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Transformation of the Glycosides from Food Materials by Probiotics and Food Microorganisms

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Abstract Glycosides are important functional materials in foods. Transformation, especially hydrolysis, of the sugar moieties tends to improve the functional properties of the administered glycosides *in vivo*. Various probiotic bacteria and edible food-grade fungi such as bifidobacteria, lactobacilli, leuconostocs, yeasts, and aspergilli are potential industrial microorganisms to transform glycosides of ginsenosides from ginseng, platycodin saponins from *Platycodon grandiflorum*, *Trignoella foenum-graecum* (TFG) saponins, and isoflavones from soybeans and *Puerariae radix*, respectively, by fermentation or enzymatic reaction. In this review, various transformation pathways bearing potential significance with respect to the changes in structure and function of the various glycosides from the food materials will be introduced. In conclusion, the proper combination of food microorganisms and transformation conditions will improve the functionality and the sensory value and reduce the cytotoxicity of the functional glycosides present in various functional food raw materials.

Key words: Transformation, glycosides, food, probiotics

Glycosides are combined forms of aglycosides and sugars. They exist as a diverse group of natural products that are ubiquitously found in plants, animals, and microorganisms. The sugar moieties including glucose, xylose, rhamnose, galactose, etc., can be linked to an aglycoside unit through O- or C-linkage with α- or β-configuration. Generally, O-glycosides are more amenable to the cleavage by enzymatic or chemical treatments than C-glycosides. Glycosides comprise important structural components of the various hormones, alkaloids, flavonoids, etc., and endow numerous biological and pharmaceutical functions for humankind. Exploitation of the biological properties of the glycosides made a remarkable contribution for the development of the pharmacological medicines and functional food products. In nature, the sugars of the glycosides make the molecules soluble in various parts of the tissues and keep them less reactive with the molecules around. These properties make the administered glycosides tend to be excreted *in vivo*, thus the bioavailability and biological activity of the glycosides are rather low in the digestive tracts of humans unless they are hydrolyzed by stomach acid or intestinal microbial enzymes. Therefore, the hydrolysis of the sugar moieties tends to improve the functional properties of the administered glycosides *in vivo*. Here, we will review some of the implications and significance of the metabolism and the metabolized products of the various glycosides with regard to their structures and functions.

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BIOTRANSFORMATION OF GLYCOSIDES BY INTESTINAL BACTERIA

Various glycosides can be hydrolyzed and transformed into aglycosides by the residential bacteria in the intestinal tracts in animals and humans. The degree of the transformation can vary depending on the chemical structures of the glycosides. Flavonoid glycosides such as isoquercitrin are readily hydrolyzed and absorbed in the rat intestine. However, the absorption of the intact glycosides were severely retarded when hydrolysis was inhibited by gluconolactone. The protective effects of the anthocyanins against cardiovascular diseases and certain forms of cancer are suggested to differ depending on the degree of the metabolism [24]. Anthocyanidin glycosides were hydrolyzed by the microflora within 2 h of incubation depending on the sugar moiety. Liberated aglycones were further transformed

into phenolic acids and was accompanied by demethylation [40].

When two different bacterial isolates from human feces were assessed, *Enterococcus casseliflavus* hydrolyzed the sugar moiety of the quercetin-3-glucoside, but did not metabolize the aglycoside further. The fermented products included quercetin, formate, acetate, lactate, and ethanol [50]. On the other hand, *Eubacterium ramulus* was capable of degrading the aromatic ring system and produced dihydroxyphenylacetic acid, acetate, and ethanol. It was of interest that *Eubacterium ramulus* did not grow on the aglycoside quercetin or the ring-fission intermediate phloroglucinol, but cleaved the flavonoid ring system when glucose was present as a cosubstrate [50].

The microbial hydrolysis of the flavonoid glycosides occurred even in the oral cavity. When quercetin 4'-glucoside was incubated with human saliva, hydrolysis to quercetin was detected within minutes. Although glucose conjugates were rapidly hydrolyzed, the glycosides with other sugars such as rutin, quercitirin, and naringin were not. The large individual variability in the metabolism of the flavonoid glycosides in the oral cavity has been observed [60].

