Japan are generally *Glycine max* (L.) Merr. cv Chiangmai 60 (CM60) and *Glycine max* (L.) Merr. cv Nanbushirome (Nanbu). Two raw soybeans (CM60 and Nanbu), a Thai black soybean (*Glycine max* (L.) Merr. cv Sukhothai 3 (SK3)) and 39 soyfood products were investigated. The information of samples was shown in Table II.2.

Formic acid (CH_2O_2) HPLC grade, acetonitrile (CH_3CN) HPLC grade, and soyasaponin Bb ($C_{48}H_{78}O_{18}$) standard reagent were purchased from Sigma-Aldrich.

2. Soyasaponin extraction

Soybean seed samples were milled with a multi-beads shocker (Yasui Kikai, Osaka, Japan) at 1,500 rpm for 30 seconds. Each part of the seeds was prepared separately for

evaluating soyasaponins of each seed organs. Soyfood products from markets were frozen in liquid nitrogen and freeze dried in vacuo for 72 hours. Dried soymilks were milled by using a multi-beads shocker (Yasui Kikai, Osaka, Japan) at 1,500 rpm for 30 seconds. Other dried samples were ground by the ultra-centrifugal mill (Retsch ZM1000; Germany). Dry powder samples were kept in airtight opaque plastic bottles until use. Soyasaponin components of soyfood samples were extracted with 5-fold volume (v/w) of 80% (v/v) aqueous methanol for 1 h at room temperature. The extracts were centrifuged by micro centrifuge at 12,000 rpm (CFM-100 Iwaki, Japan) and the supernatant were directly applied to LC-PDA/MS/MS analysis.

Table II.1 : Soyasaponin genotypes of nine soybean varieties.

Sample No.	Variety	Genotype	Soyasaponin
1	GD-50326-2	*Sg-1^a/Sg-4/Sg-6*	Aa-line
2	GD-50029-2	*Sg-1^a/sg-4/Sg-6*	
3	Norin no.3	*Sg-1^a/Sg-3/Sg-4*	
4	Shirosennari	*Sg-1^a/Sg-3/sg-4/Sg-5/sg-6*	
5	Ibarakimame No.7	*Sg-1^b/Sg-3/Sg-4*	Ab-line
6	Suzuyutaka	*Sg-1^b/Sg-5*	
7	Mikuriya-ao	*Sg-1^b/sg-3/sg-4*	
8	Tohoku No.152	*Sg-1^b/sg-5*	A0-line
9	Kinusayaka	*sg-1^0/Sg-5*	

3. Analysis of soyasaponin compositions and contents

Soyasaponin components were analyzed by ultrafast liquid chromatography system (Prominence UFLC system, Shimadzu, Japan) with a photodiode array (PDA) detector and a tandem mass spectrometer (LTQ orbitrap XL, Thermo Fisher Scientific) on a C-30 reverse phase columns (Develosil C30-UG-3, 2.0 mm I.D. x 150 mm, Nomura Chemical, Seto, Japan) at 40C. Injection volume 5 µl for soyfood extracts, respectively. Solvent A consisted of 0.1% formic acid in acetonitrile (v/v), and solvent B was 0.1% formic acid solution. A linear gradient elution of acetonitrile concentration from 10 to 90% containing a constant 0.1% formic acid was performed at a flow rate of 0.15 ml/min. Solvent A was initiated at 10% (v/v) and increased to 100% (v/v) for 90 min, and washed with 100% solvent A for 5 min. The eluent composition was returned to the initial state of 10% (v/v) solvent A for 15 min. The sample eluates were monitored by a PDA detector at UV 205 and 292 nm with a tandam mass spectrometer in the positive ion mode of electrospray ionization [ESI(+)] method. An automatic full scan mode over a mass-to-charge ratio (m/z) range from 150 – 2000 and the top-three ion-trap mode was used to acquire MS and MS/MS data, respectively. The UV and MS spectra were recorded and analyzed with Xcaribur software version 2.1 (Thermo Fisher Scientific Inc.). Soyasaponin contents were identified by MS analysis and MS/MS fragment profiles. Soyasaponin Bb was used as a standard, and the molecular absorbance coefficient for saponin Bb (ε = 5278) (Hu, Lee, Hendrich, & Murphy, 2002) was used to quantify all soyasaponin components. Limit of detection was 1 pmol per injection (0.1 µmol/100 g dry basis, DB).

Table II.2 : Name, description, soybean cultivar, cultivating source, and product source of soyfood samples.

Code no.	Sample	Description	Soybean cultivar / cultivating source	Product source
C1	SB seed	Japanese commercial SB seed	Nanbu / Japan	Japan
C2		Thai commercial SB seed	CM60 / Thailand	Thailand
C3	Black SB seed	Thai commercial black SB seed	SK3 / Thailand	Thailand
1	SB sprout	Fresh hypocotyls (root part)	- / Japan	Japan
2		Fresh cotyledons (head part)	- / Japan	Japan
3	Edamame	Fresh young SB	- / Japan	Japan
4		Boiled young SB	- / Japan	Japan
5		Fresh black young SB	- / Japan	Japan
6		Boiled black young SB	- / Japan	Japan
7	Sweet black SB	Cooked black SB (Boiled with sugar)	- / Japan	Japan
8	Soymilk A	Non-Modified Soymilk	- / Canada	Japan
9	Soymilk B		- / Hokkaido, Japan	Japan
10	Soymilk C		- / China	Japan
11	Soymilk D		- / Japan	Japan
12	Soymilk E		- / Japan	Japan
13	Soymilk F		CM60 / Thailand	Thailand
14	Soymilk G		Kinusayaka / Japan	Japan
15	Soymilk I	Modified Soymilk	- / Canada	Japan
16	Soymilk II		- / Canada	Japan
17	Soymilk III		- / Canada	Japan
18	Soymilk IV		- / Japan	Japan
19	Soymilk V		- / Japan	Japan
20	Soymilk VI		- / Japan	Japan
21	Soymilk VII		Kinusayaka / Japan	Japan

Code no.	Sample	Description	Soybean cultivar / cultivating source	Product source
22	Tofu A	Soft SB curd	- / Japan	Japan
23	Tofu B		SJ5 / Thailand	Thailand
24	Tofu C		- / Thailand	Thailand
25	Tofu D	Hard SB curd	SJ5 / Thailand	Thailand
26	Yuba A	Soymilk skin	- / Japan	Japan
27	Yuba B	Homemade soymilk skin	CM60 / Thailand	Thailand
28	Natto A	SB fermented with Bacillus spp.	- / Hokkaido, Japan	Japan
29	Natto B		- / Hokkaido, Japan	Japan
30	Natto C		- / Hokkaido, Japan	Japan
31	Natto D		- / Iwate, Japan	Japan
32	Natto E	Black SB fermented with Bacillus spp.	- / Iwate, Japan	Japan
33	Miso A	SB fermented with Aspergillus spp. (Miso paste)	- / Japan	Japan
34	Miso B		- / Japan	Japan
35	Miso C	SB fermented with Aspergillus spp. (Semi - solid sauce)	CM60 / Thailand	Thailand
36	Douchi	SB fermented with LAB spp.	- / Japan	Japan
37	Tofuyo A	SB curd fermented with white koji	CM60 / Thailand	Thailand
38	Tofuyo B	SB curd fermented with red koji SB curd	CM60 / Thailand	Thailand
39	Tofuyo C	SB curd fermented with red koji SB curd	- / Japan	Japan

Note : SB is soybean; - is unidentified cultivar; Nanbu is *Glycine max* (L.) Merr. cv Nanbushirome; CM60 is *Glycine max* (L.) Merr. cv Chiangmai 60; SK3 is *Glycine max* (L.) Merr. cv Sukhothai 3; Kinusayaka is *Glycine max* (L.) Merr. cv Kinusayaka; SJ5 is *Glycine max* (L.) Merr. cv Sorjor 5.

4. Purity evaluation

The intensity of each sample was plotted since 200 to 360 nm. Patterns of soyasaponin component are classified into two different patterns which pattern 1 expresses gr.A, gr.B and gr.E saponin, and pattern 2 expresses DDMP saponin as shown in Figure II.2. On the other hand, an example of unknown component or not pure component also was shown in Figure II.2. If sample's graph showed different pattern, it would be discarded. This method was used to calculate the purity value (%) of each saponin to obtain actual content as following formula.

Actual UV peak area = Detected UV peak area at 205nm x Purity value …(1)

Purity value = PU_{std} / PU_{samp} …(2)

Where PU_{std} is purity of saponin standard with standard deviation and PU_{samp} is purity of sample. The purity of saponin group A, B or E would be calculated by formula (3) by using saponin Bb as standard. Otherwise, the purity of DDMP saponin would be calculated by formula (4) by using DDMP saponin αg as standard.

$$PU_{(std\ or\ sample)} = (A_{220p} - A_{220b})/(A_{205p} - A_{205b}) \quad \ldots(3)$$

$$PU_{(std\ or\ sample)} = (A_{292p} - A_{292b})/(A_{205p} - A_{205b}) \quad \ldots(4)$$

Where A_{205p} is intensity at UV 205 nm of peak area; A_{205b} is intensity of base line.

A_{220p} is intensity at UV 220 nm of peak area; A_{220b} is base line just before or after the detected peak of UV220p for saponin group A, B and E.

A_{292p} is intensity at UV 292 nm of peak area; A_{292b} is base line just before or after the detected peak of UV292p for DDMP saponin.

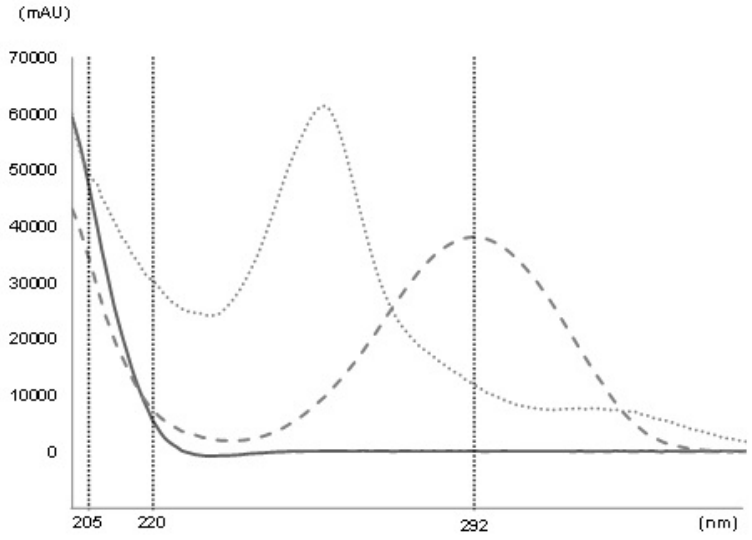


Figure II.2 : Examples of UV spectrum patterns including group A, B and E saponin component ( ); DDMP saponin component ( ) and unknown component ( ).

5. Statistical analysis

Analysis of variance (ANOVA) of the experimental data was performed and least significant difference was evaluated by the Turkey method at 95% confidence interval. All analyses were repeated in triplicate.

Result and Discussion

1. Identification of each soyasaponin component by LC-PDA/MS/MS analysis

Target 106 soyasaponin components were identified using LC-PDA/MS/MS analysis. Following soyasaponin chemical structures, soyasaponins were classified to SAG Aa-line, SAG Ab-line, SAG A0-line, DDMPs and their derivative gr.B and gr.E saponin components. The 50 soyasaponins were detected including 37 SAGs and 13 DDMPs saponin components. Molecular mass of target soyasaponins are listed in Table II.3. The detected soyasaponin compounds of nine variety soybean and soyfood samples are reported in Table II.4 - II.5 and Table II. 6 – II. 7, respectively. The identification of soyasaponins were proceeded by four steps as follows: 1) separation of soyasaponin compounds by HPLC which a reverse phase column, 2) selected ion monitoring (SIM) screening treatment of total ion current (TIC) chromatograms, 3) annotation of MS and MS/MS fragments of screened compounds, and 4) purity of soyasaponin composition were evaluated by purity evaluation. According to the identification procedures, the minor soyasaponin compounds as PSAGs and NSAGs were clearly identified. The different isomers of each soyasaponin components as their deacetylation are listed in Figure II.1 and Table II.3.

Among all soyasaponin components of soybean varieties and soyfoods, saponin Ab-line was a major SAGs in soy samples. FSAGs, saponin Ab and its derived components including mono-, di-, and triacetylated Ab were derived by MS and MS/MS analysis. For soybean seed samples, the results showed soyasaponins mainly detected in seed hypocotyls which concurred with other research (Shiraiwa, Harada, et al., 1991; Shiraiwa, Kudou, et al., 1991). Although very small amounts were detected in the seed cotyledon extracts, they were clearly identified by MS and MS/MS analysis (Figure II.3). Total ion chromatogram (TIC) of MS and MS/MS analysis of 'Suzuyutaka' cotyledon extract is shown in Figure II.3(a). Null-, mono-, di-, tri- and tetra-acetylated Ab components had m/z of 1269.61, 1311.62, 1353.63, 1359.65 and 1437.66, respectively. Selected ion monitoring (SIM) treatment of the TIC chromatogram showed extracted SIM chromatograms by m/z of 1269.61 (for nullacetyl-Ab selection) and 1437.66 (for 2,3,4,6-tetraacetyl-Ab selection) indicating a single peak at 28.66 (Figure II.3(a)) and 42.95 min (Figure II.3(b5)), respectively, while the other three SIM chromatograms (m/z 1311.62 (Figure II.3(b2)), 1353.63 (Figure II.3(b3)) and 1359.65 (Figure II.3(b4)) for mono-, di- and tri-acetylated Ab selection, respectively indicated multiple peaks. Nullacetyl- and tetraacetyl-Ab are a single compound, while mono-, di- and tri-acetylated Ab compounds can exist as structural isomers with the same molecular mass but different acetylated moieties of the molecule. They commonly contained m/z 1107.56 ([Ab—tetraacetylGlc+H]$^+$; 1436.7–330+1) and m/z 975.52 ([Ab–tetraacetylGlc–Ara+H]$^+$; 1436.7–330–132+1) peaks by MS analysis, and m/z 813.5 ([Ab–tetraacetylGlc–Ara–Glc+H]$^+$; 1436.7–330–132–162+1), m/z 615.4 ([Ab–tetraacetylGlc–Ara–Glc–Gal–2H_2O+H]$^+$; 1436.7–330–132 –162–162–36+1), and m/z 439.4 ([soyasapogenol A–2H_2O+H]$^+$; 474.4–36+1) peaks by MS/MS analysis. These results indicated that partially acetylated Ab components apparently existed in 'Suzuyutaka' seed cotyledon, although the degree of acetylation might be different from the hypocotyls. The TIC chromatogram of MS and MS/MS analysis of soyfood sample No. 26 (Yuba sample) also was showed in Figure II.4. The PSAGs also were detected as same manner with PSAGs in soybean seed sample.

Table II.3 : Molecular mass of target soyasaponins.

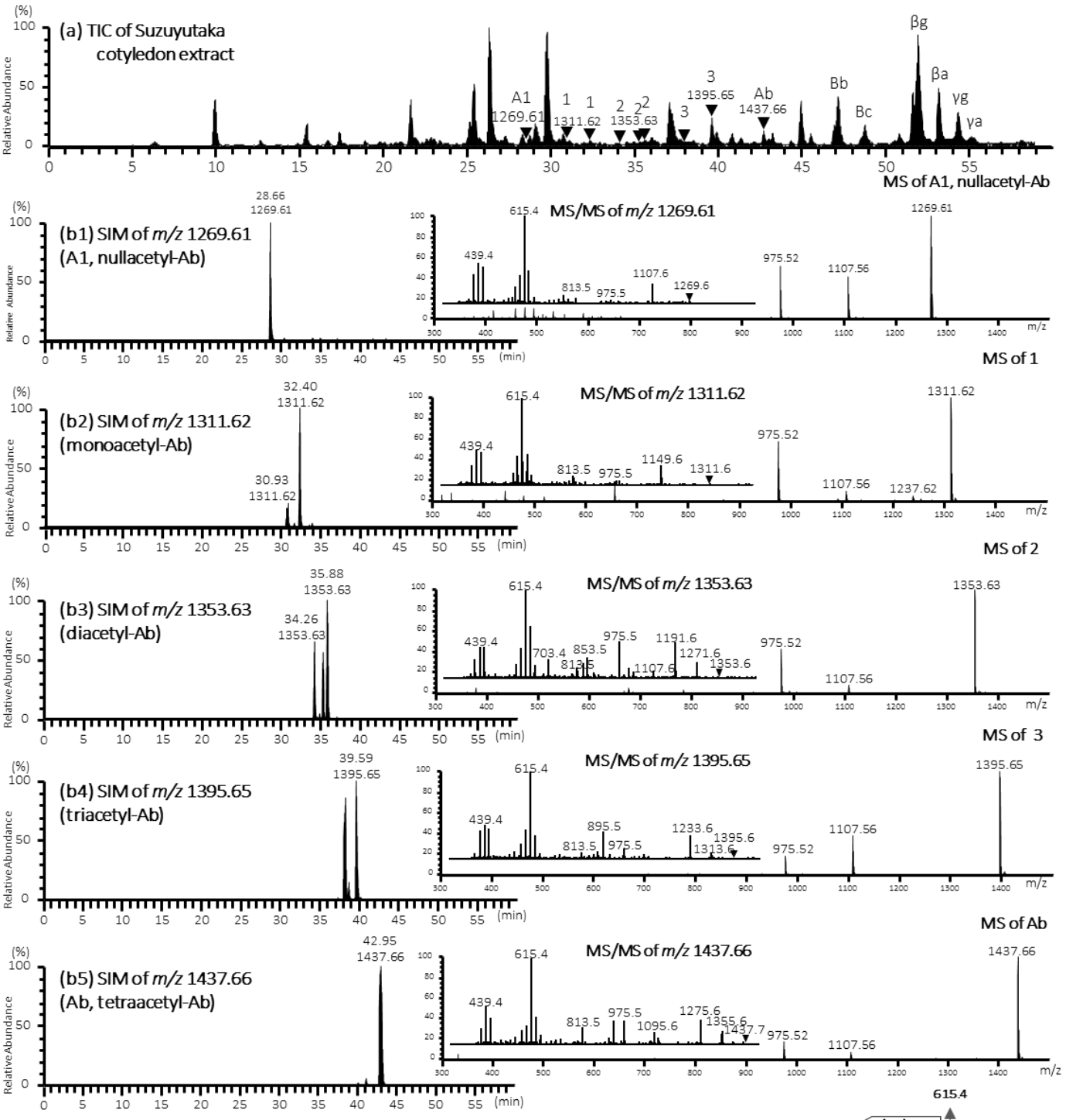
No.	Soyasaponin name	Mol. mass
	SAGs (Aa-line)	
	Fully-acetylated; FSAGs	
1	Aa (triacetyl)	1,364.6249
2	Au (triacetyl)	1,348.6300
3	Ae (triacetyl)	1,202.5720
4	Ax (triacetyl)	1,334.6143
5	Ay (triacetyl)	1,318.6194
6	Ag (triacetyl)	1,172.5615
	Deacetylated (null-acetylated); DSAGs	
7	Aa (nullacetyl) [A_4]	1,238.5932
8	Au (nullacetyl)	1,222.5983
9	Ae (nullacetyl) [A_5]	1,076.5403
10	Ax (nullacetyl)	1,208.5826
11	Ay (nullacetyl)	1,192.5877
12	Ag (nullacetyl) [A_6]	1,046.5298
	Partially acetylated; PSAGs	
13	Aa (monoacetyl, 1)	1,280.6037
14	Aa (monoacetyl, 2)	1,280.6037
15	Aa (monoacetyl, 3)	1,280.6037
16	Aa (diacetyl, 1)	1,322.6143
17	Aa (diacetyl, 2)	1,322.6143
18	Aa (diacetyl, 3)	1,322.6143
19	Au (monoacetyl, 1)	1,264.6088
20	Au (monoacetyl, 2)	1,264.6088
21	Au (monoacetyl, 3)	1,264.6088
22	Au (diacetyl, 1)	1,306.6194
23	Au (diacetyl, 2)	1,306.6194
24	Au (diacetyl, 3)	1,306.6194
25	Ae (monoacetyl, 1)	1,118.5509
26	Ae (monoacetyl, 2)	1,118.5509
27	Ae (monoacetyl, 3)	1,118.5509
28	Ae (diacetyl, 1)	1,160.5615
29	Ae (diacetyl, 2)	1,160.5615
30	Ae (diacetyl, 3)	1,160.5615

No.	Soyasaponin	Mol. mass
	SAGs (Aa-line)	
	Partially acetylated; PSAGs	
31	Ax (monoacetyl, 1)	1,250.5932
32	Ax (monoacetyl, 2)	1,250.5932
33	Ax (diacetyl, 1)	1,292.6037
34	Ax (diacetyl, 2)	1,292.6037
35	Ax (diacetyl, 3)	1,292.6037
36	Ay (monoacetyl)	1,234.5983
37	Ay (diacetyl)	1,276.6088
38	Ag (monoacetyl)	1,088.5403
39	Ag (diacetyl)	1,130.5509
	SAGs (Ab-line)	
	Fully-acetylated; FSAGs	
40	Ab (tetraacetyl)	1,436.6460
41	Ac (tetraacetyl)	1,420.6511
42	Af (tetraacetyl)	1,274.5932
43	Ad (tetraacetyl)	1,406.6354
44	Az (tetraacetyl)	1,222.5983
45	Ah (tetraacetyl)	1,244.5826
	Deacetylated (null-acetylated); DSAGs	
46	Ab (nullacetyl) [A_1]	1,268.6037
47	Ac (nullacetyl)	1,252.6088
48	Af (nullacetyl) [A_2]	1,106.5509
49	Ad (nullacetyl)	1,238.5932
50	Az (nullacetyl)	1,222.5983
51	Ah (nullacetyl) [A_3]	1,076.5403
	Partially acetylated; PSAGs	
52	Ab (monoacetyl, 1)	1,310.6143
53	Ab (monoacetyl, 2)	1,310.6143
54	Ab (diacetyl, 1)	1,352.6249
55	Ab (diacetyl, 2)	1,352.6249
56	Ab (diacetyl, 3)	1,352.6249
57	Ab (diacetyl, 3)	1,352.6249
58	Ab (triacetyl, 1)	1,394.6354

No.	Soyasaponin	Mol. mass
	SAGs (Ab-line)	
	Partially acetylated; PSAGs	
59	Ab (triacetyl, 2)	1,394.6354
60	Ac (monoacetyl)	1,294.6194
61	Ac (diacetyl, 1)	1,336.6300
62	Ac (diacetyl, 2)	1,336.6300
63	Ac (diacetyl, 3)	1,336.6300
64	Ac (triacetyl)	1,378.6405
65	Af (monoacetyl)	1,148.5615
66	Af (diacetyl, 1)	1,190.5720
67	Af (diacetyl, 2)	1,190.5720
68	Af (triacetyl, 1)	1,232.5826
69	Af (triacetyl, 2)	1,232.5826
70	Af (triacetyl, 3)	1,232.5826
71	Ad (monoacetyl)	1,280.6037
72	Ad (diacetyl, 1)	1,322.6143
73	Ad (diacetyl, 2)	1,322.6143
74	Ad (triacetyl, 1)	1,364.6249
75	Ad (triacetyl, 2)	1,364.6249
76	Az (monoacetyl)	1,264.6088
77	Az (diacetyl)	1,306.6194
78	Az (triacetyl)	1,348.6300
79	Ah (monoacetyl)	1,118.5509
80	Ah (diacetyl)	1,160.5615
81	Ah (triacetyl, 1)	1,202.5720
82	Ah (triacetyl, 2)	1,202.5720
	SAGs (A0-line)	
83	A0-ag	1,106.5509
84	A0-bg	1,090.5560
85	A0-gg	944.4981
86	A0-aa	1,076.5403
87	A0-ba	1,060.5454
88	A0-ga	914.4875

No.	Soyasaponin	Mol. mass
	DDMP saponins	
89	DDMP-αg	1,084.5454
90	DDMP-βg	1,068.5505
91	DDMP-γg	922.4926
92	DDMP-αa	1,054.5349
93	DDMP-βa	1,038.5400
94	DDMP-γa	892.4820
	Gr. B saponins (Derivative of DDMPs)	
95	Ba [V]	959.1215
96	Bb [I]	943.1221
97	Bb' [III]	796.9809
98	Bx	929.0955
99	Bc [II]	913.0961
100	Bc' [IV]	766.9549
	Group E saponins (Derivative of DDMPs)	
101	Bd	957.1056
102	Be	941.1062
103	Be'	794.9650
104	Bf	927.0797
105	Bg	911.0803
106	Bg'	764.9391

Note : Mol. Mass is Molecular mass. Highlighted compounds are newly targeted.



(c) annotation

m/z	
1437.7	[Ab+H(1)]$^+$
1107.6	[Ab-tetraacetylGlc(330)+H(1)]$^+$
975.5	[Ab-tetraacetylGlc(330)-Ara(132)+H(1)]$^+$
813.5	[Ab-tetraacetylGlc(330)-Ara(132)-Glc(162)+H(1)]$^+$
615.4	[Ab-tetraacetylGlc(330)-Ara(132)-Glc(162) -Gal(162)-2H_2O(36)+H(1)]$^+$
	= [Soyasapogenol A(474)+GlcUA(176) -2H_2O(36)+H(1)]$^+$
439.4	[Soyasapogenol A(474)-2H_2O(36)+H(1)]$^+$

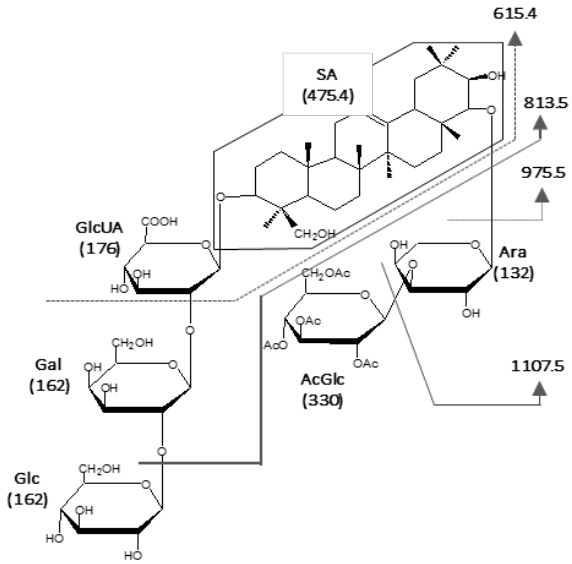


Figure II.3 : Identification of deacetylated, partially acetylated, and fully acetylated saponin Ab by LC-MS/MS analysis. (a) Total ion chromatography of the extract of Suzuyutaka cotyledon, (b) selected ion monitoring chromatography for deacetylated Ab with m/z 1269.61 (b1), mono-acetyl Ab m/z 1311.62 (b2), di-acetyl Ab m/z 1353.63 (b3), tri-acetyl Ab m/z 1395.66 (b4), and tetra-acetyl Ab m/z 1437.66 (b5), and (c) annotation of MS and MS/MS fragments of tetra-acetyl Ab.

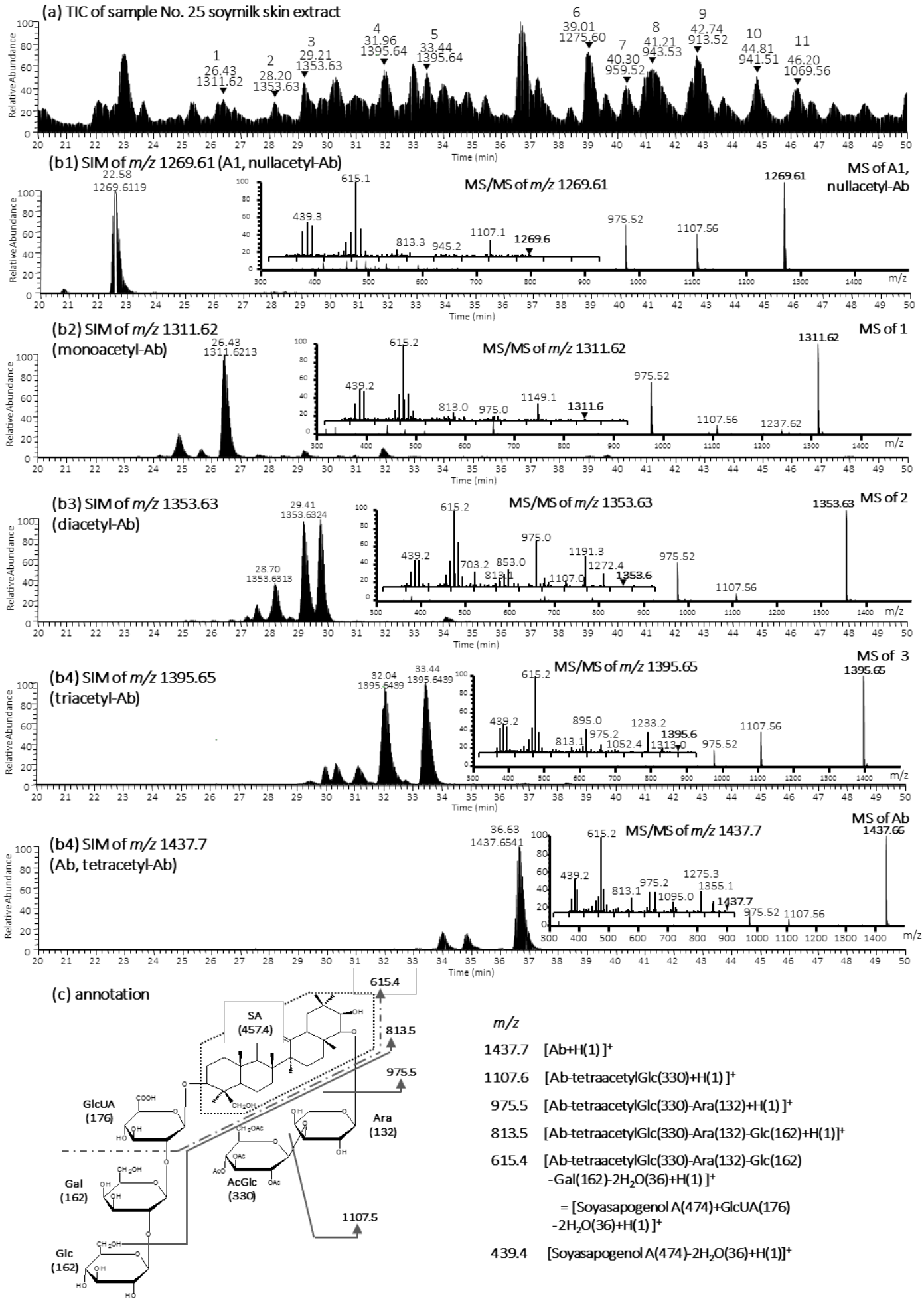


Figure II.4 : Total ion chromatography of the extract of sample No.26 yuba (a), selected ion monitoring chromatography for deacetylated Ab with m/z 1269.61 (b1), mono-acetyl Ab m/z 1311.62 (b2), di-acetyl Ab m/z 1353.63 (b3), tri-acetyl Ab m/z 1395.66 (b4), and tetra-acetyl Ab m/z 1437.66 (b5), and annotation of MS and MS/MS fragments of tetra-acetyl Ab.

Table II.4 : Weight of hypocotyls and cotyledons in 100 g dry seeds and soyasaponin contents (μmol/100 g dry basis) of nine varieties.

No.	Soyasaponin name	RT (min)	1.GD50326-2		2.GD50029-2		3.Norin No.3		4.Shirosennari		5.Ibarakimame No.7		6.Suzuyutaka		7.Mikuriya-ao		8.Tohoku No.152		9.Kinusayaka	
			hyp	cot	hyp	cot	hyp	cot	hyp	cot	hyp	cot	hyp	cot	hyp	cot	hyp	cot	hyp	cot
			2.4	84.8	3.7	79.0	1.8	92.0	1.9	91.1	1.8	92.3	2.0	90.5	2.4	89.1	2.2	89.9	2.2	89.1
1	Aa (triacetyl)	41.50	10.7	5.7	18.8	9.6	55.8	5.4	66.2	5.2	-	-	-	-	-	-	-	-	-	-
2	Au (triacetyl)	42.04	0.8	tr	tr	tr	1.1	1.1	2.8	2.7	-	-	-	-	-	-	-	-	-	-
3	Ae (triacetyl)	43.81	0.1	1.1	5.7	25.3	1.4	5.2	2.9	12.8	-	-	-	-	-	-	-	-	-	-
4	Ax (triacetyl)	42.35	5.3	5.4	tr	tr	5.6	3.5	tr	0.7	-	-	-	-	-	-	-	-	-	-
5	Ay (triacetyl)	43.10	tr	tr	tr	tr	0.1	1.7	tr	0.9	-	-	-	-	-	-	-	-	-	-
6	Ag (triacetyl)	44.36	tr	6.5	tr	tr	tr	8.8	tr	2.7	-	-	-	-	-	-	-	-	-	-
7	Aa (nullacetyl) / [A_4]	30.04	5.2	24.4	tr	-	tr	14.8	2.9	3.0	-	-	-	-	-	-	-	-	-	-
8	Au (nullacetyl)	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
9	Ae (nullacetyl) / [A_5]	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
10	Ax (nullacetyl)	31.02	tr	-	-	-	0.1	2.6	-	-	-	-	-	-	-	-	-	-	-	-
11	Ay (nullacetyl)	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
12	Ag (nullacetyl) / [A_6]	32.21	-	tr	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
13	**Aa (monoacetyl, 1)**	**31.70**	**0.3**	**2.5**	**1.1**	**-**	**tr**	**-**	**tr**	**tr**	**-**	**-**	**-**	**-**	**-**	**-**	**-**	**-**	**-**	**-**
14	**Aa (monoacetyl, 2)**	**32.86**	**0.1**	**1.7**	**-**	**-**	**0.4**	**-**	**0.9**	**0.5**	**-**	**-**	**-**	**-**	**-**	**-**	**-**	**-**	**-**	**-**
15	**Aa (monoacetyl, 3)**	**34.53**	**tr**	**-**	**0.1**	**1.7**	**1.4**	**1.5**	**2.7**	**0.8**	**-**	**-**	**-**	**-**	**-**	**-**	**-**	**-**	**-**	**-**
16	**Aa (diacetyl, 1)**	**35.73**	**0.1**	**4.9**	**0.1**	**-**	**2.9**	**1.9**	**6.1**	**2.9**	**-**	**-**	**-**	**-**	**-**	**-**	**-**	**-**	**-**	**-**
17	**Aa (diacetyl, 2)**	**36.81**	**0.8**	**3.1**	**0.2**	**5.2**	**0.3**	**-**	**1.6**	**tr**	**-**	**-**	**-**	**-**	**-**	**-**	**-**	**-**	**-**	**-**
18	**Aa (diacetyl, 3)**	**38.40**	**tr**	**-**	**-**	**-**	**4.5**	**2.9**	**0.3**	**1.2**	**-**	**-**	**-**	**-**	**-**	**-**	**-**	**-**	**-**	**-**
19	**Au (monoacetyl, 1)**	**32.19**	**0.5**	**-**	**-**	**-**	**-**	**-**	**tr**	**tr**	**-**	**-**	**-**	**-**	**-**	**-**	**-**	**-**	**-**	**-**
20	**Au (monoacetyl, 2)**	**33.23**	**-**	**1.7**	**-**	**-**	**-**	**-**	**tr**	**0.8**	**-**	**-**	**-**	**-**	**-**	**-**	**-**	**-**	**-**	**-**
21	**Au (monoacetyl, 3)**	**35.09**	**-**	**0.7**	**-**	**-**	**-**	**-**	**0.1**	**0.2**	**-**	**-**	**-**	**-**	**-**	**-**	**-**	**-**	**-**	**-**
22	**Au (diacetyl, 1)**	**36.18**	**2.2**	**-**	**-**	**-**	**-**	**-**	**-**	**tr**	**-**	**-**	**-**	**-**	**-**	**-**	**-**	**-**	**-**	**-**
23	**Au (diacetyl, 2)**	**37.41**	**0.2**	**-**	**-**	**-**	**-**	**-**	**5.1**	**-**	**-**	**-**	**-**	**-**	**-**	**-**	**-**	**-**	**-**	**-**
24	**Au (diacetyl, 3)**	**38.91**	**0.1**	**2.7**	**-**	**-**	**0.1**	**-**	**0.3**	**0.9**	**-**	**-**	**-**	**-**	**-**	**-**	**-**	**-**	**-**	**-**
25	**Ae (monoacetyl, 1)**	**33.69**	**-**	**-**	**-**	**-**	**-**	**0.3**	**tr**	**1.0**	**-**	**-**	**-**	**-**	**-**	**-**	**-**	**-**	**-**	**-**
26	**Ae (monoacetyl, 2)**	**34.48**	**-**	**-**	**-**	**-**	**1.4**	**-**	**0.4**	**3.2**	**-**	**-**	**-**	**-**	**-**	**-**	**-**	**-**	**-**	**-**
27	**Ae (monoacetyl, 3)**	**36.84**	**tr**	**3.3**	**-**	**-**	**-**	**-**	**1.6**	**tr**	**-**	**-**	**-**	**-**	**-**	**-**	**-**	**-**	**-**	**-**
28	**Ae (diacetyl, 1)**	**37.72**	**-**	**0.5**	**-**	**0.6**	**-**	**tr**	**tr**	**tr**	**-**	**-**	**-**	**-**	**-**	**-**	**-**	**-**	**-**	**-**
29	**Ae (diacetyl, 2)**	**39.28**	**-**	**-**	**-**	**1.1**	**0.6**	**0.6**	**0.6**	**0.6**	**-**	**-**	**-**	**-**	**-**	**-**	**-**	**-**	**-**	**-**
30	**Ae (diacetyl, 3)**	**40.28**	**-**	**-**	**0.7**	**8.1**	**1.2**	**0.9**	**tr**	**2.2**	**-**	**-**	**-**	**-**	**-**	**-**	**-**	**-**	**-**	**-**
31	**Ax (monoacetyl, 1)**	**33.93**	**0.1**	**-**	**-**	**-**	**0.2**	**-**	**-**	**-**	**-**	**-**	**-**	**-**	**-**	**-**	**-**	**-**	**-**	**-**
32	**Ax (monoacetyl, 2)**	**35.18**	**2.0**	**0.7**	**-**	**-**	**0.2**	**0.7**	**-**	**-**	**-**	**-**	**-**	**-**	**-**	**-**	**-**	**-**	**-**	**-**
33	**Ax (diacetyl, 1)**	**36.92**	**tr**	**3.1**	**-**	**-**	**-**	**-**	**-**	**-**	**-**	**-**	**-**	**-**	**-**	**-**	**-**	**-**	**-**	**-**
34	**Ax (diacetyl, 2)**	**37.51**	**tr**	**-**	**-**	**-**	**-**	**-**	**-**	**-**	**-**	**-**	**-**	**-**	**-**	**-**	**-**	**-**	**-**	**-**
35	**Ax (diacetyl, 3)**	**39.20**	**0.8**	**2.4**	**-**	**-**	**0.6**	**0.6**	**-**	**-**	**-**	**-**	**-**	**-**	**-**	**-**	**-**	**-**	**-**	**-**
36	**Ay (monoacetyl)**	**-**	**-**	**-**	**-**	**-**	**-**	**-**	**-**	**-**	**-**	**-**	**-**	**-**	**-**	**-**	**-**	**-**	**-**	**-**

No.	Soyasaponin name	RT (min)	1.GD50326-2		2.GD50029-2		3.Norin No.3		4.Shirosennari		5.Ibarakimame No.7		6.Suzuyutaka		7.Mikuriya-ao		8.Tohoku No.152		9.Kinusayaka	
			hyp	cot	hyp	cot	hyp	cot	hyp	cot	hyp	cot	hyp	cot	hyp	cot	hyp	cot	hyp	cot
37	**Ay (diacetyl)**	**38.51**	**-**	**-**	**-**	**-**	**-**	**1.9**	**-**	**-**	**-**	**-**	**-**	**-**	**-**	**-**	**-**	**-**	**-**	**-**
38	**Ag (monoacetyl)**	**38.16**	**-**	**13.0**	**-**	**-**	**-**	**-**	**-**	**-**	**-**	**-**	**-**	**-**	**-**	**-**	**-**	**-**	**-**	**-**
39	**Ag (diacetyl)**	**40.91**	**-**	**3.6**	**-**	**-**	**-**	**3.3**	**-**	**-**	**-**	**-**	**-**	**-**	**-**	**-**	**-**	**-**	**-**	**-**
40	Ab (tetraacetyl)	42.24	-	-	-	-	-	-	-	-	48.6	4.2	65.5	1.0	tr	tr	-	-	-	-
41	Ac (tetraacetyl)	42.80	-	-	-	-	-	-	-	-	2.0	2.2	2.3	0.8	5.7	0.3	-	-	-	-
42	Af (tetraacetyl)	44.48	-	-	-	-	-	-	-	-	1.8	6.7	2.3	6.5	16.1	1.3	-	-	-	-
43	Ad (tetraacetyl)	43.39	-	-	-	-	-	-	-	-	8.2	1.7	tr	tr	tr	tr	-	-	-	-
44	Az (tetraacetyl)	44.01	-	-	-	-	-	-	-	-	0.2	4.1	tr	0.9	tr	0.1	-	-	-	-
45	Ah (tetraacetyl)	45.21	-	-	-	-	-	-	-	-	0.1	5.3	tr	2.0	tr	0.4	-	-	-	-
46	Ab (nullacetyl) / [A_1]	28.18	-	-	-	-	-	-	-	-	1.0	tr	2.4	-	-	-	-	-	-	-
47	Ac (nullacetyl)	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
48	Af (nullacetyl) / [A_2]	32.34	-	-	-	-	-	-	-	-	-	3.2	0.1	16.0	-	0.3	-	-	-	-
49	Ad (nullacetyl)	28.93	-	-	-	-	-	-	-	-	tr	-	-	-	-	-	-	-	-	-
50	Az (nullacetyl)	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
51	Ah (nullacetyl) / [A_3]	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
52	**Ab (monoacetyl, 1)**	**31.19**	**-**	**-**	**-**	**-**	**-**	**-**	**-**	**-**	**-**	**-**	**tr**	**-**	**-**	**-**	**-**	**-**	**-**	**-**
53	**Ab (monoacetyl, 2)**	**31.92**	**-**	**-**	**-**	**-**	**-**	**-**	**-**	**-**	**0.1**	**3.9**	**0.2**	**-**	**-**	**-**	**-**	**-**	**-**	**-**
54	**Ab (diacetyl, 1)**	**30.86**	**-**	**-**	**-**	**-**	**-**	**-**	**-**	**-**	**-**	**-**	**-**	**-**	**-**	**-**	**-**	**-**	**-**	**-**
55	**Ab (diacetyl, 2)**	**33.66**	**-**	**-**	**-**	**-**	**-**	**-**	**-**	**-**	**0.6**	**0.4**	**1.1**	**-**	**-**	**-**	**-**	**-**	**-**	**-**
56	**Ab (diacetyl, 3)**	**34.81**	**-**	**-**	**-**	**-**	**-**	**-**	**-**	**-**	**0.7**	**-**	**1.0**	**tr**	**-**	**-**	**-**	**-**	**-**	**-**
57	**Ab (diacetyl, 3)**	**35.30**	**-**	**-**	**-**	**-**	**-**	**-**	**-**	**-**	**0.9**	**2.0**	**1.8**	**0.1**	**-**	**-**	**-**	**-**	**-**	**-**
58	**Ab (triacetyl, 1)**	**37.64**	**-**	**-**	**-**	**-**	**-**	**-**	**-**	**-**	**1.2**	**tr**	**1.2**	**-**	**-**	**-**	**-**	**-**	**-**	**-**
59	**Ab (triacetyl, 2)**	**39.07**	**-**	**-**	**-**	**-**	**-**	**-**	**-**	**-**	**4.9**	**0.6**	**3.1**	**4.2**	**-**	**-**	**-**	**-**	**-**	**-**
60	**Ac (monoacetyl)**	**-**	**-**	**-**	**-**	**-**	**-**	**-**	**-**	**-**	**-**	**-**	**-**	**-**	**-**	**-**	**-**	**-**	**-**	**-**
61	**Ac (diacetyl, 1)**	**33.57**	**-**	**-**	**-**	**-**	**-**	**-**	**-**	**-**	**-**	**-**	**-**	**-**	**tr**	**-**	**-**	**-**	**-**	**-**
62	**Ac (diacetyl, 2)**	**34.14**	**-**	**-**	**-**	**-**	**-**	**-**	**-**	**-**	**-**	**-**	**0.1**	**-**	**tr**	**-**	**-**	**-**	**-**	**-**
63	**Ac (diacetyl, 3)**	**35.75**	**-**	**-**	**-**	**-**	**-**	**-**	**-**	**-**	**-**	**-**	**0.2**	**0.1**	**0.4**	**0.4**	**-**	**-**	**-**	**-**
64	**Ac (triacetyl)**	**38.15**	**-**	**-**	**-**	**-**	**-**	**-**	**-**	**-**	**-**	**-**	**-**	**11.5**	**0.3**	**0.5**	**-**	**-**	**-**	**-**
65	**Af (monoacetyl)**	**33.49**	**-**	**-**	**-**	**-**	**-**	**-**	**-**	**-**	**-**	**2.7**	**-**	**tr**	**0.2**	**-**	**-**	**-**	**-**	**-**
66	**Af (diacetyl, 1)**	**36.28**	**-**	**-**	**-**	**-**	**-**	**-**	**-**	**-**	**-**	**0.6**	**-**	**-**	**tr**	**-**	**-**	**-**	**-**	**-**
67	**Af (diacetyl, 2)**	**37.27**	**-**	**-**	**-**	**-**	**-**	**-**	**-**	**-**	**-**	**-**	**0.5**	**tr**	**1.3**	**tr**	**-**	**-**	**-**	**-**
68	**Af (triacetyl, 1)**	**39.78**	**-**	**-**	**-**	**-**	**-**	**-**	**-**	**-**	**0.1**	**-**	**0.1**	**0.2**	**0.2**	**0.4**	**-**	**-**	**-**	**-**
69	**Af (triacetyl, 2)**	**41.04**	**-**	**-**	**-**	**-**	**-**	**-**	**-**	**-**	**0.4**	**1.0**	**0.7**	**tr**	**1.8**	**0.3**				
70	**Af (triacetyl, 3)**	**41.52**	**-**	**-**	**-**	**-**	**-**	**-**	**-**	**-**	**0.2**	**-**	**-**	**-**	**tr**	**-**	**-**	**-**	**-**	**-**
71	**Ad (monoacetyl)**	**32.77**	**-**	**-**	**-**	**-**	**-**	**-**	**-**	**-**	**tr**	**-**	**-**	**-**	**-**	**-**	**-**	**-**	**-**	**-**
72	**Ad (diacetyl, 1)**	**33.23**	**-**	**-**	**-**	**-**	**-**	**-**	**-**	**-**	**tr**	**tr**	**-**	**-**	**-**	**-**	**-**	**-**	**-**	**-**
73	**Ad (diacetyl, 2)**	**-**	**-**	**-**	**-**	**-**	**-**	**-**	**-**	**-**	**-**	**-**	**-**	**-**	**-**	**-**	**-**	**-**	**-**	**-**
73	**Ad (triacetyl, 1)**	**38.76**	**-**	**-**	**-**	**-**	**-**	**-**	**-**	**-**	**0.2**	**-**	**-**	**-**	**-**	**-**	**-**	**-**	**-**	**-**
74	**Ad (triacetyl, 2)**	**40.06**	**-**	**-**	**-**	**-**	**-**	**-**	**-**	**-**	**1.0**	**tr**	**-**	**-**	**-**	**-**	**-**	**-**	**-**	**-**