After fermentation of ellagic acid and related polyphenols such as punicalagin, and the ellagitannin from walnut extracts, the metabolite urolithin A (3,8-dihydroxy-6H-dibenzo[b,d]pyran-6-one) was produced in the fecal cultures from different volunteers, but with very different production rates and concentrations. This large variability in the concentration of metabolite and kinetics of metabolite production was consistent with the large variability found in the excretion of these metabolites in urine *in vivo* after human consumption of ellagitannins, and with differences in the composition of the fecal microflora [9].

Limonoids are a group of chemically related triterpenoids present in *Citrus* and *Rutaceae* species. The aglycoside forms of limonoids, especially limonin aglycoside, are involved in causing bitterness in citrus fruits. Limonoids were suggested to be absorbed directly as glucosides or after the limonoid moiety is liberated by the action of human intestinal bacterial beta-glucosidase activity *in vivo*. The aglycoside forms of limonoids were more intensely studied than the glycoside forms with respect to their anticarcinogenic activity [56].

The dietary saponins in soybean were metabolized by human intestinal bacteria. For instance, soya saponin I was converted into soya saponin III, which was sequentially transformed into soya sapogenol B. The rate of the bacterial transformation of the soya saponin was different among the subjects [18].

Glycyrrhizin (GZ) is the sweet principle of the roots of *Glycyrrhiza glabara*. GZ, a glucuronide conjugate of oleanane-type triterpene structure, is widely used for the sweetening of beverages, foods, and herbal medicines. The intestinal absorption of glycyrrhetic acid (GA), a major metabolite of GZ, was larger than that of GZ. GZ is hydrolyzed by bacteria in the stomach and large-intestinal contents. Glycyrrhetyl monoglucuronide (GAMG) is an intermediate in the hydrolysis of GL to GA. An enzyme responsible for its hydrolysis, characterized as a GAMG beta-D-glucuronidase of *Eubacterium* sp. GLH, has been isolated from human intestinal bacteria [1]. Although GZ was poorly absorbable, both GZ and GA were detected in rat plasma after oral administration of GZ, suggesting that GZ can be absorbed in both parent and metabolite forms, although their bioavailabilities were low [62].

The sennosides contained in *Cassia angustifolia* exert laxative activity. The gucosyl of sennoside B was hydrolyzed stepwise to sennidin B through sennidin-8-monoglucoside by *Bifidobacterium* sp. SEN. Coculture of *Bifidobacterium* sp. SEN and *Peptostreptococcus intermedius* produced rhein anthrone [17]. Interestingly, *Eubacterium* sp. BAR was capable of cleaving the C-glucosyl of barbaloin, which is a main laxative principle contained in aloe [17].

As described above, the biological activity and bioavailability of the various glycosides may be increased by the hydrolysis of the sugar units. Development of the detoxification process based on the use of microbial beta-glycosidases would be another potential area of industrial interest. The vicine and convicine contained in faba beans are toxic glycosides that can cause anemic symptoms in humans. Treatment of these compounds with glycosidase-producing microorganisms such as *Aspergillus*, *Fusarium*, and *Lactobacillus* resulted in the degradation of the toxic glycosides [41].

Toxic cyanogenic glycosidases such as amygdalin, linamarin, linustatin, and neolinustatin were degraded by various strains of *Lactobacillus plantarum*, *Leuconostoc mesenteroides*, and *Candida tropicalis*. These processes were suggested to remove toxic glycosides present in cassava and linseed meal [39]. However, it needs to be considered that the hydrolysis of the plethora of the glycosides can increase the toxicity and thus cause safety concerns for both humans and animals. The prudential design of the bioconversion process of the glycoside will lead to the development of new pharmaceutical or biological compounds. Although several glycosides transformed by microorganisms have been identified, investigations of the transformation pathway have frequently used inedible ones. To rectify this concern, probiotics that have been safely used for foods consumption are the most desirable since they are not only safe but also confer health promoting functions on the host [22]. Various probiotic strains have been isolated and characterized with regards to stress resistance, anticancer activity, anti-inflammation, allergy reduction, and rotavirus inhibition [28, 47, 59].

The following sections will focus on the transformation of specific glycosides from various functional food materials

as substrates with edible food microorganisms, especially probiotics.