No.	Soyasaponin name	RT (min)	1.GD50326-2		2.GD50029-2		3.Norin No.3		4.Shirosennari		5.Ibarakimame No.7		6.Suzuyutaka		7.Mikuriya-ao		8.Tohoku No.152		9.Kinusayaka	
			hyp	cot	hyp	cot	hyp	cot	hyp	cot	hyp	cot	hyp	cot	hyp	cot	hyp	cot	hyp	cot
75	**Az (monoacetyl)**	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
76	**Az (diacetyl)**	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
77	**Az (triacetyl)**	**40.72**	-	-	-	-	-	-	-	-	-	**1.1**	-	-	-	-	-	-	-	-
78	**Ah (monoacetyl)**	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
80	**Ah (diacetyl)**	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
81	**Ah (triacetyl, 1)**	**40.30**	-	-	-	-	-	-	-	-	-	**0.9**	-	-	-	-	-	-	-	-
82	**Ah (triacetyl, 2)**	**41.57**	-	-	-	-	-	-	-	-	-	**0.2**	-	-	-	-	-	-	-	-
83	A0-αg	30.23	1.5	5.2	0.8	5.0	0.2	tr	0.3	tr	0.4	10.0	0.1	7.6	-	-	-	-	49.5	8.8
84	A0-βg	30.67	1.0	-	tr	1.1	0.1	0.4	0.1	2.4	tr	1.4	tr	4.6	0.1	4.8	-	-	2.3	1.7
85	A0-γg	32.08	0.6	tr	0.2	tr	tr	0.3	-	tr	0.1	tr	-	tr	0.3	1.4	-	-	1.3	1.4
86	A0-αa	31.28	0.3	tr	0.3	1.8	0.1	tr	-	tr	0.2	tr	-	-	-	-	-	-	-	3.6
87	A0-βa	31.62	-	-	tr	tr	tr	tr	-	-	tr	0.2	-	-	-	1.6	-	-	-	0.2
88	A0-γa	32.62	-	-	-	-	-	-	-	-	tr	-	-	-	-	-	-	-	-	-
89	DDMP-αg	50.54	6.8	-	20.3	-	1.6	-	6.2	-	3.8	-	4.5	-	-	-	28.0	tr	5.6	tr
90	DDMP-βg	51.59	14.5	28.0	33.7	49.7	3.4	32.3	11.8	37.4	9.3	36.6	11.1	40.1	7.8	28.9	14.5	42.8	14.2	21.0
91	DDMP-γg	54.09	0.2	24.0	0.8	127.1	0.1	5.3	0.2	6.8	0.1	-	0.3	5.6	0.3	8.7	0.4	3.7	0.2	1.8
92	DDMP-αa	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
93	DDMP-βa	52.84	6.8	55.0	-	3.8	0.4	45.2	-	29.5	1.3	58.2	-	23.9		20.3	-	32.3	-	13.8
94	DDMP-γa	54.82	0.2	72.8	-	14.3	tr	6.7	-	5.6	tr	4.2	-	4.7	0.2	5.5	-	4.3	-	2.6
95	Ba [V]	45.95	2.5	tr	6.3	-	0.8	tr	2.1	-	0.9	-	2.5	tr	-	-	6.7	tr	1.2	tr
96	Bb [I]	46.73	6.8	2.4	17.9	4.7	2.0	7.4	6.1	7.8	3.5	8.1	3.2	13.5	2.8	8.0	5.1	9.3	3.8	6.4
97	Bb' [III]	48.31	-	1.0	tr	19.2	-	tr	0.1	-	tr	tr	0.1	tr	tr	0.6	0.1	-	tr	tr
98	Bx	47.42	tr	tr	-	tr	tr	tr	-	-	-	-	-	-	-	-	-	-	-	-
99	Bc [II]	48.35	2.8	3.3	-	-	0.3	6.4	-	4.8	0.4	10.0	-	6.3	-	2.3	-	4.2	-	2.6
100	Bc' [IV]	49.21	-	9.9	-	1.3	-	1.8	-	-		tr	-	tr	-	0.4	-	0.0	-	tr
101	Bd	50.52	1.6	-	2.4	-	0.4	-	0.7	-	0.4	-	-	-	-	-	1.3	-	0.2	-
102	Be	52.32	tr	tr	-	tr	tr	2.8	-	1.4	tr	1.6	tr	2.1	0.6	1.5	tr	2.1	tr	1.4
103	Be'	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
104	Bf	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
105	Bg	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
106	Bg'	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-

Note : RT : Retention time (min.); Soyasaponin valued: mean (n=3); tr: detected by MS analysis but not detected by PDA analysis or content was lower than limit of detection (< 0.1 μmol/100 g dry basis), - : not detected even by MS analysis, Highlighted compounds are newly detected.

Table II.5 : Soyasaponin contents (μmol/100 g dry basis) for each of the nine variety soybean samples.

Category	1.GD50326-2		2.GD50029-2		3.Norin No.3		4.Shirosennari		5.Ibarakimame No.7	
	hyp (2.4 g)	cot (84.8 g)	hyp (3.7 g)	cot (79.0 g)	hyp (1.8 g)	cot (92.0 g)	hyp (1.9 g)	cot (91.1 g)	hyp (1.8 g)	cot (92.3 g)
FSAGs (A)	16.9±0.5	18.7±0.5	24.1±0.3	34.9±0.3	64.1±0.3	25.8±0.2	71.9±0.1	25.1±0.2	60.9±0.2	24.2±0.2
PSAGs (PA)	7.2±0.4	44.0±1.5	2.2±0.4	16.7±0.0	14.0±0.6	14.7±0.0	19.8±0.1	14.5±0.1	10.3±0.0	13.5±0.0
NSAGs (NA)	5.2±0.1	24.4±0.8	tr	-	0.1±0.0	17.4±0.0	2.9±0.1	3.0±0.0	1.0±0.0	3.2±0.0
A0	3.4±0.2	5.2±0.2	1.2±0.0	tr	tr	0.7±0.0	0.4±0.0	2.4±0.0	0.7±0.0	11.6±0.1
Subtotal SAGs	*32.6±0.6*	*92.3±0.5*	*27.9±0.6*	*59.4±0.3*	*78.5±1.0*	*58.6±0.5*	*95.1±1.1*	*44.9±0.3*	*72.9±0.3*	*52.5±0.2*
Total SAGs	124.9±2.0		87.3±0.9		137.1±1.6		140.0±0.6		125.4±0.5	
DDMP (D)	28.5±0.5	179.7±0.4	54.9±0.3	194.9±0.5	5.6±0.3	89.5±0.4	18.3±0.3	79.3±0.4	14.5±0.1	98.9±0.6
Gr. B (B)	12.0±0.1	16.6±0.2	24.2±0.3	25.2±0.1	3.2±0.7	15.6±0.5	8.3±0.0	12.6±0.4	4.9±0.2	18.1±0.1
Gr. E (E)	1.6±0.1	tr	2.4±0.1	tr	0.4±0.0	2.8±0.0	0.7±0.0	1.4±0.0	0.4±0.0	1.6±0.0
DDMPs (D+B+E)	*42.2±0.8*	*196.3±0.9*	*81.5±0.8*	*220.1±0.6*	*9.2±1.0*	*108.0±0.9*	*27.2±0.3*	*93.4±0.8*	*19.7±0.3*	*118.6±0.8*
Total DDMPs	238.5±1.7		301.6±1.5		117.1±1.9		120.6±1.0		138.3±0.9	
Subtotal saponins	*74.8±2.0*	*288.6±1.5*	*109.4±1.3*	*279.5±0.9*	*87.6±0.8*	*166.6±1.4*	*122.3±1.8*	*138.3±1.1*	*92.6±0.6*	*171.2±1.2*
Total saponins	363.5±3.5		388.9±2.3		254.2±3.1		260.6±1.9		263.8±1.5	

Category	6.Suzuyutaka		7.Mikuriya-ao		8.Tohoku No.152		9.Kinusayaka	
	hyp (2.0 g)	cot (90.5 g)	hyp (2.4 g)	cot (89.1 g)	hyp (2.2 g)	cot (89.9 g)	hyp (2.2 g)	cot (89.1 g)
FSAGs (A)	70.1±0.3	11.2±0.1	21.9±0.1	2.1±0.0	-	-	-	-
PSAGs (PA)	10.0±0.1	16.1±0.2	4.3±0.1	1.6±0.0	-	-	-	-
NSAGs (NA)	2.6±0.0	16.0±0.1	-	0.3±0.0	-	-	-	-
A0	0.1±0.0	12.3±0.3	0.4±0.0	7.7±0.2	-	-	53.1±0.6	15.7±0.1
Subtotal SAGs	*82.7±0.4*	*55.6±0.4*	*26.5±0.2*	*11.7±0.3*	-	-	*53.1±0.6*	*15.7±0.1*
Total SAGs	138.3±0.4		38.2±0.1		-		68.8±0.4	
DDMP (D)	15.9±0.0	74.3±0.4	8.3±0.1	63.4±0.3	42.9±0.4	83.1±0.5	20.0±0.2	39.1±0.4
Gr. B (B)	5.8±0.1	19.8±0.1	2.8±0.1	11.1±0.0	11.9±0.1	13.6±0.1	5.0±0.1	9.0±0.1
Gr. E (E)	tr	2.1±0.0	0.6±0.0	1.5±0.0	1.3±0.0	2.1±0.0	0.2±0.0	1.4±0.0
DDMPs (D+B+E)	*21.8±0.1*	*96.2±0.2*	*11.7±0.1*	*76.0±0.1*	*56.0±0.5*	*98.8±0.4*	*25.2±0.3*	*49.5±0.2*
Total DDMPs	118.0±0.7		87.7±0.2		154.8±0.3		74.7±0.6	
Subtotal saponins	*104.5±0.6*	*151.7±0.2*	*38.1±0.2*	*87.8±0.1*	*56.0±0.5*	*98.8±0.4*	*78.3±0.4*	*65.2±0.3*
Total saponins	256.2±1.4		125.9±0.7		154.8±0.3		143.5±0.9	

Note : Seed coats were removed, soyasaponin value: mean ± SD (n=3), not detected by MS analysis.

Table II.6 : Soyasaponin contents (μmol/100 g dry basis) in soyfood samples (code no. C1-11)

No.	Soyasaponin names	Code no. of soyfood samples													
		C1	C2	C3	1	2	3	4	5	6	7	8	9	10	11
1	Aa (triacetyl)	-	-	-	-	-	-	-	-	-	-	-	-	-	-
2	Au (triacetyl)	-	-	-	-	-	-	-	-	-	-	-	-	-	-
3	Ae (triacetyl)	-	-	-	-	-	-	-	-	-	-	-	-	-	-
4	Ax (triacetyl)	-	-	-	-	-	-	-	-	-	-	-	-	-	-
5	Ay (triacetyl)	-	-	-	-	-	-	-	-	-	-	-	-	-	-
6	Ag (triacetyl)	-	-	-	-	-	-	-	-	-	-	-	-	-	-
7	Aa (nullacetyl) / [A_4]	-	-	-	-	-	-	-	-	-	-	-	-	-	-
8	Au (nullacetyl)	-	-	-	-	-	-	-	-	-	-	-	-	-	-
9	Ae (nullacetyl) / [A_5]	-	-	-	-	-	-	-	-	-	-	-	-	-	-
10	Ax (nullacetyl)	-	-	-	-	-	-	-	-	-	-	-	-	-	-
11	Ay (nullacetyl)	-	-	-	-	-	-	-	-	-	-	-	-	-	-
12	Ag (nullacetyl) / [A_6]	-	-	-	-	-	-	-	-	-	-	-	-	-	-
13	**Aa (monoacetyl, 1)**	-	-	-	-	-	-	-	-	-	-	-	-	-	-
14	**Aa (monoacetyl, 2)**	-	-	-	-	-	-	-	-	-	-	-	-	-	-
15	**Aa (monoacetyl, 3)**	-	-	-	-	-	-	-	-	-	-	-	-	-	-
16	**Aa (diacetyl, 1)**	-	-	-	-	-	-	-	-	-	-	-	-	-	-
17	**Aa (diacetyl, 2)**	-	-	-	-	-	-	-	-	-	-	-	-	-	-
18	**Aa (diacetyl, 3)**	-	-	-	-	-	-	-	-	-	-	-	-	-	-
19	**Au (monoacetyl, 1)**	-	-	-	-	-	-	-	-	-	-	-	-	-	-
20	**Au (monoacetyl, 2)**	-	-	-	-	-	-	-	-	-	-	-	-	-	-
21	**Au (monoacetyl, 3)**	-	-	-	-	-	-	-	-	-	-	-	-	-	-
22	**Au (diacetyl, 1)**	-	-	-	-	-	-	-	-	-	-	-	-	-	-
23	**Au (diacetyl, 2)**	-	-	-	-	-	-	-	-	-	-	-	-	-	-
24	**Au (diacetyl, 3)**	-	-	-	-	-	-	-	-	-	-	-	-	-	-
25	**Ae (monoacetyl, 1)**	-	-	-	-	-	-	-	-	-	-	-	-	-	-
26	**Ae (monoacetyl, 2)**	-	-	-	-	-	-	-	-	-	-	-	-	-	-
27	**Ae (monoacetyl, 3)**	-	-	-	-	-	-	-	-	-	-	-	-	-	-
28	**Ae (diacetyl, 1)**	-	-	-	-	-	-	-	-	-	-	-	-	-	-
29	**Ae (diacetyl, 2)**	-	-	-	-	-	-	-	-	-	-	-	-	-	-
30	**Ae (diacetyl, 3)**	-	-	-	-	-	-	-	-	-	-	-	-	-	-
31	**Ax (monoacetyl, 1)**	-	-	-	-	-	-	-	-	-	-	-	-	-	-
32	**Ax (monoacetyl, 2)**	-	-	-	-	-	-	-	-	-	-	-	-	-	-
33	**Ax (diacetyl, 1)**	-	-	-	-	-	-	-	-	-	-	-	-	-	-
34	**Ax (diacetyl, 2)**	-	-	-	-	-	-	-	-	-	-	-	-	-	-
35	**Ax (diacetyl, 3)**	-	-	-	-	-	-	-	-	-	-	-	-	-	-

No.	Soyasaponin names	Code no. of soyfood samples													
		C1	C2	C3	1	2	3	4	5	6	7	8	9	10	11
36	**Ay (monoacetyl)**	-	-	-	-	-	-	-	-	-	-	-	-	-	-
37	**Ay (diacetyl)**	-	-	-	-	-	-	-	-	-	-	-	-	-	-
38	**Ag (monoacetyl)**	-	-	-	-	-	-	-	-	-	-	-	-	-	-
39	**Ag (diacetyl)**	-	-	-	-	-	-	-	-	-	-	-	-	-	-
40	Ab (tetraacetyl)	65.4	36.7	93.4	131.6	11.3	59.2	4.7	63.7	67.4	tr	10.7	30.5	2.8	16.2
41	Ac (tetraacetyl)	12.1	22.4	21.9	0.7	0.7	9.4	9.5	11.5	11.7	-	2.1	-	-	4.8
42	Af (tetraacetyl)	28.2	18.3	26.6	tr	78.0	10.9	4.9	13.8	4.8	-	8.2	18.1	-	9.0
43	Ad (tetraacetyl)	-	-	18.5	-	-	-	-	-	-	-	-	-	-	-
44	Az (tetraacetyl)	-	-	-	-	-	-	-	-	-	-	-	tr	-	tr
45	Ah (tetraacetyl)	0.7	8.8	10.4	-	-	-	-	-	-	-	2.0	3.2	-	2.3
46	Ab (nullacetyl) / [A_1]	2.7	-	-	-	-	-	-	-	-	5.1	-	-	-	-
47	Ac (nullacetyl)	-	-	-	-	-	-	-	-	-	-	-	-	-	-
48	Af (nullacetyl) / [A_2]	-	-	-	-	-	-	-	-	-	0.6	-	-	-	-
49	Ad (nullacetyl)	-	-	-	0.3	4.4	1.7	2.2	0.9	1.7	-	-	-	1.5	-
50	Az (nullacetyl)	-	-	-	-	-	-	-	-	-	-	-	-	-	-
51	Ah (nullacetyl) / [A_3]	-	-	-	-	-	-	-	-	-	-	-	-	-	-
52	**Ab (monoacetyl, 1)**	-	-	-	-	-	-	-	-	-	**2.6**	-	-	-	-
53	**Ab (monoacetyl, 2)**	-	-	-	-	-	-	-	-	-	-	-	-	-	-
54	**Ab (diacetyl, 1)**	**tr**	-	-	**0.1**	**1.8**	**2.1**	**0.4**	**tr**	**0.7**	**2.9**	**3.6**	**16.1**	-	-
55	**Ab (diacetyl, 2)**	-	-	-	-	-	-	-	-	-	-	-	-	-	-
56	**Ab (diacetyl, 3)**	-	-	-	-	-	-	-	-	-	-	-	-	-	-
57	**Ab (diacetyl, 3)**	-	-	-	-	-	-	-	-	-	-	-	-	-	-
58	**Ab (triacetyl, 1)**	**tr**	-	-	**15.9**	**tr**	**1.7**	**tr**	**tr**	**tr**	-	-	**9.0**	**3.5**	**1.8**
59	**Ab (triacetyl, 2)**	-	-	-	-	-	-	-	-	-	-	-	-	-	-
60	**Ac (monoacetyl)**	-	-	-	-	-	-	-	-	-	-	-	-	-	-
61	**Ac (diacetyl, 1)**	-	-	-	-	-	-	-	-	-	-	-	-	-	-
62	**Ac (diacetyl, 2)**	-	-	-	-	-	-	-	-	-	-	-	-	-	-
63	**Ac (diacetyl, 3)**	-	-	-	-	-	-	-	-	-	-	-	-	-	-
64	**Ac (triacetyl)**	-	-	-	-	-	-	-	-	-	-	**0.8**	**1.1**	-	-
65	**Af (monoacetyl)**	-	-	-	-	-	-	-	-	-	**1.7**	**1.7**	-	**3.7**	**3.4**
66	**Af (diacetyl, 1)**	-	-	-	-	-	**0.5**	-	-	-	-	-	**0.5**	-	-
67	**Af (diacetyl, 2)**	-	-	-	-	-	-	-	-	-	-	-	-	-	-
68	**Af (triacetyl, 1)**	-	-	-	**2.5**	**2.6**	**tr**	**0.3**	**0.4**	**1.0**	-	-	-	-	-
69	**Af (triacetyl, 2)**	-	-	-	-	-	-	-	-	-	-	-	-	-	-
70	**Af (triacetyl, 3)**	-	-	-	-	-	-	-	-	-	-	-	-	-	-
71	**Ad (monoacetyl)**	-	-	-	**tr**	**tr**	**tr**	**tr**	**tr**	**tr**	-	-	-	-	-

No.	Soyasaponin names	Code no. of soyfood samples													
		C1	C2	C3	1	2	3	4	5	6	7	8	9	10	11
72	Ad (diacetyl 1)	-	-	-	1.7	1.4	1.0	1.5	0.7	0.9	-	-	-	-	-
73	Ad (diacetyl 2)	-	-	-	0.7	tr	0.9	1.8	3.0	tr	-	-	-	-	-
74	Ad (triacetyl, 1)	-	-	18.7	-	-	-	-	-	-	-	-	-	-	-
75	Ad (triacetyl, 2)	-	-	-	-	-	-	-	-	-	-	-	-	-	-
76	Az (monoacetyl)	-	-	-	tr	tr	tr	tr	tr	5.1	-	-	-	-	-
77	Az (diacetyl)	-	-	-	tr	tr	1.5	1.9	1.6	2.2	-	-	-	-	-
78	Az (triacetyl)	-	-	-	-	-	-	-	-	-	-	-	-	-	-
79	Ah (monoacetyl)	-	-	-	1.0	tr	0.6	0.7	0.9	1.3	-	-	-	-	-
80	Ah (diacetyl)	-	-	-	tr	tr	0.6	0.6	0.7	0.8	-	-	-	-	-
81	Ah (triacetyl, 1)	-	-	-	-	-	-	-	-	-	-	-	-	-	-
82	Ah (triacetyl, 2)	-	-	-	-	-	-	-	-	-	-	-	-	-	-
83	A0-αg	tr	17.0	66.4	-	-	-	-	-	-	-	-	-	-	-
84	A0-βg	-	tr	4.9	-	-	-	-	-	-	-	-	2.5	0.1	0.4
85	A0-γg	-	tr	7.6	-	-	-	-	-	-	-	-	-	-	-
86	A0-αa	-	-	-	-	-	-	-	-	-	-	-	-	-	-
87	A0-βa	-	-	-	-	-	-	-	-	-	-	-	-	-	-
88	A0-γa	-	-	-	-	-	-	-	-	-	-	-	-	-	-
89	DDMP-αg	40.0	15.9	16.5	21.4	7.6	15.4	14.1	18.4	18.9	-	3.1	5.6	-	-
90	DDMP-βg	453.9	145.6	147.1	62.0	227.3	210.5	195.6	270.3	230.9	-	106.6	144.2	35.4	181.2
91	DDMP-γg	44.6	20.0	18.4	7.6	247.6	4.9	4.3	14.7	11.7	-	4.2	3.1	-	7.6
92	DDMP-αa	-	-	-	-	-	-	-	-	-	-	-	-	-	-
93	DDMP-βa	233.7	67.8	67.8	6.2	74.9	146.3	109.5	165.2	143.7	-	59.2	90.3	7.4	104.3
94	DDMP-γa	7.0	6.8	6.4	1.5	90.3	3.0	2.4	0.8	5.2	-	2.2	1.8	-	1.7
95	Ba [V]	-	-	4.0	5.9	1.0	1.6	0.8	1.6	1.9	5.2	2.3	8.4	-	1.4
96	Bb [I]	13.4	12.2	24.4	58.0	22.6	32.8	40.9	41.9	58.3	166.0	90.9	191.4	82.7	289.6
97	Bb' [III]	-	-	-	-	-	-	-	-	-	-	-	-	tr	-
98	Bx	-	-	-	-	-	-	-	-	-	-	-	-	-	-
99	Bc [II]	79.2	11.4	11.3	tr	tr	1.1	14.9	0.9	9.9	-	45.5	120.1	37.8	143.2
100	Bc' [IV]	-	-	-	-	-	-	-	-	-	-	-	-	-	3.3
101	Bd	-	-	-	tr	tr	tr	tr	tr	tr	-	-	-	-	-
102	Be	-	-	-	-	-	-	-	-	-	-	-	2.2	-	0.8
103	Be'	-	-	-	-	-	-	-	-	-	-	-	-	-	-
104	Bf	-	-	-	-	-	-	-	-	-	-	-	-	-	-
105	Bg	-	-	-	-	-	-	-	-	-	-	-	-	-	-
106	Bg'	-	-	-	-	-	-	-	-	-	-	-	-	-	-

Note : Soyasaponin value: mean (n=3); tr: detected by MS analysis but not detected by PDA analysis or content lower than limit of detection (< 0.1 μmol/100 g dry basis), not detected by MS analysis. Highlighted compounds are newly detected.

Table II.6 : Soyasaponin contents (μmol/100 g dry basis) in soyfood samples (code no. 12-25)

No.	Soyasaponin names	Code no. of soyfood samples													
		12	13	14	15	16	17	18	19	20	21	22	23	24	25
1	Aa (triacetyl)	-	-	-	-	-	-	-	-	-	-	58.8	-	-	-
2	Au (triacetyl)	-	-	-	-	-	-	-	-	-	-	-	-	-	-
3	Ae (triacetyl)	-	-	-	-	-	-	-	-	-	-	23.0	-	-	-
4	Ax (triacetyl)	-	-	-	-	-	-	-	-	-	-	-	-	-	-
5	Ay (triacetyl)	-	-	-	-	-	-	-	-	-	-	-	-	-	-
6	Ag (triacetyl)	-	-	-	-	-	-	-	-	-	-	0.8	-	-	-
7	Aa (nullacetyl) / [A_4]	-	-	-	-	-	-	-	-	-	-	-	-	-	-
8	Au (nullacetyl)	-	-	-	-	-	-	-	-	-	-	-	-	-	-
9	Ae (nullacetyl) / [A_5]	-	-	-	-	-	-	-	-	-	-	-	-	-	-
10	Ax (nullacetyl)	-	-	-	-	-	-	-	-	-	-	-	-	-	-
11	Ay (nullacetyl)	-	-	-	-	-	-	-	-	-	-	-	-	-	-
12	Ag (nullacetyl) / [A_6]	-	-	-	-	-	-	-	-	-	-	-	-	-	-
13	**Aa (monoacetyl, 1)**	-	-	-	-	-	-	-	-	-	-	-	-	-	-
14	**Aa (monoacetyl, 2)**	-	-	-	-	-	-	-	-	-	-	-	-	-	-
15	**Aa (monoacetyl, 3)**	-	-	-	-	-	-	-	-	-	-	-	-	-	-
16	**Aa (diacetyl, 1)**	-	-	-	-	-	-	-	-	-	-	-	-	-	-
17	**Aa (diacetyl, 2)**	-	-	-	-	-	-	-	-	-	-	-	-	-	-
18	**Aa (diacetyl, 3)**	-	-	-	-	-	-	-	-	-	-	-	-	-	-
19	**Au (monoacetyl, 1)**	-	-	-	-	-	-	-	-	-	-	-	-	-	-
20	**Au (monoacetyl, 2)**	-	-	-	-	-	-	-	-	-	-	-	-	-	-
21	**Au (monoacetyl, 3)**	-	-	-	-	-	-	-	-	-	-	-	-	-	-
22	**Au (diacetyl, 1)**	-	-	-	-	-	-	-	-	-	-	-	-	-	-
23	**Au (diacetyl, 2)**	-	-	-	-	-	-	-	-	-	-	-	-	-	-
24	**Au (diacetyl, 3)**	-	-	-	-	-	-	-	-	-	-	-	-	-	-
25	**Ae (monoacetyl, 1)**	-	-	-	-	-	-	-	-	-	-	-	-	-	-
26	**Ae (monoacetyl, 2)**	-	-	-	-	-	-	-	-	-	-	-	-	-	-
27	**Ae (monoacetyl, 3)**	-	-	-	-	-	-	-	-	-	-	-	-	-	-
28	**Ae (diacetyl, 1)**	-	-	-	-	-	-	-	-	-	-	-	-	-	-
29	**Ae (diacetyl, 2)**	-	-	-	-	-	-	-	-	-	-	-	-	-	-
30	**Ae (diacetyl, 3)**	-	-	-	-	-	-	-	-	-	-	-	-	-	-
31	**Ax (monoacetyl, 1)**	-	-	-	-	-	-	-	-	-	-	-	-	-	-
32	**Ax (monoacetyl, 2)**	-	-	-	-	-	-	-	-	-	-	-	-	-	-
33	**Ax (diacetyl, 1)**	-	-	-	-	-	-	-	-	-	-	-	-	-	-
34	**Ax (diacetyl, 2)**	-	-	-	-	-	-	-	-	-	-	-	-	-	-
35	**Ax (diacetyl, 3)**	-	-	-	-	-	-	-	-	-	-	-	-	-	-

No.	Soyasaponin names	Code no. of soyfood samples													
		12	13	14	15	16	17	18	19	20	21	22	23	24	25
36	**Ay (monoacetyl)**	-	-	-	-	-	-	-	-	-	-	-	-	-	-
37	**Ay (diacetyl)**	-	-	-	-	-	-	-	-	-	-	-	-	-	-
38	**Ag (monoacetyl)**	-	-	-	-	-	-	-	-	-	-	-	-	-	-
39	**Ag (diacetyl)**	-	-	-	-	-	-	-	-	-	-	-	-	-	-
40	Ab (tetraacetyl)	2.1	15.9	-	0.3	-	-	-	-	-	-	94.5	16.0	20.4	12.4
41	Ac (tetraacetyl)	-	2.0	-	-	-	-	-	-	-	-	7.1	3.4	3.1	1.1
42	Af (tetraacetyl)	2.3	2.3	-	-	-	-	-	-	-	-	15.1	3.2	2.1	0.7
43	Ad (tetraacetyl)	-	-	-	-	-	-	-	-	-	-	-	-	-	-
44	Az (tetraacetyl)	-	-	-	-	-	-	-	-	-	-	-	-	-	-
45	Ah (tetraacetyl)	-	-	-	-	-	-	-	-	-	-	-	-	-	-
46	Ab (nullacetyl) / [A_1]	20.5	-	-	4.7	-	-	-	-	-	-	-	-	-	-
47	Ac (nullacetyl)	-	-	-	-	-	-	-	-	-	-	-	-	-	-
48	Af (nullacetyl) / [A_2]	-	-	-	2.0	0.9	1.3	-	4.4	-	-	-	-	-	-
49	Ad (nullacetyl)	-	3.5	-	-	-	-	-	-	-	-	3.1	2.8	2.4	6.2
50	Az (nullacetyl)	-	-	-	-	-	-	-	-	-	-	-	-	-	-
51	Ah (nullacetyl) / [A_3]	-	-	-	-	-	-	-	-	-	-	1.3	-	-	-
52	**Ab (monoacetyl, 1)**	**tr**	**-**	**-**	**8.2**	**-**	**-**	**-**	**-**	**-**	**-**	**-**	**-**	**-**	**-**
53	**Ab (monoacetyl, 2)**	**-**	**-**	**-**	**-**	**-**	**-**	**-**	**-**	**-**	**-**	**-**	**-**	**-**	**-**
54	**Ab (diacetyl, 1)**	**9.5**	**0.3**	**-**	**5.4**	**-**	**0.9**	**-**	**-**	**-**	**-**	**0.5**	**0.5**	**0.2**	**1.1**
55	**Ab (diacetyl, 2)**	**-**	**-**	**-**	**-**	**-**	**-**	**-**	**-**	**-**	**-**	**-**	**-**	**-**	**-**
56	**Ab (diacetyl, 3)**	**-**	**-**	**-**	**-**	**-**	**-**	**-**	**-**	**-**	**-**	**-**	**-**	**-**	**-**
57	**Ab (diacetyl, 3)**	**-**	**-**	**-**	**-**	**-**	**-**	**-**	**-**	**-**	**-**	**-**	**-**	**-**	**-**
58	**Ab (triacetyl, 1)**	**-**	**3.7**	**-**	**-**	**1.8**	**-**	**-**	**0.7**	**-**	**-**	**-**	**2.5**	**5.7**	**3.4**
59	**Ab (triacetyl, 2)**	**-**	**-**	**-**	**-**	**-**	**-**	**-**	**-**	**-**	**-**	**-**	**-**	**-**	**-**
60	**Ac (monoacetyl)**	**-**	**-**	**-**	**-**	**-**	**-**	**-**	**-**	**-**	**-**	**-**	**-**	**-**	**-**
61	**Ac (diacetyl, 1)**	**-**	**-**	**-**	**-**	**-**	**-**	**-**	**-**	**-**	**-**	**-**	**-**	**-**	**-**
62	**Ac (diacetyl, 2)**	**-**	**-**	**-**	**-**	**-**	**-**	**-**	**-**	**-**	**-**	**-**	**-**	**-**	**-**
63	**Ac (diacetyl, 3)**	**-**	**-**	**-**	**-**	**-**	**-**	**-**	**-**	**-**	**-**	**-**	**-**	**-**	**-**
64	**Ac (triacetyl)**	**-**	**-**	**-**	**-**	**0.4**	**0.4**	**-**	**0.4**	**-**	**-**	**-**	**-**	**-**	**-**
65	**Af (monoacetyl)**	**0.7**	**-**	**-**	**2.0**	**0.2**	**-**	**1.4**	**1.3**	**0.5**	**-**	**-**	**-**	**-**	**-**
66	**Af (diacetyl, 1)**	**-**	**1.0**	**-**	**0.6**	**-**	**0.2**	**2.0**	**-**	**0.6**	**-**	**-**	**-**	**-**	**-**
67	**Af (diacetyl, 2)**	**-**	**-**	**-**	**-**	**-**	**-**	**-**	**-**	**-**	**-**	**-**	**-**	**-**	**-**
68	**Af (triacetyl, 1)**	**-**	**tr**	**-**	**-**	**-**	**-**	**-**	**-**	**-**	**-**	**-**	**0.3**	**0.6**	**0.6**
69	**Af (triacetyl, 2)**	**-**	**-**	**-**	**-**	**-**	**-**	**-**	**-**	**-**	**-**	**-**	**-**	**-**	**-**
70	**Af (triacetyl, 3)**	**-**	**-**	**-**	**-**	**-**	**-**	**-**	**-**	**-**	**-**	**-**	**-**	**-**	**-**
71	**Ad (monoacetyl)**	**-**	**tr**	**-**	**-**	**-**	**-**	**-**	**-**	**-**	**-**	**-**	**4.2**	**2.2**	**8.8**

No.	Soyasaponin names	Code no. of soyfood samples													
		12	13	14	15	16	17	18	19	20	21	22	23	24	25
72	**Ad (diacetyl, 1)**	**-**	**0.2**	**-**	**-**	**-**	**-**	**-**	**-**	**-**	**-**	**-**	**1.3**	**0.7**	**2.9**
73	**Ad (diacetyl, 2)**	**-**	**-**	**-**	**-**	**-**	**-**	**-**	**-**	**-**	**-**	**-**	**-**	**-**	**-**
74	**Ad (triacetyl, 1)**	**-**	**-**	**-**	**-**	**-**	**-**	**-**	**-**	**-**	**-**	**-**	**-**	**-**	**-**
75	**Ad (triacetyl, 2)**	**-**	**-**	**-**	**-**	**-**	**-**	**-**	**-**	**-**	**-**	**-**	**-**	**-**	**-**
76	**Az (monoacetyl)**	**-**	**1.0**	**-**	**-**	**-**	**-**	**-**	**1.2**	**-**	**-**	**-**	**0.6**	**0.6**	**0.6**
77	**Az (diacetyl)**	**-**	**-**	**-**	**-**	**-**	**-**	**-**	**-**	**-**	**-**	**-**	**0.1**	**0.1**	**0.9**
78	**Az (triacetyl)**	**-**	**-**	**-**	**-**	**-**	**-**	**-**	**-**	**-**	**-**	**-**	**-**	**-**	**-**
79	**Ah (monoacetyl)**	**-**	**-**	**-**	**1.7**	**-**	**-**	**-**	**-**	**-**	**-**	**-**	**4.0**	**2.6**	**5.8**
80	**Ah (diacetyl)**	**-**	**-**	**-**	**-**	**-**	**-**	**-**	**-**	**-**	**-**	**-**	**-**	**0.2**	**0.7**
81	**Ah (triacetyl, 1)**	**-**	**-**	**-**	**-**	**-**	**-**	**-**	**-**	**-**	**-**	**-**	**-**	**-**	**-**
82	**Ah (triacetyl, 2)**	**-**	**-**	**-**	**-**	**-**	**-**	**-**	**-**	**-**	**-**	**-**	**-**	**-**	**-**
83	A0-αg	-	-	31.2	-	-	-	-	-	-	20.5	1.8	-	-	-
84	A0-βg	-	-	-	-	-	-	-	-	-	-	-	-	-	-
85	A0-γg	-	-	-	-	-	-	-	-	-	-	-	-	-	-
86	A0-αa	-	-	-	-	-	-	-	-	-	-	-	-	-	-
87	A0-βa	-	-	-	-	-	-	-	-	-	-	-	-	-	-
88	A0-γa	-	-	-	-	-	-	-	-	-	-	-	-	-	-
89	DDMP-αg	-	5.5	-	1.1	-	-	-	-	-	-	-	7.8	8.9	8.9
90	DDMP-βg	2.5	7.2	91.4	69.1	62.7	5.4	53.2	10.8	62.5	49.0	192.6	3.5	14.1	6.8
91	DDMP-γg	-	0.6	5.2	1.0	0.2	-	3.0	-	5.8	-	11.1	2.7	0.2	0.5
92	DDMP-αa	-	-	-	-	-	-	-	-	-	-	-	-	-	-
93	DDMP-βa	-	7.9	55.4	39.7	39.6	10.4	23.6	3.7	30.2	30.3	77.9	2.2	9.0	3.8
94	DDMP-γa	-	3.2	1.3	-	-	-	-	-	-	-	1.9	1.5	0.2	0.1
95	Ba [V]	14.7	6.2	-	4.2	1.5	-	-	-	-	-	11.2	4.9	5.7	6.8
96	Bb [I]	326.9	106.3	124.2	141.2	124.0	28.6	67.1	71.7	76.8	75.5	331.3	119.2	146.7	135.8
97	Bb' [III]	13.3	-	2.7	-	-	-	-	-	-	-	-	-	-	-
98	Bx	-	-	-	-	-	-	-	-	-	-	-	-	-	-
99	Bc [II]	133.6	74.6	67.3	94.5	82.8	13.8	31.4	38.0	45.5	53.4	106.5	52.0	67.0	55.9
100	Bc' [IV]	0.9	-	4.0	3.2	4.3	-	-	-	-	-	1.4	-	-	-
101	Bd	-	0.6	-	-	-	-	-	-	-	-	-	1.1	0.8	1.0
102	Be	17.7	-	-	-	-	-	-	-	-	-	10.5	-	-	-
103	Be'	-	-	-	-	-	-	-	-	-	-	-	-	-	-
104	Bf	-	-	-	-	-	-	-	-	-	-	-	-	-	-
105	Bg	-	-	-	-	-	-	-	-	-	-	-	-	-	-
106	Bg'	-	-	-	-	-	-	-	-	-	-	-	-	-	-

Note : Soyasaponin value: mean (n=3); tr: detected by MS analysis but not detected by PDA analysis or content lower than limit of detection (< 0.1 μmol/100 g dry basis), not detected by MS analysis. Highlighted compounds are newly detected.

Table II.6 : Soyasaponin contents (μmol/100 g dry basis) in soyfood samples (code no.26-39)

No.	Soyasaponin names	Code no. of soyfood samples													
		26	**27**	**28**	**29**	**30**	**31**	**32**	**33**	**34**	**35**	**36**	**37**	**38**	**39**
1	Aa (triacetyl)	-	-	-	-	-	-	-	-	-	-	-	-	-	-
2	Au (triacetyl)	-	-	-	-	-	-	-	-	-	-	-	-	-	-
3	Ae (triacetyl)	-	-	-	-	-	-	-	-	-	-	-	-	-	-
4	Ax (triacetyl)	-	-	-	-	-	-	-	-	-	-	-	-	-	-
5	Ay (triacetyl)	-	-	-	-	-	-	-	-	-	-	-	-	-	-
6	Ag (triacetyl)	-	-	-	-	-	-	-	-	-	-	-	-	-	-
7	Aa (nullacetyl) / [A_4]	-	-	-	-	-	-	-	-	29.5	-	-	-	-	-
8	Au (nullacetyl)	-	-	-	-	-	-	-	-	-	-	-	-	-	-
9	Ae (nullacetyl) / [A_5]	-	-	-	-	-	-	-	-	-	-	-	-	-	-
10	Ax (nullacetyl)	-	-	-	-	-	-	-	-	-	-	-	-	-	-
11	Ay (nullacetyl)	-	-	-	-	-	-	-	-	-	-	-	-	-	-
12	Ag (nullacetyl) / [A_6]	-	-	-	-	-	-	-	-	-	-	-	-	-	-
13	**Aa (monoacetyl, 1)**	-	-	-	-	-	-	-	-	-	-	-	-	-	-
14	**Aa (monoacetyl, 2)**	-	-	-	-	-	-	-	-	-	-	-	-	-	-
15	**Aa (monoacetyl, 3)**	-	-	-	-	-	-	-	-	-	-	-	-	-	-
16	**Aa (diacetyl, 1)**	-	-	-	-	-	-	-	-	-	-	-	-	-	-
17	**Aa (diacetyl, 2)**	-	-	-	-	-	-	-	-	-	-	-	-	-	-
18	**Aa (diacetyl, 3)**	-	-	-	-	-	-	-	-	-	-	-	-	-	-
19	**Au (monoacetyl, 1)**	-	-	-	-	-	-	-	-	-	-	-	-	-	-
20	**Au (monoacetyl, 2)**	-	-	-	-	-	-	-	-	-	-	-	-	-	-
21	**Au (monoacetyl, 3)**	-	-	-	-	-	-	-	-	-	-	-	-	-	-
22	**Au (diacetyl, 1)**	-	-	-	-	-	-	-	-	-	-	-	-	-	-
23	**Au (diacetyl, 2)**	-	-	-	-	-	-	-	-	-	-	-	-	-	-
24	**Au (diacetyl, 3)**	-	-	-	-	-	-	-	-	-	-	-	-	-	-
25	**Ae (monoacetyl, 1)**	-	-	-	-	-	-	-	-	-	-	-	-	-	-
26	**Ae (monoacetyl, 2)**	-	-	-	-	-	-	-	-	-	-	-	-	-	-
27	**Ae (monoacetyl, 3)**	-	-	-	-	-	-	-	-	-	-	-	-	-	-
28	**Ae (diacetyl, 1)**	-	-	-	-	-	-	-	-	-	-	-	-	-	-
29	**Ae (diacetyl, 2)**	-	-	-	-	-	-	-	-	-	-	-	-	-	-
30	**Ae (diacetyl, 3)**	-	-	-	-	-	-	-	-	-	-	-	-	-	-
31	**Ax (monoacetyl, 1)**	-	-	-	-	-	-	-	-	-	-	-	-	-	-
32	**Ax (monoacetyl, 2)**	-	-	-	-	-	-	-	-	-	-	-	-	-	-
33	**Ax (diacetyl, 1)**	-	-	-	-	-	-	-	-	-	-	-	-	-	-
34	**Ax (diacetyl, 2)**	-	-	-	-	-	-	-	-	-	-	-	-	-	-
35	**Ax (diacetyl, 3)**	-	-	-	-	-	-	-	-	-	-	-	-	-	-

No.	Soyasaponin names	Code no. of soyfood samples													
		26	27	28	29	30	31	32	33	34	35	36	37	38	39
36	**Ay (monoacetyl)**	-	-	-	-	-	-	-	-	-	-	-	-	-	-
37	**Ay (diacetyl)**	-	-	-	-	-	-	-	-	-	-	-	-	-	-
38	**Ag (monoacetyl)**	-	-	-	-	-	-	-	-	-	-	-	-	-	-
39	**Ag (diacetyl)**	-	-	-	-	-	-	-	-	-	-	-	-	-	-
40	Ab (tetraacetyl)	125.0	0.8	23.2	33.0	34.1	48.2	65.1	-	7.9	2.1	12.7	3.6	2.5	11.3
41	Ac (tetraacetyl)	12.3	1.2	-	-	-	-	4.4	-	-	1.7	tr	tr	tr	tr
42	Af (tetraacetyl)	34.0	1.3	2.2	3.0	2.5	2.1	3.3	-	-	0.7	tr	tr	tr	tr
43	Ad (tetraacetyl)	-	-	-	-	-	-	-	-	-	-	-	-	-	-
44	Az (tetraacetyl)	-	-	-	-	-	-	-	-	-	-	-	-	-	-
45	Ah (tetraacetyl)	-	-	-	0.5	tr	tr	0.6	-	-	-	-	-	-	-
46	Ab (nullacetyl) / [A_1]	-	-	2.1	5.3	7.8	22.8	7.8	24.8	-	-	-	-	-	-
47	Ac (nullacetyl)	-	-	-	-	-	-	-	-	-	-	-	-	-	-
48	Af (nullacetyl) / [A_2]	-	-	-	-	-	-	-	-	-	-	-	-	-	-
49	Ad (nullacetyl)	tr	8.7	-	-	-	-	-	-	-	7.5	11.6	12.6	12.9	11.5
50	Az (nullacetyl)	-	-	-	-	-	-	-	-	-	-	-	-	-	-
51	Ah (nullacetyl) / [A_3]	-	-	-	-	-	-	-	-	6.4	-	-	-	-	-
52	**Ab (monoacetyl, 1)**	**tr**	-	-	-	**5.4**	-	-	-	**1.6**	-	-	-	-	-
53	**Ab (monoacetyl, 2)**	-	-	-	-	-	-	-	-	-	-	-	-	-	-
54	**Ab (diacetyl, 1)**	**5.2**	**0.1**	**2.5**	**11.2**	**17.5**	**32.5**	**14.7**	-	-	-	**8.5**	**1.9**	**12.3**	**3.3**
55	**Ab (diacetyl, 2)**	-	-	-	-	-	-	-	-	-	-	-	-	-	-
56	**Ab (diacetyl, 3)**	-	-	-	-	-	-	-	-	-	-	-	-	-	-
57	**Ab (diacetyl, 3)**	-	-	-	-	-	-	-	-	-	-	-	-	-	-
58	**Ab (triacetyl, 1)**	**27.9**	**2.6**	-	-	-	**13.2**	-	-	-	**7.2**	**0.9**	**19.1**	**1.6**	**1.9**
59	**Ab (triacetyl, 2)**	-	-	-	-	-	-	-	-	-	-	-	-	-	-
60	**Ac (monoacetyl)**	-	-	-	-	-	-	-	-	-	-	-	-	-	-
61	**Ac (diacetyl, 1)**	-	-	-	-	-	-	-	-	-	-	-	-	-	-
62	**Ac (diacetyl, 2)**	-	-	-	-	-	-	-	-	-	-	-	-	-	-
63	**Ac (diacetyl, 3)**	-	-	-	-	-	-	-	-	-	-	-	-	-	-
64	**Ac (triacetyl)**	-	-	-	-	-	-	-	-	-	-	-	-	-	-
65	**Af (monoacetyl)**	**3.0**	-	**9.1**	**10.2**	**14.8**	**21.8**	**16.3**	-	**tr**	-	-	-	-	-
66	**Af (diacetyl, 1)**	-	**7.0**	-	-	-	-	-	-	-	-	**1.1**	**3.3**	-	-
67	**Af (diacetyl, 2)**	-	-	-	-	-	-	-	-	-	-	-	-	-	-
68	**Af (triacetyl, 1)**	-	**5.3**	-	-	-	-	-	-	-	**tr**	**0.1**	**0.1**	**1.7**	**0.3**
69	**Af (triacetyl, 2)**	-	-	-	-	-	-	-	-	-	-	-	-	-	-
70	**Af (triacetyl, 3)**	-	-	-	-	-	-	-	-	-	-	-	-	-	-
71	**Ad (monoacetyl)**	-	**3.8**	-	-	-	-	-	-	-	**tr**	**68.3**	-	**74.2**	**64.2**

No.	Soyasaponin names	Code no. of soyfood samples													
		26	27	28	29	30	31	32	33	34	35	36	37	38	39
72	**Ad (diacetyl 1)**	**-**	**0.1**	**-**	**-**	**-**	**-**	**-**	**-**	**-**	**-**	**2.0**	**4.6**	**11.5**	**1.9**
73	**Ad (diacetyl 2)**	**-**	**0.2**	**-**	**-**	**-**	**-**	**-**	**-**	**-**	**-**	**-**	**-**	**18.7**	**-**
74	**Ad (triacetyl, 1)**	**-**	**-**	**-**	**-**	**-**	**-**	**-**	**-**	**-**	**-**	**-**	**-**	**-**	**-**
75	**Ad (triacetyl, 2)**	**-**	**-**	**-**	**-**	**-**	**-**	**-**	**-**	**-**	**-**	**-**	**-**	**-**	**-**
76	**Az (monoacetyl)**	**-**	**-**	**-**	**-**	**-**	**-**	**-**	**-**	**-**	**tr**	**tr**	**43.6**	**-**	**1.5**
77	**Az (diacetyl)**	**-**	**0.4**	**-**	**-**	**-**	**-**	**-**	**-**	**-**	**-**	**-**	**-**	**-**	**18.0**
78	**Az (triacetyl)**	**-**	**-**	**-**	**-**	**-**	**-**	**-**	**-**	**-**	**-**	**-**	**-**	**-**	**-**
79	**Ah (monoacetyl)**	**-**	**-**	**-**	**-**	**-**	**-**	**-**	**-**	**-**	**-**	**2.8**	**3.2**	**17.8**	**-**
80	**Ah (diacetyl)**	**-**	**0.1**	**-**	**-**	**-**	**-**	**-**	**-**	**-**	**-**	**0.9**	**0.2**	**8.8**	**-**
81	**Ah (triacetyl, 1)**	**-**	**-**	**-**	**-**	**-**	**-**	**-**	**-**	**-**	**-**	**-**	**-**	**-**	**-**
82	**Ah (triacetyl, 2)**	**-**	**-**	**-**	**-**	**-**	**-**	**-**	**-**	**-**	**-**	**-**	**-**	**-**	**-**
83	A0-αg	1.8	-	-	-	-	-	-	-	-	-	-	-	-	-
84	A0-βg	-	-	-	-	-	-	-	-	-	-	-	-	-	-
85	A0-γg	-	-	-	-	-	-	-	-	-	-	-	-	-	-
86	A0-αa	-	-	-	-	-	-	-	-	-	-	-	-	-	-
87	A0-βa	-	-	-	-	-	-	-	-	-	-	-	-	-	-
88	A0-γa	-	-	-	-	-	-	-	-	-	-	-	-	-	-
89	DDMP-αg	-	1.7	-	-	-	-	-	-	-	1.0	2.8	7.7	6.6	8.2
90	DDMP-βg	36.8	1.2	-	-	-	-	-	-	-	3.4	10.2	15.8	2.0	13.2
91	DDMP-γg	3.1	2.4	-	-	-	-	-	-	-	0.2	1.6	0.4	0.7	2.1
92	DDMP-αa	-	-	-	-	-	-	-	-	-	-	-	-	-	-
93	DDMP-βa	8.1	9.6	-	-	-	-	-	-	-	1.6	2.8	21.7	1.4	8.3
94	DDMP-γa	-	1.6	-	-	-	-	-	-	-	7.2	1.7	0.4	1.4	4.1
95	Ba [V]	21.1	9.8	32.0	16.8	28.1	66.1	80.5	48.0	18.4	40.4	16.5	4.4	5.8	5.6
96	Bb [I]	536.9	180.3	463.2	435.6	505.6	714.2	580.8	256.4	398.0	94.9	151.7	101.5	122.2	113.4
97	Bb′ [III]	22.6	-	92.2	58.3	84.5	51.8	37.4	-	-	-	-	-	-	-
98	Bx	-	-	-	-	-	-	-	-	-	-	-	-	-	-
99	Bc [II]	132.5	87.0	123.2	186.2	190.3	290.4	278.7	121.9	189.0	28.9	97.9	-	69.1	66.8
100	Bc′ [IV]	3.7	-	20.6	8.0	20.1	14.4	13.0	-	7.3	-	-	-	-	-
101	Bd	-	1.3	-	-	-	-	-	-	-	0.1	0.2	0.4	0.7	0.7
102	Be	24.7	-	2.5	2.5	-	-	-	-	-	-	-	-	-	-
103	Be′	-	-	2.6	2.6	-	-	-	-	-	-	-	-	-	-
104	Bf	-	-	-	-	-	-	-	-	-	-	-	-	-	-
105	Bg	-	-	-	-	-	-	-	-	-	-	-	-	-	-
106	Bg′	-	-	-	-	-	-	-	-	-	-	-	-	-	-

Note : Soyasaponin value: mean (n=3); tr: detected by MS analysis but not detected by PDA analysis or content lower than limit of detection (< 0.1 μmol/100 g dry basis), not detected by MS analysis. Highlighted compounds are newly detected.

Table II.7 : Total soyasaponin content (µmol/100 g dry basis) in soyfood samples.

Soyasaponin composition	Dry SB seed			SB sprout		Young SB	
	C1	**C2**	**C3**	**1**	**2**	**3**	**4**
FSAGs	107.0 ± 0.0	86.2 ± 15.0	170.7 ± 0.4	132.2 ± 6.7	90.0 ± 2.9	79.5 ± 1.1	19.0 ± 2.2
PSAGs	-	-	17.3 ± 1.9	21.9 ± 10.7	5.7 ± 0.2	9.0 ± 1.1	7.1 ± 0.0
NSAGs	2.7 ± 0.87	-	-	0.3 ± 0.4	4.4 ± 0.9	1.7 ± 0.4	2.2 ± 0.4
A0-line group A saponin	-	17.0 ± 0.7	78.9 ± 1.5	-	-	-	-
Total of SAGs	*109.8 ± 4.5*	*103.1 ± 15.7*	*266.9 ± 3.1*	*154.5 ± 17.8*	*100.1 ± 4.0*	*90.2 ± 0.3*	*28.3 ± 2.6*
DDMP	779.3 ± 0.0	256.1 ± 0.0	256.2 ± 0.1	98.8 ± 0.9	647.7 ± 8.6	380.1 ± 3.4	326.3 ± 2.7
Gr. B	92.6 ± 6.1	23.6 ± 2.2	39.7 ± 5.8	63.9 ± 4.7	23.5 ± 2.2	35.5 ± 4.5	56.6 ± 5.1
Gr. E	-	-	-	-	tr	-	tr
Total of DDMPs	*871.8 ± 14.6*	*279.7 ± 2.2*	*295.9 ± 5.7*	*162.7 ± 5.6*	*671.2 ± 11.4*	*415.6 ± 1.1*	*382.9 ± 3.0*
Total saponin contents	981.6 ± 18.3	382.9 ± 17.9	562.8 ± 23.5	317.2 ± 23.5	771.3 ± 15.4	505.8 ± 0.7	411.2 ± 5.5

Soyasaponin composition	Young black SB		Cooked black SB	Non-modified soymilk			
	5	**6**	**7**	**8**	**9**	**10**	**11**
FSAGs	89.0 ± 1.3	83.9 ± 2.8	tr	23.0 ± 0.8	51.9 ± 0.1	2.8 ± 0.0	32.2 ± 1.6
PSAGs	7.2 ± 0.9	12.1 ± 1.4	7.2 ± 0.9	6.1 ± 0.2	26.6 ± 0.5	7.2 ± 0.4	5.2 ± 0.1
NSAGs	0.9 ± 0.3	1.76 ± 0.1	5.7 ± 0.2	-	-	1.5 ± 0.1	-
A0-line group A saponin	-	-	-	-	2.5 ± 0.2	0.1 ± 0.0	0.4 ± 0.0
Total of SAGs	*97.2 ± 1.9*	*97.7 ± 4.4*	*12.9 ± 1.1*	*29.1 ± 1.0*	*81.0 ± 0.1*	*11.6 ± 0.4*	*37.8 ± 1.5*
DDMP	469.3 ± 4.6	410.4 ± 2.6	tr	175.2 ± 0.2	245.2 ± 0.8	42.8 ± 0.1	294.8 ± 0.4
Gr. B	44.4 ± 3.7	70.1 ± 1.9	239.2 ± 0.4	138.7 ± 0.0	319.9 ± 0.0	120.5 ± 0.1	437.4 ± 0.3
Gr. E	tr	-	-	-	2.2 ± 0.3	tr	0.8 ± 0.1
Total of DDMPs	*513.7 ± 0.6*	*480.5 ± 0.7*	*239.2 ± 0.4*	*314.0 ± 0.2*	*567.3 ± 1.1*	*163.3 ± 0.2*	*733.0 ± 0.9*
Total saponin contents	610.9 ± 2.5	578.2 ± 3.7	252.2 ± 1.5	343.1 ± 1.2	648.3 ± 1.0	174.8 ± 0.7	770.8 ± 2.4

Soyasaponin composition	Non-modified soymilk			Modified soymilk			
	12	**13**	**14**	**15**	**16**	**17**	**18**
FSAGs	4.4 ± 0.1	20.3 ± 0.1	-	0.3 ± 0.0	-	-	-
PSAGs	10.2 ± 0.4	6.1 ± 1.0	-	18.0 ± 1.1	2.3 ± 0.5	1.5 ± 0.1	3.4 ± 0.3
NSAGs	20.5 ± 0.0	3.5 ± 0.3	-	6.7 ± 0.9	0.9 ± 0.0	1.3 ± 0.0	-
A0-line group A saponin	-	-	31.2 ± 2.0	-	-	-	-
Total of SAGs	*35.2 ± 0.5*	*29.9 ± 1.3*	*31.2 ± 2.0*	*25.0 ± 0.2*	*3.2 ± 0.6*	*2.8 ± 0.1*	*3.4 ± 0.3*
DDMP	2.5 ± 0.1	24.4 ± 0.2	153.3 ± 0.6	110.9 ± 0.3	102.6 ± 0.7	15.9 ± 0.5	79.8 ± 0.4
Gr. B	489.3 ± 1.3	187.2 ± 0.6	198.2 ± 0.6	243.2 ± 0.6	212.6 ± 2.4	42.4 ± 0.7	98.6 ± 1.3
Gr. E	17.7 ± 0.8	0.6 ± 0.0	-	-	-	-	-
Total of DDMPs	*509.5 ± 2.0*	*212.2 ± 0.8*	*351.5 ± 1.2*	*354.1 ± 0.3*	*315.2 ± 3.1*	*58.3 ± 0.1*	*178.4 ± 1.7*
Total saponin contents	544.6 ± 1.5	242.1 ± 0.5	382.7 ± 3.1	379.1 ± 0.5	318.4 ± 3.7	61.1 ± 0.3	181.8 ± 1.9

Group of soy food sample could be divided to raw soy foods, thermal processed soy foods and fermented soy foods. The polymorphic soyasaponin composition was varied on food process as shown in Table II.4. Group of raw soy food samples included dry soybean seed, soybean sprout and raw young soybean. Thermal processed samples included boiled young soybean, cooked soybean, soymilk, tofu and yuba. Yuba is a protein-lipid film which forms during heating soymilk. Further information, soymilk samples included non-modified and modified soymilk samples. The non-modified soymilk samples are 100% soymilk which may be added some sugar and flavor. The modified (or adjusted) soymilk may be added acidity regulator, antioxidant, emulsifier or stabilizer and the additional allowance content will follow the Codex Alimentarius international food standard (FAO, 2017). Fermented soyfood samples natto, miso, douchi and tofuyo which varied on microorganism types and fermented conditions. Natto is a traditional Japanese food which fermented cooked soybean with *Bacillus subtilis* var. natto for 18-24 h. Miso is seasoning in paste form which produced by fermenting cooked soybean with *Aspergillus oryzae* then adding salt and some other ingredients may be added such rice or barley. The miso might be fermented for one week or several years. Douchi is a traditional Chinese food which is a fermented black soybean with *Aspergillus* spp. and *Lactobacillus* spp. for 3-4 days then washed and added other seasoning and maturing about 15 days.

2. SAGs composition relating undesirable taste characteristics in soybean and soyfood samples

Chemical structures and nomenclatures of soyasapogenol A glycosides (SAGs) and DDMPs are shown in Figure II.1. SAGs can be divided into three lines; Aa-line, Ab-line and A0-line with the sugar chain at the C-22 position as Xyl-Ara-, Glc-Ara-, and Ara-, respectively. The C-3 position sugar chains (No. 1 to No. 6 in Figure II.1(A)) attach to the R1 position. Degree of acetylation of the C-22 terminal sugar (R2) varied as shown in Figure II.1(B). For example, Aa-line components were composed of xylose residue which has three hydroxyl moieties in the molecule as triacetyl (fully acetylated)-, diacetyl-, monoacetyl-, and nullacetyl (deacetylated)-SAGs. On the other hand, the Ab-line contained glucose which has four hydroxyl moieties in the molecule as tetraacetyl (fully acetylated)-, triacetyl-, diacetyl-, monoacetyl-, and nullacetyl (deacetylated)- SAGs. Illustrations of the chemical structures of some isomers with fully-, partially-, and null-acetylated xylose or glucose residues attached to the arabinose residue at the C-22 position of Aa-line and Ab-line, respectively are shown in Figure II.1(B). Nomenclature of representative SAGs (fully acetylated Aa-, fully acetylated Ab- and A0-line) and DDMP saponins including their derivatives are shown in Figure II.1(C).

2.1 Nine soybean variety samples

The 88 SAGs compounds were targeted, and consisted of Aa-, Ab- and A0-line saponins as numbers 1 − 39, 40 − 81 and 82 − 88 in Table II.2, respectively. This soyasaponin identification confirmed their polymorphism as phenotypes of soyasaponin composition for soybean samples. Among nine soybean varieties, ‘GD-50326-2’, ‘GD-50029-2’, ‘Norin No. 3’ and ‘Shirosennari’ were Aa-line saponin soybeans while ‘Ibarakimame No. 7’, ‘Suzuyutaka’ and ‘Mikuriya-ao’ were Ab-line saponin soybeans. The A0-line saponin soybeans consisted of ‘Tohoku No.152’ and ‘Kinusayaka’. Figure II.5 shows UV chromatograms of the major soyasaponin components at 205 nm in seed hypocotyls and cotyledons of nine soybean varieties. Figure II.5(1A) belonged to ‘GD50326-2’, the first and second peak showed NSAGs and FSAGs, respectively as the major soyasaponin components often detected in soybean. The UV chromatograms showed a new detection of PSAGs (Figure II.5(2A) – (7A)). Monoacetyl and

diacetyl-gr.A saponin Aa were detected in hypocotyls of 'GD50029-2' soybean at retention time 35.24 and 36.81 min, respectively (Figure II.5(2A)). PSAGs were detected in minor amounts in cotyledons. Total PSAGs in hypocotyls and cotyledons ranged from 2.2 ± 0.4 to 19.8 ± 0.1 and 1.6 ± 0.0 to 44.0 ± 1.5 µmol/100 g dry samples, respectively (Table II.5).

Among the nine soybean varieties, 'Shirosennari' (*G. max*) gave the highest SAGs while 'Tohoku No.152' did not contain SAGs. 'Kinusayaka' contained only A0 saponin which does not present undesirable tastes. For FSAGs with undesirable tastes, G. max was composed of higher FSAGs than *G. soja*. FSAGs in G. max hypocotyls were higher than in cotyledons while FSAGs in *G. soja* hypocotyls were lower than in its cotyledons. The profiles of SAGs related directly to the taste characteristics of soybean based products.

FSAGs were reported as a major cause of undesirable taste characteristics including bitterness, astringency, roughness, dry mouth feel and green beany flavors. FSAGs were also reported as located only on seed hypocotyls (Shiraiwa et al., 1991b). Thus, seed hypocotyls were suggested for removal during soy food production, especially soymilk preparation, to reduce these undesirable characteristics (Asano et al., 1987). Shiraiwa et al. (1991b) reported that group A saponin Aa or Ab was detected only in seed hypocotyls and not located in other plant organs. However, this research found that LC-PDA/MS/MS analysis demonstrated the existence of group A saponin Ab and other FSAGs components in seed cotyledons at low concentrations. However, FSAGs present the strongest undesirable characteristics among other soy functional compounds even at low concentrations. Table II.4 shows FSAGs ranging from 0 – 97.0 µmol in 100 g dry soybean seeds (an average of 88.7 g of seed cotyledons). Average FSAG contents in the cotyledons of *G. soja* and *G. max* were 26.4 and 12.6 µmol/100 g dry seeds, respectively. FSAGs in seed hypocotyls of *G. soja* and *G. max* were 20.7 and 41.3 µmol/100 g dry seeds, respectively. The ratio of FSAGs in seed hypocotyls to cotyledons in *G. soja* and *G. max* were different. *G. soja* hypocotyls contained higher FSAGs than its cotyledons. On the other hand, FSAGs in *G. max* cotyledons were lower than in its seed hypocotyls by 2.5 to 10 times. This low concentration of FSAGs in seed cotyledons might affect the taste characteristics of soy food products. Okubo et al. reported that FSAGs at 0.01 µmol/L presented a high intensity of bitter taste (Okubo et al., 1992). Seed cotyledons might present a bitter taste in soy products even though the seed hypocotyls were removed.

To compare the bitterness in soy products, a model soymilk product was used to assess the relation between FSAG concentration and bitterness. For example, 90 g of seed cotyledons were used to prepare 500 mL of soymilk as a common ratio of production. On average, 90 g of seed cotyledons contain about 10 µmol of FSAGs (average FSAGs in G. max cotyledons was 12.6 µmol/100 g dry soybean seed) which decreases with degradation during soymilk production. At 50% recovery, FSAG content in soymilk would be 5 µmol/500 mL soymilk or 10 µmol/L. From this hypothesis, the soymilk would present a high intensity of bitter taste even if it was prepared from seed cotyledons. Therefore, removing seed hypocotyls cannot get rid of all the bitterness or undesirable characteristics from soybean based food products. Removing the seed hypocotyls from the soybean would also decrease the health functional compounds. Therefore, this method is not recommended for healthy soy food production.

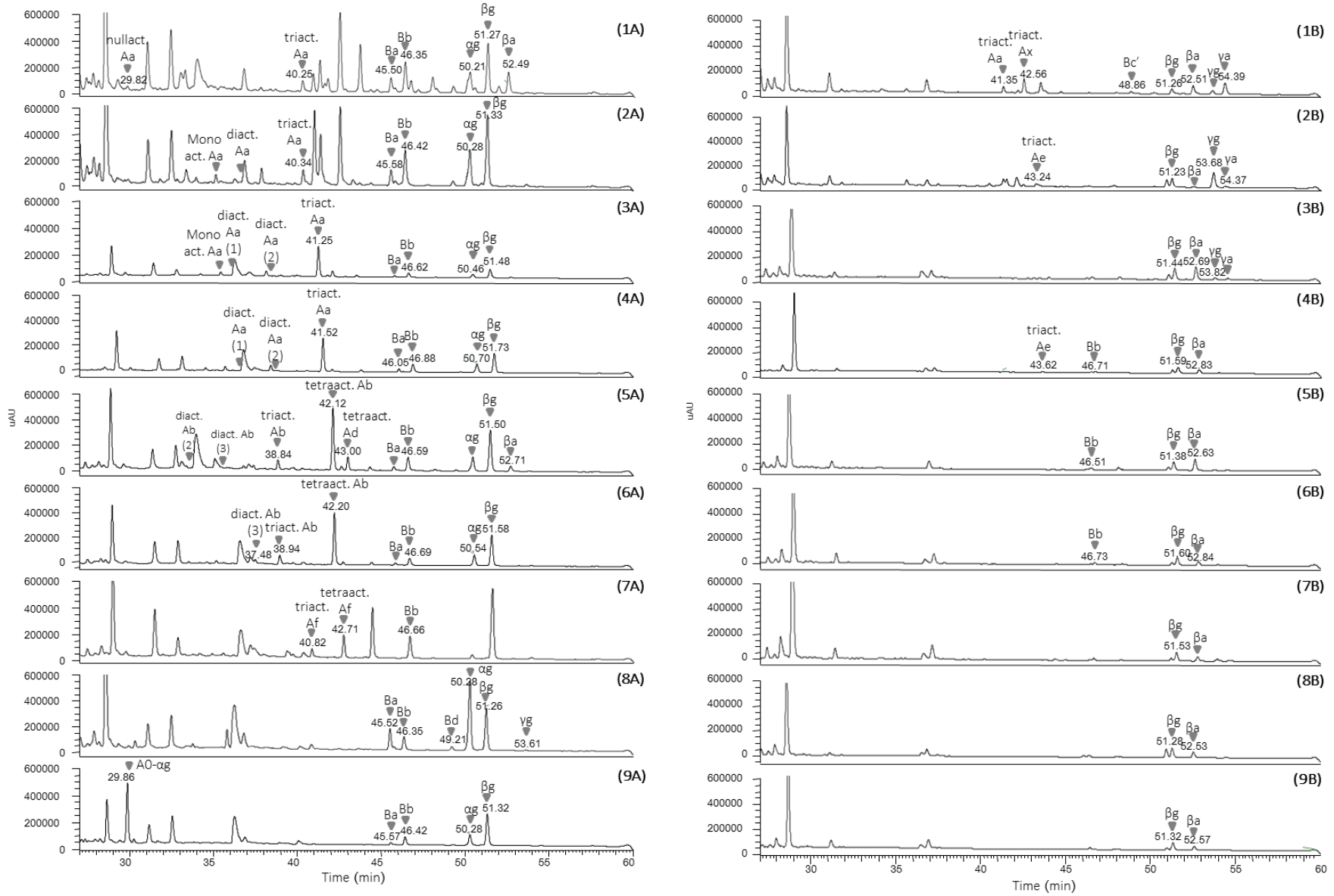


Figure II.5 : UV chromatograms (at 205 nm) of major saponin composition in seed hypocotyls (A) and cotyledons (B) of nine soybean varieties including GD50326-2 (1), GD50029-2 (2), Norin No.3 (3), Shirosennari (4), Ibarakimame No.7 (5), Susuyutaka (6), Mikuriya-ao (7), Tohoku No.152 (8), and Kinusayaka (9).

The dominant Sg-1a and Sg-1b genes controlled the producing groups Aa-line and Ab-line saponin of common soybean, respectively. On the other hand, 'Tohoku No. 152' is a genetically modified soybean which lacks the genotypes to produce group A saponin. 'Kinusayaka' is a genetically modified A0-series saponin phenotype soybean which produces only A0 groups as A0-αg, A0-βg, A0-γg, A0-αa, A0-βa and A0-γa. Total saponin A0 group of 'Kinusayaka' was detected at 68.8 ± 0.4 μmol/100 g dry seeds from 143.5 ± 0.9 μmol of total saponin compounds. SAGs were not detected among the nine soybean varieties in 'Tohoku No. 152'. 'Kinusayaka' seeds contained A0-line saponins in both hypocotyls and cotyledons and FSAGs, PSAGs, or null acetylated-SAGs were not detected. 'Tohoku No. 152' carries the sg-5 recessive trait (Yano et al., 2017) which cannot produce soyasapogenol A, and 'Kinusayaka' has the sg-10 recessive trait which cannot attach acetylxylose nor acetylglucose at the terminal second position of the C-22 sugar chain. These two varieties were developed to improve the undesirable taste characteristics of soy foods by introducing a recessive trait, thereby eliminating acetylated-SAGs from soybean seeds. Since saponin A0-line presents non-undesirable tastes, 'Kinusayaka' does not present these tastes. However, some authors reported antioxidative effects of gr.A saponin higher than gr.B saponin (Nishida et al., 1993). Thus, elimination of gr.A saponin causing undesirable taste will also reduce the bioactive activity of those soybeans. Moreover, genetic modification to remove undesirable soyasaponins (SAGs) reduces the composition of the DDMP groups. However, despite the low DDMP content, health functionalities of these soybeans are low. Genetically modified soybean products have good taste characteristics but low health advantages.

Among all soyasaponin compositions, partially acetylated SAGs (PSAGs) were newly detected in this research. These minor components have never been previously reported. This might be because HPLC, which required standard reagent, was normally used to identify soyasaponin components while PSAGs were not extracted as purified components and used as standard. On the other hand, the LC-MS/MS technique can specify total complexity of soyasaponin compounds. The PSAGs were identified by MS and MS/MS analysis and quantified by UV absorbance at 205 nm. However, some soyasaponin components showed a single UV peak, some showed shoulder peaks, and some did not show peaks by UV 205 nm monitoring but were detected by MS screening. Only single and shoulder peaks were quantified and evaluated. Some soyasaponins could be identified by MS but not detected by UV, because of very low amount and these are shown as trace in Table II.3. Results revealed the existence of PSAG in both hypocotyls and cotyledons at 0 – 41.0% of total SAGs. The PSAG percentages of G. soja and G. max were similar, thus PSAG content did not depend on soybean varieties. Nevertheless, PSAG percentage of cotyledons was higher than hypocotyls. PSAGs in hypocotyls ranged from 0.0 – 20.9 in 100 μmol of total SAGs, while cotyledons ranged from 0 – 47.6. PSAG contents ranged from 0 – 51.2 μmol in 100 g dry soybean seeds. FSAGs were reported as the major cause of undesirable taste characteristics, while null-acetylated SAGs did not adversely affect the taste (Okubo et al., 1992; Yoshiki, Kudou, & Okubo, 1998), while PSAGs were not mentioned. The degree of acetylation of C-22 terminal sugar might also impact on the intensity of undesirable taste characteristics; consequently, PSAGs might present these tastes. However, no information existed concerning the degree of bitterness, astringency and taste characteristics of partially acetylated SAGs in soy food. The PSAG content in cotyledons could present undesirable taste characteristics in soybean products. Undesirable taste also depends on the aglycone structure and soyasapogenol A is bitterer than soyasapogenol B and E. In this research, a certain amount of partially acetylated SAGs were detected in soybean cotyledons.

PSAGs may be responsible for the bitterness and astringency of soy foods, especially the taste characteristics of soymilk.

Soyfood samples

The variation of soyfood product presented polymorphic soyasaponin compositions. FSAGs were detected as major soyasaponin in both raw and thermal processed samples which averaged 125.7 and 32.1 µmol/100g DB, respectively. On the other hand, major SAGs in fermented sample was PSAGs which averaged 49.7 µmol/100g DB. NSAGs were highest detected in fermented samples then thermal process and raw samples. The average NSAGs in fermented, thermal processed and raw soyfoods was 14.3, 3.47 and 1.7 µmol/100g DB. Moreover, thermal processed soyfood samples; boiled young soybean and non-modified soymilk samples contained FSGAs as majority with 19.0 – 83.9 µmol/100g DB. But modified soymilk composed very low content of FSAGs while PSAGs and NSAGs content ranged 1.1 – 18.0 and 0 – 0.67 µmol/100g DB, respectively. The average of total SAGs saponin content of boiled young soybean, cooked soybean, non-modified soymilk, modified soymilk, tofu and yuba was 63.0, 36.5, 9.14, 82.6 and 118.9 µmol/100g DB, respectively. For fermented soyfood samples, natto sample mainly composed FSGAs and PSAGs while douchi and tofuyo composed PSAGs as major SAGs. The long-term fermented soyfood as miso mainly composed NSAGs. The average of total SAGs saponins of natto, miso, douchi and tofuyo was 87.48, 29.73, 92.3 and 128.3 µmol/100g DB, respectively.

Among three types of soyfoods samples, raw sample contained highest total SAGs and FSAGs which could be predicted that they might presented most undesirable taste. Fermented soyfood samples contained higher total SAGs but most SAGs were PSAGs while thermal processed samples mainly composed FSAGs. Thus, the undesirable taste might be degraded by thermal process and fermentation. The degradation of SAGs could be explained by the ration of FSAGs and De-SAGs which this ratio of raw, thermal processed and fermented samples was 10.6, 2.3 and 0.3.

According to dominant producing saponin genes, SAGs could be classified to Aa-line, Ab-line and A0-line (Takada et al., 2012) which Aa-line and Ab-line saponins are detected in common soybean. Three dry soybean samples; 'Nanbu', 'CM60' and 'SK3' were Ab-line saponin soybean as stated by the detected group A saponin Ab (in Table II.3). Even though group A saponin A0 were detected in 'CM60' and 'SK3' which normally not occur in soybean seed. However, saponin Aa and Ab is produced though A0 in the biosynthetic pathway of soyasaponin production which A0 is a precursor of saponin Aa and Ab. Therefore, saponin Aa-line and Ab-line soybean can produce group A saponin A0 which due to cultivating conditions or during storage at high temperature or humidity. Thus saponin A0-αg, A0-βg and A0-γg could be detected in Thai soybean sample such 'CM60' and 'SK3'.

Between bioactive compounds in soybean, SAGs were reported as a major cause of undesirable taste characteristics including bitterness, astringency, and green beany flavors. To eliminate the undesirable taste characteristics of SAGs, A0-line soybean breed was developed which lacked the genotypes to produce gr.A saponin such as 'Kinusayaka' soybean (Kikuchi, Tsukamoto, Tabuchi, Adachi, & Okubo, 1999; Takahashi, Li, Tsukamoto, & Wang, 2016). Thus, some soyfood product use 'Kinusayaka' as raw material especially soymilk such sample no. 14 and 21. These 'Kinusayaka' soymilk samples composed only group A saponin A0, so these samples would not present undesirable taste from FSAGs. Beside the saponin genetic of soybean, chemical structure of SAGs also plays an important role on taste characteristics. FSAGs present high intensity of bitter taste while NSAGs do not present bitterness (Okubo et

al., 1992). However, PSAGs were not mentioned about their taste characteristics but they might present some undesirable tastes. Moreover, the minor compounds as PSAGs in soyfood samples have never been previously reported. They were newly detected and reported in this research.

According the polymorphic soyasaponin contents in soyfoods, raw soyfoods contained highest amount of FSAGs as shown in Table II.4 then followed by thermal processed and fermented samples, respectively. The average of total FSAGs of soyfoods was 125.7 µmol/100 g DB (sample no. 1 and 2 were summed as one sample). PSAGs and NSAGs content in raw soyfoods averaged 10.18 and 1.67 µmol/100 g DB. The ratio of FSAGs and deacetylated SAGs (PSAGs and NSAGs) content was 10.6 which FSAGs were highly consisted of because they were not degraded by any food process. For thermal processed samples, the ratio of FSAGs and deacetylated SAGs content was 2.28 which quite lower than fresh samples. Furthermore, the ratio of FSAGs and deacetylated SAGs content of fermented soyfoods only was 0.34. These ratio data of each soyfood group could reflect the degradation of SAGs and relate to taste characteristic. The degradation might be caused by thermal process and/or enzymatic reaction due to microorganism activities. For an example, the FSAGs of young soybean (sample no. 3) was reduced to 23% after boiling. In addition, the FSAGs almost was not detected in fermented food samples especially long-term fermented products such miso, douchi and tofuyo. On the other side, NSAGs almost not detected in raw samples and thermal processed samples. The degradative effect during fermentation in soyfood on soyasaponin degradation was agreeable with other research (Kim et al., 2014). Again, they did not classify and mention about the minor compounds of soyasaponin.

For soymilk samples, they could be classified to non-modified soymilk and modified soymilk. The non-modified soymilk is 100% soymilk without other ingredient adding (except sugar and flavors) whilst modified (or adjusted) soymilk may be added some agent such as emulsifier and/or stabilizing agent. An objective of modification in soymilk is to improve and stable soymilk viscosity by using emulsification which might affect taste characteristic. Beside soymilk viscosity, taste characteristics of soymilk is the most concerned issue. According FSAGs is a major cause of undesirable taste consequently eliminate FSAGs is an important point. Genetically modified technology was used to improve soybean taste characteristics such as ‘Kinusayaka’ and ‘L-Star’ due to their gr.A saponin and LOXs deficient producing, respectively (Chitisankul et al., 2015; Yumoto et al., 2007). Moreover, the hypocotyls of soybeans commonly are removed during soymilk preparation to reduce undesirable taste (Asano et al., 1987). Because FSAGs mainly locates in hypocotyl part of soybean seed (Chitisankul et al., 2018) similar to sample no. 1, fresh hypocotyl of soybean sprout which contained FSAGs higher than its cotyledon (sample no. 2). However, PSAGs and NSAGs were detected in fresh soybean sprout almost 13% (including hypocotyl and cotyledon sample) of total SAGs which normally not much detected in fresh sample. The detection of degradative SAGs might be explained by the enzymatic reaction during the soybean germination as sprout (Chitisankul, Murakami, Tsukamoto, & Shimada, 2019). Nevertheless, the SAGs in thermal processed sampled could be degraded as de-attachment of C-22 terminal sugar. Furthermore, the fermentation process of natto, miso etc. include both heat treatment and fermentation. Hence several treatments in fermented samples, FSAGs were degraded to PSAGs and NSAGs.

3. DDMPs relating health beneficial properties

3.1 Nine soybean variety samples

The DDMPs included six different chemical structures of gr.B and gr.E saponin as DDMP-αg, DDMP-βg, DDMP-γg, DDMP-αa, DDMP-βa and DDMP-γa. DDMP-βg is commonly detected in soybean (Kudou et al., 1993; Shiraiwa, Harada, & Okubo, 1991). Total DDMP saponin content ranged from 74.7 ± 0.6 to 301.6 ± 1.5 μmol/100 g dry sample (Tables 3 and 4). Gr.B saponin was composed of Ba, Bb, Bb', Bx, Bc and Bc', with Bb mainly detected in both hypocotyls and cotyledons. Bd, Be and gr.E saponin were detected in small amounts ranging from 0.2 – 28 μmol/100 g dry sample (Tables 3 and 4). Bd saponins were only detected in the hypocotyls of soybean. Total DDMPs, including gr.B and gr.E saponin were mainly detected in the cotyledons of soybean, while SAGs were more prevalent in the hypocotyls. Among the nine soybean varieties, total DDMPs were 87.7 ± 0.2 to 301.6 ± 1.5 μmol/100 g dry sample. *G. soja* contained a higher amount of DDMPs than *G. max*. For total soyasaponin compositions, *G. soja* or wild soybean consisted of 363.5 and 388.9 μmol/100 g dry sample for 'GD-50326-2' and 'GD-50029-2', respectively. 'Kinusayaka' (*G. max*) had the lowest total soyasaponin content at 143.5 ± 0.9 μmol/100 g dry sample.

DDMPs composed of DDMP saponin and its derivatives, group B and E saponins, were reported to possess beneficial health functionalities. The DDMP content of *G. soja* was higher than *G. max*. Average DDMP compositions of *G. soja* and *G. max* were 270.1 and 115.9 μmol in 100 g dry soybean seeds, respectively. On the other hand, the DDMP content of seed cotyledons was higher than hypocotyls by 2 to 12 times. Hypocotyls and cotyledons comprised 9.2 – 81.5 and 76.0 – 220.1 μmol DDMPs in 100 g dry soybean seeds, respectively. Results revealed that wild soybean (*G. soja*) contained higher health functionalities than commercial soybean (*G. max*). Thus, consumption of wild soybean is healthier than commercial soybean. However, wild soybean is composed of high FSAGs which cause undesirable bitter taste. Because of this adverse effect of FSAGs on the taste characteristics of soy food products and the health promoting benefits of DDMP saponins, genetic modification by breeding was developed to control soyasaponin composition and content in soybean seeds. 'Tohoku No. 152' soybean possesses no SAGs components as a result of introducing the recessive gene *sg-5*, and 'Kinusayaka' soybean does not contain FSAGs and PSAGs as a result of introducing the recessive gene *sg-1*^{0}. Thus, the undesirable tastes due to fully and partially acetylated SAGs are eliminated (Tables 3 and 4). The DDMP contents of 'Tohoku No.152' and 'Kinusayaka' were 74.7 and 154.8 μmol in 100 g dry soybean seeds, respectively. Although genetic modification through soybean breeding can improve food characteristics by eliminating undesirable soyasaponins, food processing has an important effect on the quality of soy food products such as taste characteristics and health functionalities.

3.2 Soyfood samples

The DDMP-saponin (DDMPs) component includes DDMP, gr.B and gr.E saponins. Gr.B was interested as nutraceutical properties as above mentions. Their ratio of DDMP and its derivative compounds varied on type of food products which related to their degradation by food process. DDMP were mainly detected in raw soyfood; dry soybean seeds, soybean sprout and raw young soybean whilst their derivative compounds were detected very low amount. DDMP and gr.B saponin in raw soyfood samples averaged 481.3 and 53.8 μmol/100g DB, respectively. The ratio of DDMP and gr.B saponin of raw soyfood samples was 8.9. On the other hand, DDMP saponin content of processed soyfoods, thermal and fermented process were lower than raw soyfood samples. DDMP and gr.B saponin of thermal processed soyfoods

averaged 112.8 and 228.4 µmol/100g DB while these contents of fermented soyfood averaged 10.5 and 532.1 µmol/100g DB. Gr.B was the major soyasaponin components in processed food samples instead of DDMP especially fermented food. The ratio of DDMP and group B saponin of thermal processed and fermented soyfoods was 0.5 and 0. About 98% of DDMPs in fermented food was gr.B saponin which was interested of nutraceutical soyasaponin.

Between all soyfood samples, gr.E saponin was not detected in raw soyfoods but it was detected in processed soyfood samples, both thermal processed and fermented samples. Gr.E saponin in thermal processed and fermented samples averaged 2.6 and 1.0 µmol/100g DB, respectively which was much lower than gr.B saponin.

The health beneficial properties of gr.B saponins were severally reported such anti-hyperlipidemic and anti-cancer properties, furthermore, better absorbed than other soyasaponins. By the way, nutraceutical effect of gr.E saponin was not reported yet. Therefore, gr.B saponin content is considered as health promoting component in soyfood products. The degradation of DDMP could be activated by thermal and enzymatic treatment. Previous research revealed that heat would degrade DDMP to gr.B saponin whilst enzymatic reaction might degrade it to both gr.B and gr.E saponin (Chitisankul et al., 2015).

Between soyasaponin content, DDMPs were major soyasaponin in soy samples. Food processing plays an important role on DDMP degradation and conversion DDMP into gr.B saponin. Thus non processed samples, raw samples composed highest amount DDMP. According free radical scavenging properties of DDMP (Yoshiki & Okubo, 1995), raw soy samples might express high antioxidative capacity but they would be decreased during food processed treatment. The thermal processed samples contained less DDMP contents especially soymilk products. Soymilk preparing process included soaking, extracting, heating and/or homogenizing method, thus DDMP could be degraded during these processes. The cooking effect on soyasaponin components was revealed that pressure cooking could reduce saponin content in pigeon pea about 28 – 38% and 22 – 27% for soaking method (Duhan, Khetarpaul, & Bishnoi, 2001). The degradation of saponin component was confirmed that the isothermal condition (80 – 130 °C) strongly affected to reduce saponin component (Tarade, Rekha, Jayram, & Pandit, 2006). The different degradation of soyasaponin related to the chemical structure changing during processing such thermal and pH conditions (Rickert, Meyer, Hu, & Murphy, 2004). As some soymilk preparation method, soybeans would be extracted by room temperature water then pass-through thermal process, as stated by this method enzymatic reaction would occur then degrade and change chemical structure of soyasaponins. The unstable structure of saponin compound would be easily to degrade by heating. Lipoxygenase is one of most effective enzymes that can degrade saponin component and change the chemical structure (Chitisankul et al., 2015; Shimada et al., 2008). Moreover, the lipoxygenase reaction also interrupts maltol production during thermal degradation of DDMPs (Chitisankul et al., 2015) which would result in reducing natural sweetness in soymilk. Thus 'L-star' was developed to eliminate this lipoxygenase action.

Among all samples, fermented soyfood product presented interesting results due to health beneficial effects. The soyasaponin components in fermented samples were degraded to different chemical structure and remained in higher soyasaponin contents whereas soyasaponins in other samples were depleting. Natto samples contained highest amount of gr.B saponin which averaged 878.3 µmol/100g DB following by miso, tofuyo and douchi respectively. Even though, raw samples contained highest amount of DDMP but less gr.B saponin which was better absorbed than DDMP (Kamo et al., 2014). Thus gr.B saponins were reported as health beneficial soyasaponins, so natto may be promoted as healthy foods. These

polymorphic soyasaponin component may results from the variant of raw materials and processes especially microorganism activities. Natto is produced by the fermentation of bacteria, *Bacillus* spp. (Toshiro, 2012); miso is produced by combination of fungi which including mold, *Aspergillus* spp. and yeast, *Zygosaccharomyces* spp. (Yamabe, Hattori, Kaneko, Endo, & Takita, 2007); douchi is produced by *lactobacillus* spp. (Liu et al., 2012) and tofuyo is produced by fungi or yeast in salt-brine or rice-wine mixture (Yasuda, 2011). Because of high content of health functional soyasaponin in these fermented products particularly natto might be recommended to daily intake for preventing chronic diseases.

During soyfood processing, many factors degrade soyasaponin components and change their chemical structure (Chitisankul et al., 2015) which directly impacts on taste, quality, and health functionalities. Soyasaponin components are degraded by thermal processes and enzymatic reactions such as heat treatment and fermentation. Thus, quality improvement of soy food could be controlled by food processing methods as well as genetic modification.

Conclusion

The nine soybean varieties with different soyasaponin phenotypes were analyzed for soyasaponin composition using LC-PDA-MS/MS. Two categories of soyasaponins were identified as group A saponin and DDMP saponin, detected at about 75 and 13 components, respectively. Complexities of soyasaponin composition depend on hypocotyl and cotyledon seed organs and soybean varieties. Accordingly, FSAGs are an important factor regarding undesirable taste characteristics of soybean based foods. Genetic modification was used to eliminate gr.A saponin producing genes, with seed hypocotyls removed to discard FSAGs. However, PSAGs have never previously been mentioned and were newly detected in this research. The PSAGs may still present some undesirable tastes though not as strong as FSAGs. Moreover, SAGs were also detected in seed cotyledons in small amounts. Among the nine different soyasaponin phenotypes of soybean varieties analyzed, 'Tohoku No.152' did not contain any SAGs while 'Kinusayaka' contained only A0-line SAGs. Although soybean quality can be genetically improved by technology, soyasaponin composition and content are reduced by degradation during food processing.

FSAGs was mainly detected in raw samples then lower in processed samples particularly fermented process. As stated by variation of SAGs in each samples, raw samples composed highest undesirable taste soyasaponin content following by thermal processed and fermented samples. In addition, the food process could affect nutraceutical properties in soyfood products with conversion of DDMP into gr.B saponin, the health functional soyasaponin. And fermented food as natto composed highest amount of gr.B saponin composition among all soyfoods. The understanding of soyasapogenol compositions in soybean is concerned since choosing raw material and process for health functional soyfood research and development. Hence food process as thermal treatment and fermentation could derive FSAGs into partial- or de-acetylated SAGs, and degrade DDMP into active gr.B saponin. Those effects could decrease undesirable taste and promote nutraceutical effects in target products which might meet the need of consumers.

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CHAPTER III

The effects of soymilk preparation on DDMP saponin degradation and maltol production

Introduction

Soymilk is a common plant-based milk which known as nutritious alternative milk due to the complete amino acid and its active compound. There are many good reasons of soymilk intake such as low cost protein source, good for lactose intolerant consumer, high protein digestibility and free from cholesterol. Soymilk can be prepared by several methods which are traditional method, defatted soy meal method, soy protein isolated or concentrate method, whole bean method, and extruder method. The traditional method is generally used to prepare soymilk for both home scale production and commercial production. There are two different methods of the traditional soymilk preparations which are hot extraction (extracts the soymilk after cooking), and cool extraction (extracts the soymilk before cooking) (Shurtleff and Aoyagi, 1979). The different soymilk preparation can affect to taste characteristic and health beneficial promotes.

Among changing soy active compounds, soyasaponins also are altered to different chemical structure which directly affect to soymilk flavor and taste. The degradation of soyasaponin was reported that it could be degraded by many factors such as heat treatments, pH changes, or enzymatic reactions (Heng et al., 2006; Serventi et al., 2013). DDMPs degrade into group B and E saponins, and maltol by heat treatment (Kudou et al., 1992; Kudou et al., 1993). And these reactions would affect the taste and health beneficial characteristics of soybean based foods. Higher amount of maltol would express preferred sweetness (Zhang, Li, Lo, & Guo, 2010) and enrich the quality of soy foods. DDMPs are reported to show antimutagenic activities (Berhow, Wagner, Vaughn, & Plewa, 2000) and radical scavenging activities (Yoshiki & Okubo, 1995). Whereas group B saponins may decrease and prevent colon cancer (Ellington, Berhow, & Singletary, 2005; Ellington, Berhow, & Singletary, 2006; Tsai, Chen, Chien, Huang, & Lin, 2010) and may be effective against Alzheimer's disease (Hong et al., 2014). Thus, the different chemical structures of soyasaponins contribute to different functionality for human health.

The previous chapter reported that soyasaponin composition is altered by the various process production. This chapter, the effect of each soymilk process preparation on soyasaponin and taste characteristic were investigated. The experiments were performed by practical soymilk preparation and model radical reaction system. In the practical soymilk preparation, a common soybean variety and a special variety which genetically eliminated three lipoxygenase isozymes (LOXs) in soybean seeds were used. In the radical reaction models, purified DDMP-saponin βg, AAPH [2,2'-azobis(2-amidinopropane) dihydrochloride] and DPPH (2,2-diphenyl-1-picrylhydrazyl) were applied to simulate LOXs-mediated DDMPs degradation.

Objective

- To investigate the effect of soymilk preparation on the DDMPs saponin degradation including the preparation of group B, group E saponin which relating health promoting properties of soy products.
- To investigate the effect of soymilk preparation on taste characteristic due to maltol production.

Material and method

1. Samples and reagents

Two soybean varieties were used; a common soybean *Glycine max* (L.) Merr. cv. Fukuyutaka (305 g/1000 seeds) and a genetically modified LOXs-deficient soybean *Glycine max* (L.) Merr. cv. L-Star (289 g/1000 seeds). 'L-Star' is a progeny of the cross between var. Murayutaka which was derived from 'Fukuyutaka', and a 'Fukuyutaka BC2' line which is three LOXs-deficient (Takahashi et al., 2003). Although 'Fukuyutaka' and 'L-Star' have some genetic differences other than LOXs, they are genetically closely related.

All soybeans were provided by the National Institute of Crop Science, National Agriculture and Food Research Organization (NARO), Tsukuba, Japan. Nordihydroguaiaretic acid (NDGA), 2,2'-azobis (2-amidinopropane) dihydrochloride (AAPH), and 2,2-diphenyl-1-picrylhydrazyl (DPPH) were purchased from Sigma-Aldrich, Japan.

2. Crude soyasaponin extracts from soybean seeds

Soybean seeds were crushed by a hammer in cotton bag then ground with a mill (HL-2053, PHILIPS) for 20 sec for 3 times. Soybean powders (about 0.2 g) were extracted with 19-fold volume of 800 mL/L absolute methanol at room temperature for 1 h with stirring every 10 min. The samples were centrifuged at 1,200 x*g* (Kubota 5310, Japan) for 15 min to obtain crude soyasaponin extract. After filtering the supernatant through a 0.45 µm membrane filter, it was directly used for the analysis.

3. Raw soymilk and soymilk preparation, and crude soyasaponin and maltol extraction

3.1 Raw soymilk preparation

About 10 g of soybean seeds were soaked in 5-fold volume of distilled water for 18 h at 20 °C. They were ground to slurry in a homogenizer (Homogenizer ED-5, Nihonseiki Kaisha LTD., Japan) for 2 min in the uncontrolled atmosphere or under nitrogen gas, and then raw soymilk was filtered through muslin cloth.

3.2 Soymilk preparation

Raw soymilks from 'Fukuyutaka' and 'L-Star' prepared by the aforementioned raw soymilk preparation process were heated in a screw cap test tube for 10, 20, 40 and 60 min in boiling water.

3.3 Crude soyasaponin and maltol extraction

2.0 g of raw soymilk or soymilk was mixed with 2.0 mL of methanol then stirred for 1 h. After centrifugation at 1,200 x g (Kubota 5310, Japan) for 15 min, the supernatant (crude soyasaponin and maltol extract) was filtered through a 0.45 µm membrane filter. The crude extracts were directly used for the analysis.

4. NDGA and APPH treatment in raw soymilk preparation

4.1 NDGA treatment

'Fukuyutaka' raw soymilk was used in this treatment. NDGA ethanol solution (100 mmol/L) was added to the 18 h soaked soybean just before grinding at different final concentrations (0.4, 1.0 and 2.0 mmol/L). Raw soymilk and crude soyasaponin extracts were prepared by the aforementioned normal procedure.

4.2 AAPH treatment

'L-Star' soybean was used to prepare raw soymilk. AAPH aqueous solution (500 mmol/L) was added to the 18 h soaked soybean just before grinding at different final

concentrations (2.5, 5.0, 7.5 and 10.0 mmol/L). Raw soymilk and crude soyasaponin extracts were prepared by the aforementioned normal procedure.

5. Soyasaponin purification

DDMP-saponin βg was purified from soybean seed hypocotyls, it was conducted with a method described by Kudou et al. (1992) with modification. A sample of soybean hypocotyls (30 g) was ground to a fine powder (about 40 μm) and extracted with 300 mL of 800 mL/L aqueous methanol for 1 h at room temperature. The solution was filtered through paper filter (Toyo Roshi Ltd., Tokyo, Japan). The filtrate was evaporated under vacuum at 35 °C, and the concentrates were dissolved in 30 mL of water, and then 30 mL of 2-butanol was added to the solution. The 2-butanol layer was collected and evaporated. The concentrates were dissolved in 10 mL of methanol containing 0.1 g/L ethylenediaminetetraacetic acid disodium salt (EDTA-2Na) and centrifuged at 1,200 x g for 15 min. The supernatant was loaded onto an ODS column (Imtakt Unison US C-18, 20x250 mm i.d., Imtakt Corp., Kyoto, Japan) using acetonitrile: 2-propanol: water: acetic acid (400: 80: 519: 1) which contained 0.1 g/L EDTA-2Na as mobile phase. The solvent flow rate was 9 mL/min. The crude fraction containing DDMP-saponin βg was evaporated and freeze dried. The crude DDMP-saponin βg fraction dissolved in 10 mL of methanol was loaded onto the ODS column in the same manner and recovered. The purified DDMP-saponin βg was stored at -20 °C before using. The purified DDMP-saponin βg was evaluated by LC-MS/MS with the same manner to analyze soyasaponin composition and contents.

6. Thermal and radical treatments of purified DDMP-saponin βg

6.1 Thermal treatment of DDMP-saponin βg

Purified DDMP-saponin βg was dissolved in water (246 mg/L; 0.23 mmol/L solution) and heated at 65 °C. Soyasaponin composition and maltol production were investigated at 0, 50, 100, 150, and 200 min heating. They were centrifuged at 19,000 x*g* for 5 min then supernatant was collected for analysis.

6.2 Radical treatment of purified DDMP-saponin βg

The purified DDMP-saponin βg was treated by two different treatments as following:

6.2.1 AAPH radical treatment

Purified DDMP-saponin βg was dissolved in methanol (0.47 mmol/L solution) as saponin solution. AAPH was dissolved in water to obtain different concentration of AAPH solutions; 50, 100, and 200 mmol/L. The same volumes of the soyasaponin solution and AAPH solution (1:1) were mixed (total 50 μL) to make a reaction mixture of saponin βg (0.24 mmol/L) and a final concentration of AAPH solution as 25, 50, and 100 mmol/L. The reaction mixtures were kept at 37 ºC for 120 min then directly used for analysis.

6.2.2 DPPH radical treatment

Purified DDMP-saponin βg was dissolved in ethanol (1.41 mmol/L solution) as soyasaponin solution. DPPH was dissolved in ethanol to obtain a 0.25 mmol/L DPPH solution. The soyasaponin solution and DPPH solution (ratio 1:2) was mixed to make a reaction mixture (total 60 μL) of saponin βg 0.47 mmol/L and DPPH 0.17 mmol/L. The reaction mixtures were kept in dark at 25 °C for 30 min then directly used for analysis.

7. Analysis of soyasaponin composition and contents

Soyasaponins were detected and analyzed by high-performance liquid chromatography with a photodiode array detector and a tandem mass spectrometer (LC-PDA/MS/MS, Agilent HP1200 series, Agilent Technologies, Santa Clara, CA; HCTultra, Bruker Daltonics Inc. Bremen, Germany). Elution was performed with an ODS column (TSK-gel ODS-100V, 150 mm x 2.0 mm i.d., Tosoh Corp, Tokyo, Japan). The solvents were: A) water and formic acid (1000:1, mL/mL); and B) acetonitrile and formic acid (1000:1, mL/mL). A linear gradient was performed from 5 to 100% solvent B in 0-30 min, and washed the column with 100% solvent B in 30-35 min, and reconditioning with 5% solvent B in 35-50 min. In the case of DPPH radical treatment, the linear gradient condition was changed from 5-85% solvent B in 0-30 min. The solvent flow rate was 0.15 mL/min and 10 µL of samples were injected. To acquire MS and MS/MS data, sample ionization was performed with the positive ion mode of the electrospray ionization (ESI) method; Capillary voltage 4.0kV, Capillary exit voltage 121V, Dry temperature 250 °C, Nitrogen dry gas 10.0 L/min, Nitrogen nebulizer pressure 50.0 psi, and Full scan mass spectra 50-1700 m/z. Purified saponin Bb was used as a standard, and the molecular absorbance coefficient of saponin Bb at 205 nm in methanol (ε= 5,278) (Hu, Lee, Hendrich, & Murphy, 2002) was used to calculate the content of independent soyasaponin components.

8. Analysis of maltol

Maltol was detected and analyzed by high-performance liquid chromatography with a photodiode array detector and a tandem mass spectrometer (LC-PDA/MS/MS) similar to soyasaponin components. Maltol was identified by the molecular ions ($[M+H]^+$=127) and comparison with retention time of maltol standard and quantified by a standard curve of a maximum UV absorption at 275 nm.

9. Statistical analysis

Analysis of variance (ANOVA) of the experimental data was performed and least significant difference test was used to evaluate by Tukey method at 95% confidence interval. The samples were repeat analyzed for three times.

Results and discussion

1. Soyasaponin composition and maltol production in soymilk preparation

1.1 Degradation of DDMP-saponins during raw soymilk preparation

Two different soybean varieties were used in this research; a common soybean var. 'Fukuyutaka' and a soybean var. 'L-Star' which genetically lacks the ability to produce three lipoxygenase isozymes (LOXs). There is no significant difference between 'L-Star' and 'Fukuyutaka' with respect to soyasaponin compositions and contents; similar amounts of DDMPs as major compounds and small amount of group B saponins were detected in both soybeans as shown in Table III.1.

During raw soymilk preparation after 2 min grinding, DDMPs were not detected and 33.1 µmol of group B saponins were detected in 'Fukuyutaka' raw soymilk while 38.9 µmol of DDMPs remained in 'L-Star' raw soymilk (Table III.1). From this result, we assumed that LOXs radical reactions in 'Fukuyutaka' seeds degraded DDMPs during grinding. Oxygen might have activated LOXs activities, so that raw soymilk was prepared by grinding under nitrogen gas condition to reduce oxygen supply. However, 2 min grinding under nitrogen gas condition did not prevent DDMPs degradation in Fukuyataka raw soymilk, and 33.9 µmol/100 g raw soymilk

of group B saponins were also detected. Next, the grinding duration was reduced to 30 sec. DDMPs remained 7.8 μmol/100g raw soymilk but largely degraded into group B saponins. The grinding water might have contained oxygen so that nitrogen gas treatment was not sufficient to prevent DDMPs degradation by LOXs reactions even in a shorter grinding period.

Kudou et al. reported that maltol is produced from degradation of DDMPs through thermal treatment (Kudou et al., 1992; Kudou et al., 1993). DDMPs in 'Fukuyutaka' raw soymilk samples were degraded completely, and maltol could not be generated as shown in Table III.1. Therefore, we assumed that LOX has a strong affect on the degradation of DDMPs during grinding process.

Table III.1: Soyasaponin and maltol contents of var. Fukuyataka and L-Star soybean seeds; and their raw soymilks prepared by grinding conditions

Sample	Grinding Condition	Content (μmol/100g sample)			
		DDMPs	Group B	Total saponin	Maltol
Fukuyutaka SB		280.8 ± 8.4 [a]	5.6 ± 0.2 [a]	286.4 ± 8.5 [a]	-
L-Star SB		284.8 ± 5.8 [a]	5.8 ± 0.5 [a]	290.6 ± 6.3 [a]	-
Fukuyutaka RSM	2 min	nd	33.1 ± 0.6 [a]	33.1 ± 0.6 [b]	nd
L-Star RSM	2 min	38.9 ± 0.8 [b]	6.3 ± 0.1 [c]	45.2 ± 0.7 [a]	0.5
Fukuyutaka RSM	2 min under N_2 gas	nd	33.9 ± 0.6 [a]	33.9 ± 0.6 [b]	nd
Fukuyutaka RSM	30 sec under N_2 gas	7.8 ± 0.2 [a]	25.2 ± 0.7 [b]	33.0 ± 0.6 [b]	0.8

Note : SB is soybean seeds. RSM is raw soymilk. Saponin value is mean ± SD (n = 3); maltol: n = 1. DDMPs includes αg, βg, βa, γg and γa DDMP; Group B includes Ba, Bb, Bc, Bb' and Bc'; Total saponin includes DDMPs saponin and group B saponin. Detection limit is 0.1 μmol/100 g sample. nd is not detected.- is not measured. The different superscript presented the significant different between rows.

To confirm these three hypotheses; 1) LOXs mediated radical reaction in 'Fukuyutaka' seeds degrades DDMPs during grinding, 2) DDMPs degrade to group B saponins and some other components by LOXs mediated radical reaction in 'Fukuyutaka' raw soymilk, and 3) LOX has a strong affect to inhibit maltol production relates to the degradation of DDMPs during grinding process, we conducted the following experiments.

1.1.1 NDGA treatment

'Fukuyutaka' raw soymilk was used to investigate efficiency of the radical scavenging reagent (NDGA) which inhibits radical reaction during grinding process. As shown in Figure III.1(A), DDMPs in 'Fukuyutaka' raw soymilk were completely degraded without adding NDGA. Increasing the concentration of NDGA corresponded to the increased inhibition of DDMPs degradation.

Since the LOX reaction induces oxidative reaction of polyenic fatty acid (Kühn & Borchert, 2002), the free radical from this reaction seems to be affected the DDMPs degradation. Therefore, in order to disrupt oxidative chain reaction, NDGA radical scavenger was used. The results were consistent with this assumption.

1.1.2 AAPH treatment

'L-Star' raw soymilk was used to study radical degradation of DDMPs. AAPH radical might induce oxygen insertion to produce peroxy radical. This reaction might be similar to the LOX reaction which contributes to depletion of DDMP-saponins. DDMPs in 'L-Star' sample have decreased for 16.4 μmol from 39.0 μmol to 22.6 μmol (42.1% degradation) when AAPH 2.5 mmol/L was added, while group B saponin were

increased from 8.5 µmol to 12.9 µmol (newly generated 4.4 µmol), as shown in Figure III.1(B). Total soyasaponins were also decreased from 47.5 µmol to 35.5 µmol which 12 µmol (25.3%) was disappeared. AAPH radical activated DDMPs degradation into group B saponin and other compounds. This result suggests that the degradation of DDMPs and production of group B saponins occurred due to the LOXs mediated radical reaction to DDMPs.

1.2 Degradation of DDMP-saponins and maltol production during soymilk preparation

Raw soymilks were heat treated in a screw cap test tube in boiling water for 10, 20, 40, and 60 min to investigate the changes in DDMPs, group B saponins, and maltol content. In 'Fukuyutaka' raw soymilk (at 0 min heating time), DDMPs and maltol were not detected, while 33.1 µmol/100 g raw soymilk of group B saponins were detected; and in 100 g 'L-Star' raw soymilk, 38.9, 6.3, and 0.5 µmol of DDMPs, group B saponins, and maltol, were detected respectively as shown in Figure III.1(C) – 'Fukuyutaka', and Figure III.1(D) – 'L-Star'.

In 'Fukuyutaka' soymilk, very small amount of maltol (1.2 µmol) was detected after 60 min heating. Meanwhile in 'L-Star' soymilk, DDMPs were degraded at a steady rate (decreased to 13.8 µmol at the end of the 60 min heating) while group B saponins and maltol were generated at a steady rate throughout the heat treatment (33.3 and 11.3 µmol detected at the end of the 60 min heat treatment, respectively).

The heat treatment was assumed to generate maltol from DDMPs degradation (Kudou et al., 1992; Kudou et al., 1993). Due to the lack of LOXs in 'L-Star' soybean, most of DDMP-saponins remained in raw soymilk, and therefore DDMPs were decomposed to group B saponins and maltol during heat treatment.

2. Soyasaponin composition and maltol production using a purified DDMP-saponin βg with model systems

2.1 Heat treatment of DDMP-saponin βg

The purified DDMP-saponin βg solution contained 0.23, 0.05 and 0.02 mmol/L of DDMP-saponin βg, group B saponin Bb, and group E saponin Be, respectively. During the heat treatment (60, 120, 180 and 240 min), DDMP-saponin βg (0.23 mmol/L) was degraded steadily. Saponin Bb, Be and maltol were newly generated and reached to 0.20, 0.04 and 0.07 mmol/L, respectively at 180 min heat treatment. But saponin Bb was partially degraded at 240 min heat treatment (Figure III.1(E)). A higher conversion rate of DDMP-saponin βg into saponin Bb, Be, and maltol was detected during the first 180 min of heat treatment. Kudou et al. reported that thermal degradation of DDMPs produced group B, group E saponins, and maltol (Kudou et al., 1992; Kudou et al., 1993); however, in this study, the amount of group E saponin Be detected was less than expectation, group E may not be produced only by heat treatment.

This result corresponded to the results of the previous research (Fujino et al., 2009) which concluded that the quantity of group B saponin Bb and maltol produced by heat treatments were not equal. After the 240 min reaction, saponin Bb and maltol content was 0.18 and 0.08 mmol/L, respectively. Maltol was detected much lower amount than group B saponin Bb production; it is possible that maltol may be evaporated and/or degraded during thermal treatment because it is a volatile and unstable compound.

2.2 APPH treatment of DDMP-saponin βg

AAPH radical reaction in DDMP-saponin βg solution was used to investigate similarity of the radical degradation of DDMPs by LOXs reaction. This model simulates the peroxydative

activity of LOXs enzymes. As DDMP-saponin βg (0.24 mmol/L) reacted with AAPH (25, 50 and 100 mmol/L), it was dose dependently decreased to 0.03 mmol/L, and saponin Bb and Be were dose dependently increased to 0.17 and 0.05 mmol/L, respectively by 100 mmol/L of AAPH. However, maltol was not generated in this reaction (Figure III.1(F)).

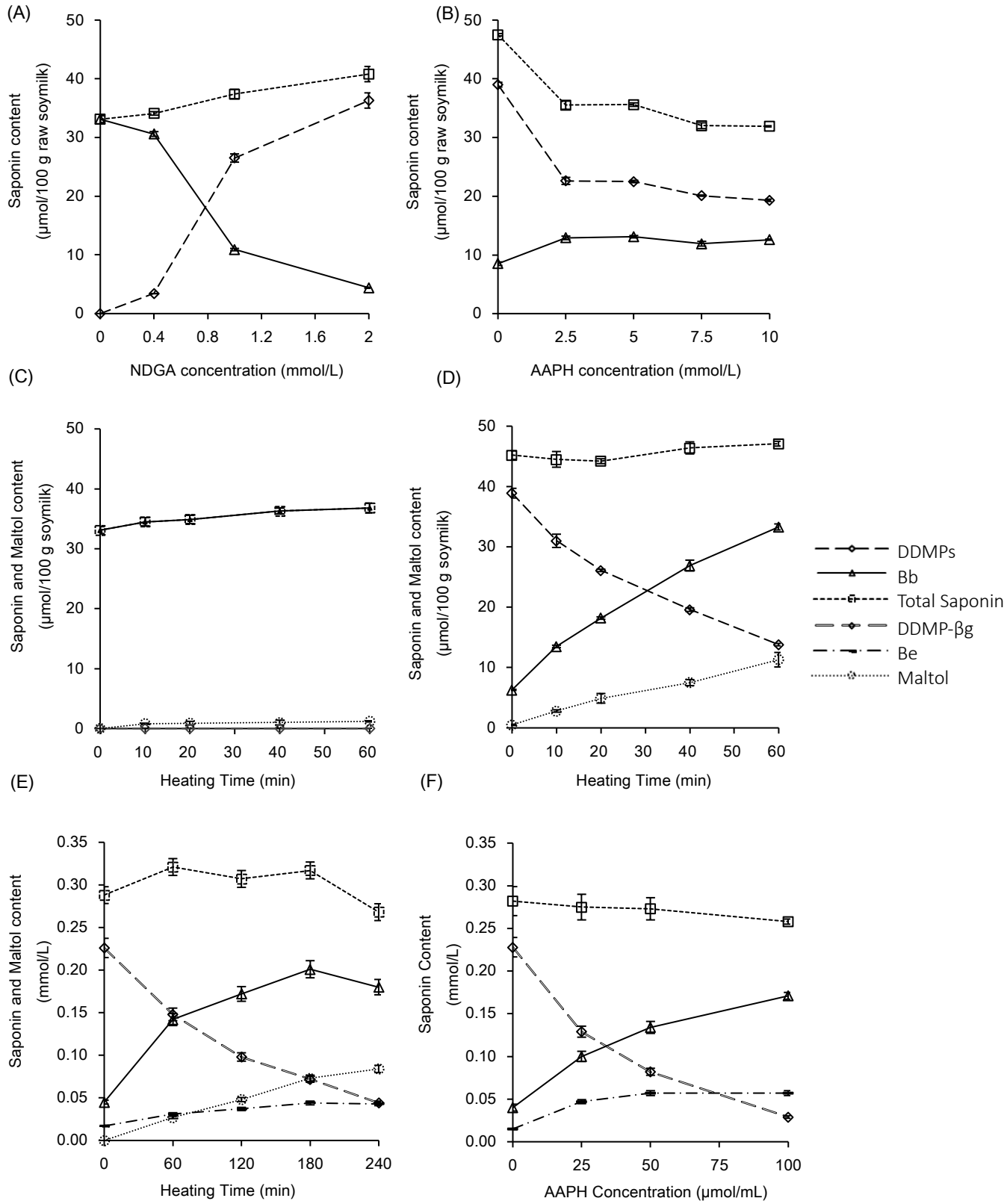

Figure III.1 : Changes of soyasaponin and/or maltol content in various experiment conditions. (A) Soyasaponin content (µmol/100 g raw soymilk) of 'Fukuyutaka' raw soymilk with NDGA radical scavenging treatment, (B) Soyasaponin content (µmol/100 g raw soymilk) of 'L-Star' raw soymilk with AAPH radical treatment, (C, D) Soyasaponins and maltol content (µmol/100 g soymilk) of 'Fukuyutaka' (C) and 'L-Star' (D) soybeans at different heating time (min), (E) Soyasaponin and maltol content (mmol/L) during thermal treatment (65 °C) of purified DDMP-saponin βg with thermal treatment at different time (min), (F) Soyasaponin content (mmol/L) by radical degradation of purified DDMP-saponin βg with AAPH radical treatment (mmol/L) (120 min reaction time)

Note : Detection limit of soyasaponin and maltol for (A) to (D) is 0.1 µmol/100 g sample, and that of (E) and (F) is 0.001 mmol/L.

Figure III.2 shows UV chromatograms of purified DDMP-saponin βg compound and derivatives of before and after reaction with AAPH radical compound. Figure III.2(A) shows UV chromatogram of purified DDMP-saponin βg solution (containing 0.24 mmol/L), which included saponin Bb (0.05 mmol/L) and Be (0.02 mmol/L). DDMP-saponin βg absorbs both UV 205 and 292 nm which are specific to DDMP moiety of DDMPs (at 26.2 min), while saponin Bb and Be absorb only UV 205 nm at 24.7 min and 25.6 min, respectively. Figure III.2(B) shows UV chromatograms of AAPH solution without purified DDMP-saponin βg solution, and a peak (P1) was detected at 15.5 min, and the MS/MS fragment pattern of P1 was m/z = 72, 86, 155, 170, 187, 214 and 429; this fragment did not reflect the chemical structure of AAPH radical reagent. This result suggests that P1 component was produced during AAPH radical reaction and had no relation to soyasaponin components. Figure III.2(C) shows UV chromatograms of the reaction mixture of purified DDMP-saponin βg and AAPH solutions after 120 min. DDMP-saponin βg has decreased, while saponins Bb and Be, have increased. In addition, an unknown peak (P2) appeared at 18.5 min. P2 absorbed UV 205 nm but not 292 nm, so it was considered not to contain DDMP moiety. The amount of P2 component continuously increased to 120 min, then gradually decreased until 200 min (data not shown).

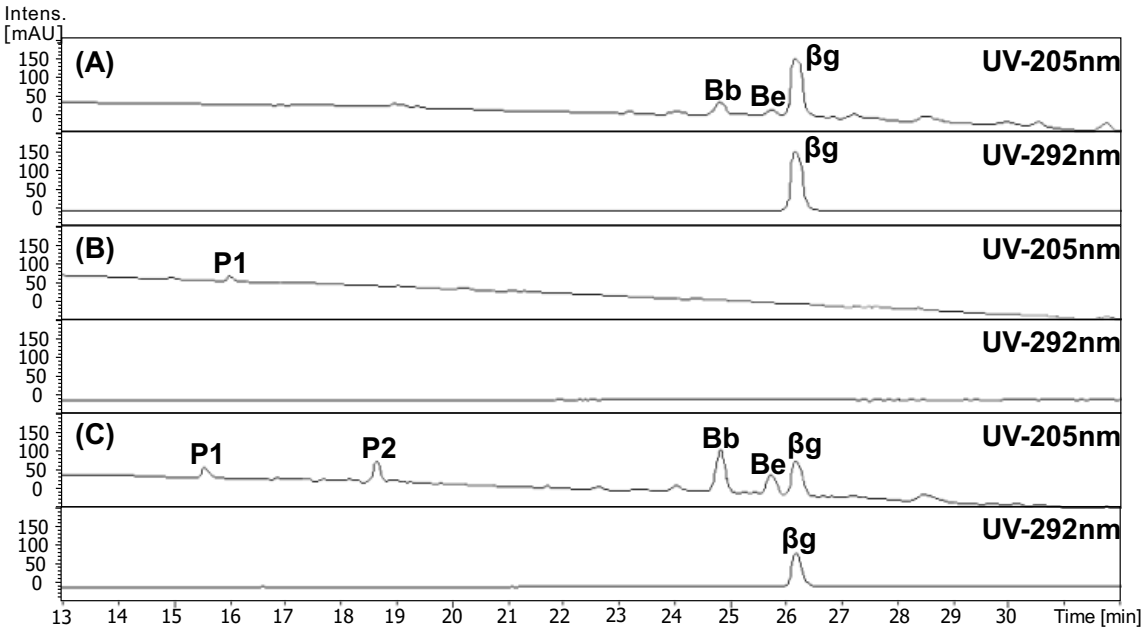

Figure III.2 : UV chromatograms of the reaction mixture with and without DDMP-saponin βg and/or AAPH radical reagent. (A) With DDMP-saponin βg, without AAPH radical reagent; (B) Without DDMP-saponin βg, with AAPH radical reagent; and (C) With both of DDMP-saponin βg and AAPH radical reagent (after 120 min reaction).

The molecular mass $[M+H]^+$ of DDMP-saponin βg is m/z 1069.5 and the MS/MS fragment pattern shows m/z 923.5, 725.4, 599.3 and 423.4 (Figure III.3(A)). These fragments denote the consequent detachment of rhamnose (-146), galactose+$2H_2O$ (-146-162-18-18), DDMP (-146-162-18-18-126), and glucuronic acid (-146-162-18-18-126-176), respectively, from DDMP-saponin βg molecule. On the other hand, the molecular mass [M+H]+ of P2 was m/z 1085.5, 16 Da higher than DDMP-saponin βg (m/z 1069.5) and the MS/MS fragment pattern showed m/z 939.4, 741.4, 599.3 and 423.4 (Figure III.3(B)). The comparison of MS/MS fragments of DDMP-saponin βg (Figure III.3(A)) and P2 (Figure III.3(B)) was considered that these fragments of P2 could denote the consequent detachment of rhamnose (-146), galactose

+ $2H_2O$ (-146-162-18-18), DDMP+ one oxygen atom [-146-162-18-18-(126+16)], and glucuronic acid [-146-162-18-18-(126+16)-176], respectively, from the P2 molecule. Thus, P2 component would have an additional oxygen atom at DDMP moiety of DDMP-saponin βg. During the radical degradation of DDMP-saponin βg, maltol was not generated. AAPH radical probably caused degradation of DDMP moiety of DDMP-saponin βg by inducing an oxygen atom and resulted in the inhibition of maltol production.

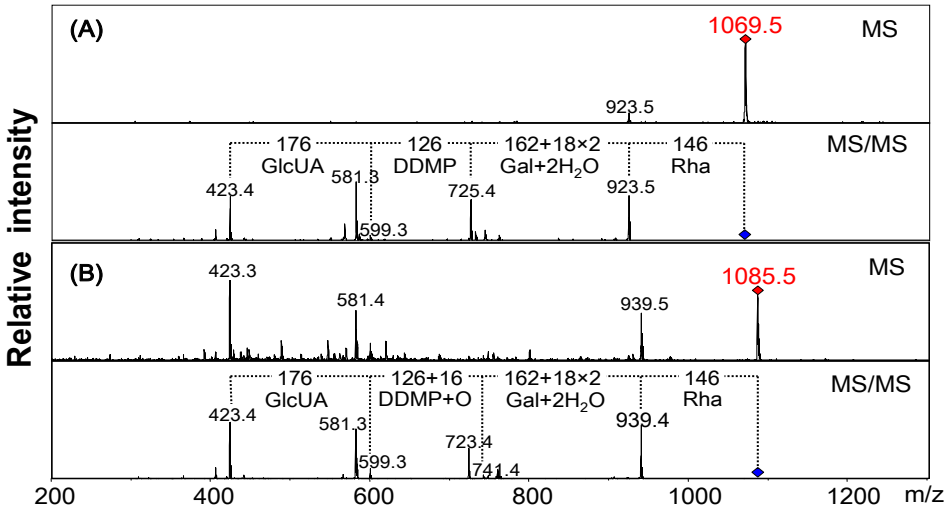

Figure III.3 : MS and MS/MS spectra of DDMP-saponin βg (A) and P2 component (B).

Table III.2 : Soyasaponin content of purified DDMP-saponin βg before and after reacted with DPPH radical for 30 min

Soyasaponins	Soyasaponin Content (µmol/mL)*	
	Before	After
DDMP-saponin βg	0.47 ± 0.01 [a]	0.14 ± 0.05 [b]
Group B saponin Bb	0.11 ± 0.01 [b]	0.15 ± 0.06 [a]
Group E saponin Be	0.04 ± 0.00 [b]	0.11 ± 0.01 [a]
Total soyasaponins	0.62 ± 0.02 [a]	0.41 ± 0.05 [b]

The different superscript presented the significant different between columns. *Mean ± SD (n=3)

DPPH treatment of DDMP-saponin βg: DPPH solution is a free radical reagent which can withdraw hydrogen atoms from the donor compounds. Purified DDMP-saponin βg (contents were shown in Table III.2) was treated by DPPH-radical reagent to examine the similarity to the radical degradation of DDMP-saponins by LOXs reaction. This model simulates the dehydrogenation activity of LOXs enzymes. DDMP-saponin βg was treated with DPPH radical solution for 30 min. Figure III.4(A) shows UV chromatograms at 205 and 292 nm of purified DDMP-saponin βg solution, which initially contained certain amounts of saponin Bb and Be. DDMP-saponin βg absorbs both of UV 205 and 292 nm (at 28.8 min), and saponin Bb (27.3 min) and Be (28.2 min) absorb only UV 205 nm. Figure III.4(B) shows UV chromatograms of DPPH solution and a peak, which seemed to be a component derived from DPPH that was detected at 28.0 min. Figure III.4(C) shows UV chromatograms of the reaction mixture of purified DDMP-saponin βg and DPPH solutions after 30 min. DDMP saponin βg decreased for

0.33 mmol/L, group B saponins Bb did not change, but group E saponin Be had been increased from 0.04 to 0.11 mmol/L. Maltol was not generated during this reaction.

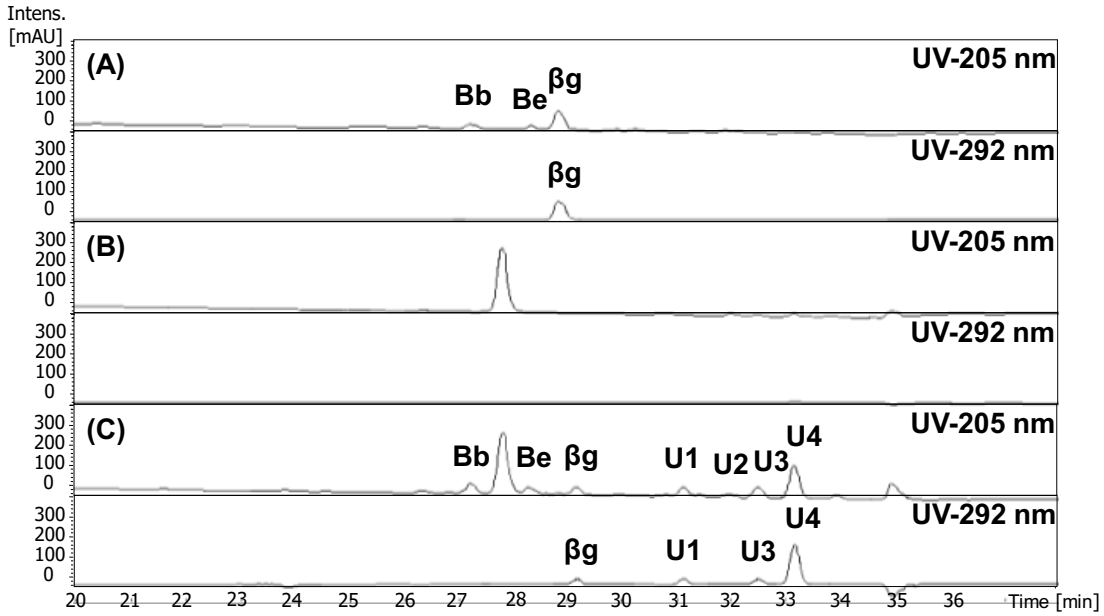

Figure III.4 : UV chromatograms of the reaction mixture with and without DDMP-saponin βg and/or DPPH radical reagent. (A) With DDMP-saponin βg, without DPPH radical reagent; (B) Without DDMP-saponin βg, with DPPH radical reagent; and (C) With both of DDMP-saponin βg and DPPH radical reagent (after 30 min reaction). Numbers 1 to 4 are unknown components. Peaks 1, 3 and 4 showed UV absorption at 292 nm as same as DDMP-saponin βg.

Four unknown peaks (U1 to U4), of which U2 absorbed only UV 205 and three other peaks (U1, U3 and U4) absorbed both of UV 205 and 292 nm which are specific to DDMP moiety, but these compounds were eluted later than DDMP-saponin βg. In this elution condition, the components eluted later than DDMP- saponin βg would be more hydrophobic than DDMP-saponin βg. From these results, it could be assumed that these unknown components were degrading compounds of DDMP-saponin βg, which aglycone (soyasapogenol B) and/or attached sugars were dehydrogenated by DPPH radical reaction. Hereby, DPPH radical could degrade DDMP-saponin βg differently from AAPH radical reaction and group E saponin Be might be generated from radical dehydrogenation of DDMP-saponin βg.

The reaction of AAPH radical is represented as the production of group B saponins by degradation of DDMPs. Meanwhile, DPPH radical might produce group E saponins from DDMPs through dehydrogenation of DDMP conjugated soyasapogenol B at the C-22 positions to produce soyasapogenol E, which has a ketone form at the C-22 position.

Conclusion

According to various soymilk preparing process, the soymilk quality will be varied due to thermal treatment and enzymatic reaction. LOXs in soybean seeds contain metal ions which can induce both peroxidation and dehydrogenation reactions (Heng et al., 2006). These radical activities of LOXs in soybean seeds are imitated by AAPH and DPPH radical treatments. This chapter revealed that LOXs in soybean seeds can degrade DDMPs and directly affect the

amount of maltol production. Thus, during soybean based food production, LOXs activities strongly affect not only increased grassy beany flavor (Baysal & Demirdöven, 2007; Whitehead, Muller, & Dean, 1995) but also decreased maltol sweet flavor. Although manufacturers may use the same soybeans as raw materials, their products would have different tastes and flavors depending on individual processing conditions. These different conditions would affect the compositions of DDMPs and their derivatives; group B saponin, group E saponin components and maltol (Figure III.5).

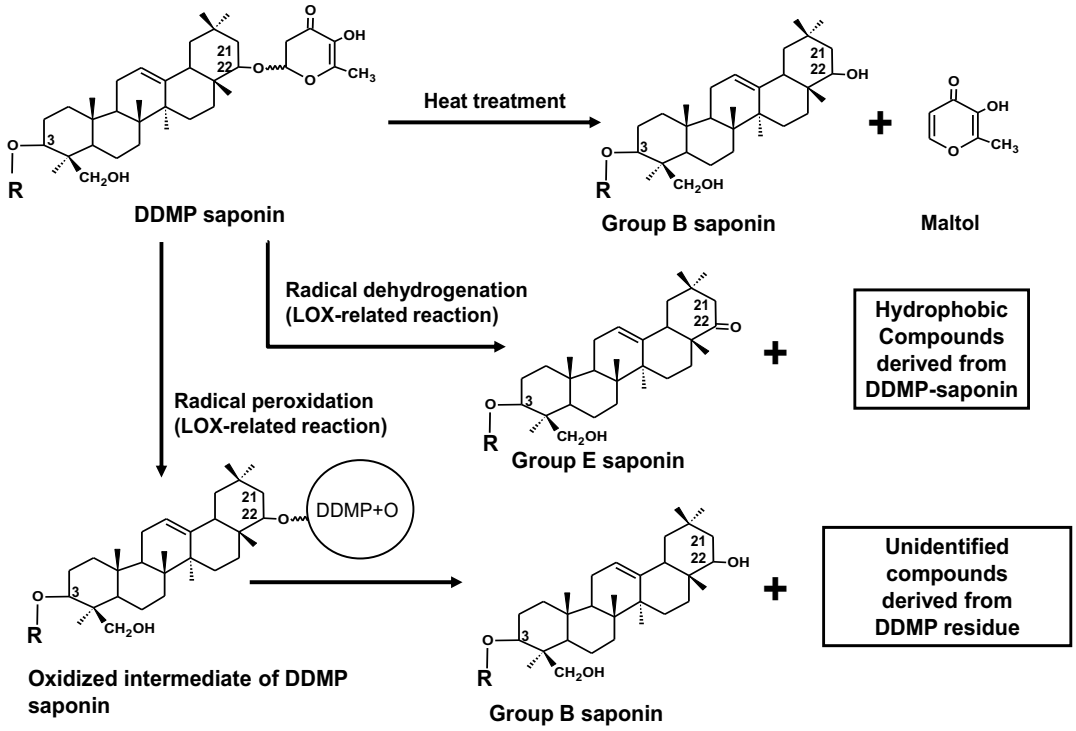

Figure III.5 : Estimated Scheme of DDMP-saponin degradation by heating and grinding during soymilk preparation.

Maltol contents in soy foods, which can be generated by heat degradation of DDMP-saponins, are affected by processing conditions. On the other hand, the radical degradative reaction of DDMPs by LOXs in soybean seeds can inhibit maltol production.

Beside taste characteristic effected, health promoting property of soyfood product would be altered. The composition and content of DDMPs saponin would be changed due to the degradation by thermal and enzymatic treatment. Thus, DDMPs contents and LOXs activities in raw materials should be a matter of concern for controlling the flavor and health promoting characteristics, which are derived from soyasaponin components of soybean base foods.

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SUMMARY

Introduction and purpose

Soybean is recognized as nutritious food because it contains high protein, healthy fatty acid, and health functional active compounds such as isoflavones and soyasaponins. The health promoting effect of soybean was reported to reduce risks of cardiovascular diseases and other non-communicable diseases (NCDs); therefor, soybean has nutraceutical properties. The soyasaponin is an effective compound for taste characteristic of soyfood and health benefits. However, soybean products are not accepted as much as it should be due to the undesirable taste characteristic, especially bitter taste and astringent flavor, which are primarily caused by soyasaponin.

The soyasaponins have been classified into two groups based on their aglycone structure: Group A and DDMP (2,3-dihydro-2,5-dihydroxy-6-methyl-4H-pyran-4-one) saponins. Group A saponins (gr.A) composed of soyasapogenol A glycosides (SAGs) and usually two sugar chains attached at the C-3 and C-22 positions. DDMP saponins (DDMPs) are DDMP conjugated soyasapogenol B glycosides at the C-22 positions which degrade to group B (gr.B) and group E (gr.E) saponins. Gr.B and gr.E saponins are composed of soyasapogenol B and soyasapogenol E as the aglycone, respectively. Gr.A saponin was reported as a major cause of undesirable taste characteristic. And gr.B saponin could promote health benefits such as anti-hyperlipidemic activity and suppression of human colon cancer cell proliferation.

This research aimed to evaluate nutraceutical property and investigate soy active components, especially soyasaponin components in various soybean varieties, soybean seed organ, soybean-based food, and the processing effect on soyasaponin composition.

Materials and methods

I. Antioxidant (AO) capacities of soyfoods, and long-term soaking effect on soy active compounds

The antioxidant activity of various soyfood products were evaluated along with antioxidative capacity of soyasaponin and isoflavone including their glycoside and aglycone forms. The purified isoflavone components included the glycoside forms; malonyldaidzin, malonylgenistin, daidzin and genistin, and the aglycone forms; daidzein and genistein. The purified soyasaponins included the glycoside forms; DDMP saponin g and group B saponin Bb, and the aglycone forms; soyasapogenol A and soyasapogenol B. The Japanese soyfood samples included non-fermented and fermented food. The antioxidant capacity of all soy active compounds and soybean based food samples were evaluated by hydrophilic-oxygen radical absorbance capacity (H-ORAC assay). Moreover, the soaking effect on soy active compounds of two Thai soybean varieties (general soybean, Glycine max (L.) Merr. cv. Chiang-Mai 60; and black soybean, Glycine max (L.) Merr. cv. Sukhothai 3) also were analyzed. The isoflavone, soyasaponin, amino acid and free sugar content, and H-ORAC value were investigate during soaking treatment in both soybean varieties.

II. The effect of soybean variety and food processing on soyasaponin composition complexities relating health beneficial properties and undesirable taste characteristics

The 106 soyasaponin components, fully-acetylated soyasapogenol A glucoside (FSAGs), partially-acetylated SAGs (PSAGs), deacetylated SAGs (DSAGs), and DDMPs were targeted to identify in 9 different soybean varieties, soybean seed organ (hypocotyls and cotyledons), and 39 soyfood products by using LC-PDA/MS/MS analysis. The 9 soybean varieties having different seed saponin composition included 2 wild soybean collections (Glycine soja) ‘GD50326-2’ and

'GD50029-2', and 7 Japanese domestic soybean varieties (Glycine max) 'Norin No. 3', 'Shirosennari', 'Ibarakimame No. 7', 'Suzuyutaka', 'Mikuriya-ao', 'Tohoku No. 152', and 'Kinusayaka'. The 39 soyfood products were collected from Thai and Japanese market, including raw soybean seed, fresh soybean sprout, cooked soybean, soymilk (non-modified and modified soymilk), tofu, yuba, natto, miso, douchi, and tofuyo.

III. The effects of soymilk preparation on DDMP saponin degradation and maltol production

The soymilk producing process was used as food processing model to investigate the degradation of DDMP saponin to gr.B saponin, and the maltol production (natural sweet compound). Two soybean varieties were used as raw materials, which were Glycine max (L.) Merr. cv. Fukuyutaka and a genetically modified LOXs-deficient soybean Glycine max (L.) Merr. cv. L-Star. The grinding process and heat process of soymilk preparation were evaluated the effect on DDMP and maltol. During grinding process, 'Fukuyutaka' raw soymilk was treated by nordihydroguaiaretic acid (NDGA) reagent as free radical scavenging, while 2,2'-azobis (2-amidinopropane) dihydrochloride (AAPH) was added to 'L-star' raw soymilk as the free radical. Moreover, the purified DDMP saponin βg was used for investigating the radical treatment and heating treatment effect. The purified DDMP solution was treated by heating and free radical, AAPH and 2,2-diphenyl-1-picrylhydrazyl (DPPH).

Results

I. Antioxidant (AO) capacities of soyfoods, and long-term soaking effect on soy active compounds

The soy active compound showed different antioxidant capacity, and isoflavone promoted higher antioxidant capacity than soyasaponin. The different chemical structure also presented the different capacity, isoflavone aglycone promoted higher AO capacity than glycoside while soyasaponin glycoside promoted higher AO capacity than aglycone. Among all soybean based food products, fermented soyfoods showed higher AO capacity than non-fermented samples especially soy miso product following by natto, soy sauce, rice miso, and tempe, respectively.

The soaking treatment which is pre-treatment process for soyfood preparation also affected the soy active composition and AO capacity. The isoflavone and soyasaponin composition were changed after soaking for 12h due to degrade the glycoside to aglycone. The 12h soaking could enhance folate content and total amino acid content especially gamma-aminobutyric acid (GABA) which promote nervous system. However, the 12h soaking decreased total free sugar and good taste amino acid.

II. The effect of soybean variety and food processing on soyasaponin composition complexities relating health beneficial properties and undesirable taste characteristics

The soyasaponin content of nine different soybean varieties were evaluated by using LC-PDA/MS/MS analysis. The identification of soyasaponin was proceeded by 1) separation of soyasaponin compounds by HPLC with ODS column, 2) screening of total ion chromatograms (TIC) by selected ion monitoring (SIM), and 3) annotation by MS and MS/MS fragment pattern. The soyasaponin content were determined by UV absorvance after evaluation of the purity of soyasaponin peaks. According to the identification procedures, not only fully-acetylated soyasapogenol A glucoside (FSAGs) but also the minor saponin compounds as partially acetylated soyasapogenol A glycosides (PSAGs) and null acetylated soyasapogenol A glycosides (NSAGs) were clearly identified. Nine soybean varieties were classified into three different soyasaponin lines; Aa-line, Ab-line, and A0-line, which agreed with the previous research. In the previous research, gr.A saponins had been reported to locate only in the seed hypocotyls.

However, gr.A saponin components FSAGs, PSAGs and NSAGs were detected even in seed cotyledons in this research. Thus, the cotyledons might present undesirable taste characteristics.

As stated in the soyasaponin composition investigation of 39 soyfood samples above, the composition was varied in different soyfood samples. The FSAGs, the major cause of undesirable taste characteristics, were highest in raw soybean samples following by fresh soyfood samples and thermal processed samples. The PSAGs and NSAGs were highly detected in fermented soyfood samples. On the other hand, DDMPs saponins were mainly detected in raw soybean seeds, fresh samples, and thermal processed samples, while gr.B saponins, which is the derivative compounds, were detected in fermented samples and highly processed samples (modified soymilk products).

III. The effects of soymilk preparation on DDMP saponin degradation and maltol production

The soymilk preparation showed the different effects for 'Fukuyataka' and 'L-star' on raw soymilk which related to lipoxygenase enzyme (LOXs) activity. The LOXs reaction could be activated during grinding process and reacted with fatty acid, then produce grassy flavor and free radicals which might affect in DDMP saponin degradation. The DDMP saponins in raw soymilk of 'Fukuyutaka' after 2-minute grinding, were completely degraded and gr.B saponins were produced. The NDGA reagent was used as free radical scavenging reagent during 'Fukuyataka' raw soymilk preparation, the high NDGA concentration could inhibit the DDMP saponin degradation. On the other hand, AAPH radical was added to 'L-star' raw soymilk then the DDMP saponins were continually decreased along with higher AAPH concentration. Beside DDMP saponin degradation, maltol production also was concerned because maltol is expected to be produced during soymilk production. The maltol, natural sweet flavor, could be produced by thermal degradation of DDMP saponin. The 'Fukuyataka' raw soymilk was heated after 2-minute grinding, the maltol was produced in low amount while 'L-star' heated soymilk showed higher maltol contents.

To confirm the DDMP saponin degradation by LOXs or radical reaction, purified DDMP saponin βg was treated by AAPH radical as LOXs peroxidative reaction, and DPPH radical as LOXs dehydrogenated reaction. The result showed that DDMP saponin βg was degraded into gr. B saponin and some unknown compounds were identified after radical treatment. For maltol production, maltol could be produced when purified DDMP saponin βg was treated by heat. However, there was no maltol production by radical treatment.

Conclusion and consideration

I. Antioxidant (AO) capacities of soyfoods, and long-term soaking effect on soy active compounds

As explained by screening AO capacity of soy active compounds and soyfood samples, the different soyfood samples presented the different AO capacity which might relate to different chemical structure of soy active compounds in soyfood samples. The soaking treatment also induced the changes of soy active compounds especially degradation of glycoside to aglycone form for both isoflavone and DDMP saponin. Moreover, the soaking process could enhance the GABA and folate contents in both soybeans. As this statement, the soaking treatment could promote nutraceutical properties of soybeans. However, the AO capacity of soaking soybeans was decreased after soaking. The soaking treatment also decreased total free sugar content and sweet taste amino acid content; however, bitter amino acid was increased. Even though functional compounds of soybean were increased during soaking, the good taste characteristics were decreased.

II. The effect of soybean variety and food processing on soyasaponin composition complexities relating health beneficial properties and undesirable taste characteristics

The precise soyasaponin composition in different soybean varieties and soybean seed organ were newly revealed in this research. The gr.A saponins, bitter components, were identified in seed cotyledons. The minor compounds PSAGs also were detected in both seed hypocotyls and cotyledons for all soybean varieties except 'Tohoku No.152 (SAGs deficient)' and 'Kinusayaka (A0-line)'. Thus, the existance of gr.A saponin in seed cotyledons and PSAGs in soybean might be the causes of undesirable taste of soyfood products.

Based on altered chemical structure of soyasaponin in different soyfood products, the food process might play an important role in both taste characteristic and nutraceutical properties. The fermentation process might decrease undesirable taste by deacetylating gr.A saponin, and promote health benefit by degrading DDMP saponin to gr.B saponins.

III. The effects of soymilk preparation on DDMP saponin degradation and maltol production

The quality of soymilk depended on preparation process which include enzymatic reaction of LOXs and thermal treatment. The LOXs presented strongly effect on degradation of DDMP saponin and inhibition of maltol production. The LOXs enzymatic reaction or radical reaction showed the negative effect to the quality of soymilk. On the other hand, thermal treatment positively affected to stop enzymatic reaction prior to DDMP saponin degraded to gr.B and producing maltol (the natural sweet flavor).

In addition, the chemical structure of soy active compound could be altered by food processing, which resulting in the change of functionalities and taste characteristics. As soyasaponin has been interested by many researchers, FSAGs were the major cause of undesirable tastes while gr.B soyasaponin promoted nutraceutical properties. The soyasaponin composition could be controlled by genetical modification of soybean varieties as previously studied. The precise soyasaponin composition of different soybean varieties revealed that gr.A saponin was also located in seed cotyledons which were newly reported in this research. However, the food processing played an important role in changing soyasaponin composition. As indicated in the soaking effect above, DDMP saponin could be degraded to gr.B saponin. Among various kinds of soyfood, the fermented soyfood samples and highly processed food samples (eg. modified soymilk samples), showed the lowest FSAGs and highest gr.B saponin contents in order to reduce the undesirable taste and enhance nutraceutical property, respectively. As mentioned in the soymilk preparation effect on soyasaponin contents, the enzymatic or radical reaction presented negative effect on the quality of soymilk. The thermal treatment was preferred as inhibiting enzymatic reaction and enhancing health functional gr.B saponin and natural sweet maltol production during soymilk preparation.

Finally, it can be concluded that nutraceutical properties and taste characteristic of soyfood products depend on the composition and chemical structure of soy active components and food processing treatment. Even though genetic modification can control some soy active compounds such as soyasaponins, the chemical structure of the components can be also altered by food processing treatment.

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