TRANSFORMATION OF GINSENG SAPONINS

Ginseng (the root of *Panax ginseng* C.A. Meyer; family Araliaceae) is used worldwide for the prevention of various diseases. Ginseng saponins (ginsenosides) are the principal components having pharmacological and biological activities, such as antidiabetic [53] and antitumor [42, 49] activities. Over 30 different ginsenosides have so far been isolated and identified from ginseng saponins. The main ginsenosides are glycosides that contain an aglycone with a dammarane skeleton, and include protopanaxadiol-type saponins such as ginsenosides Rb1, Rb2, Rc, and Rd, and protopanaxatriol-type saponins such as ginsenosides Re and Rg1. Among them, deglycosylated ginsenosides are known to be more readily absorbed into the bloodstream and act as active compounds [55]. Intestinal microflora can transform ginsenosides into more active forms after ingestion of ginsenosides [7, 23, 24, 33]. Protopanaxadiol ginsenosides such as Rb1, Rb2, and Rc have previously been shown to be metabolized by human intestinal bacteria to their final derivative, compound K, with the degree of the transformation of the ginsenosides and production of compound K differing between *Eubacterium* sp., *Streptococcus* sp., and *Fusobacterium* K-60 [6, 7, 55]. Bae *et al.* [5] reported that Rb1 and Rb2 were not metabolized to Rh2 by human intestinal bacteria. They suggested that Rb1 and Rb2 should be transformed into Rg3 in the stomach to be absorbed in the form of Rh2, based on their observation that human intestinal bacteria such as *Bacteroides* sp., *Fusobacterium* sp., and *Bifidobacterium* sp. were able to transform Rg3 into Rh2. Otherwise, Rb1 and Rb2 were presumed to be metabolized to compound K in the human intestine. In addition, Tawab *et al.* [55] reported that the protopanaxatriol ginsenosides Re and Rg1 were metabolized to F1 or Rh1, which reached the systemic circulation after oral administration in humans. The efficiency of conversion and the transformation pathways may differ greatly owing to the diversity of the resident microflora between individuals.

Recently, the transformation pathways of Rb1, Rb2, and Rc with enzymes from various food microorganisms such as *Bifidobacterium*, *Lactobacillus*, and *Aspergillus* were characterized [10, 11]. *Bifidobacterium* and *L. delbrueckii* initially cleaved the glucose unit at C-20 of Rb1 and the arabinose unit of Rb2 and Rc at C-20 (Figs. 1–3). In contrast, *A. niger* cleaved glucose at C-3 better than the arabinose unit of Rb2 and Rb3, while it initially hydrolyzed glucose at C20 of Rb1. It was of interest that *L. delbrueckii*

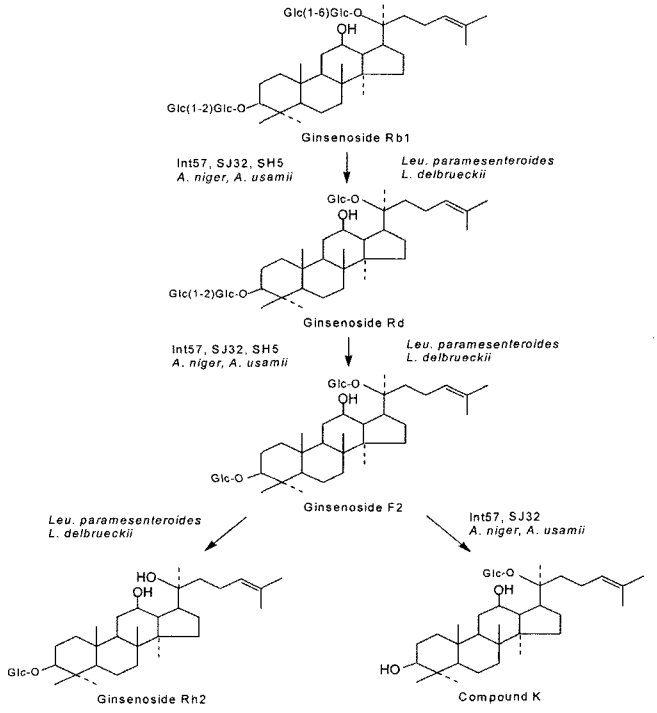

Fig. 1. Proposed transformation pathways of Rb1 by cell extracts from various food microorganisms [11].

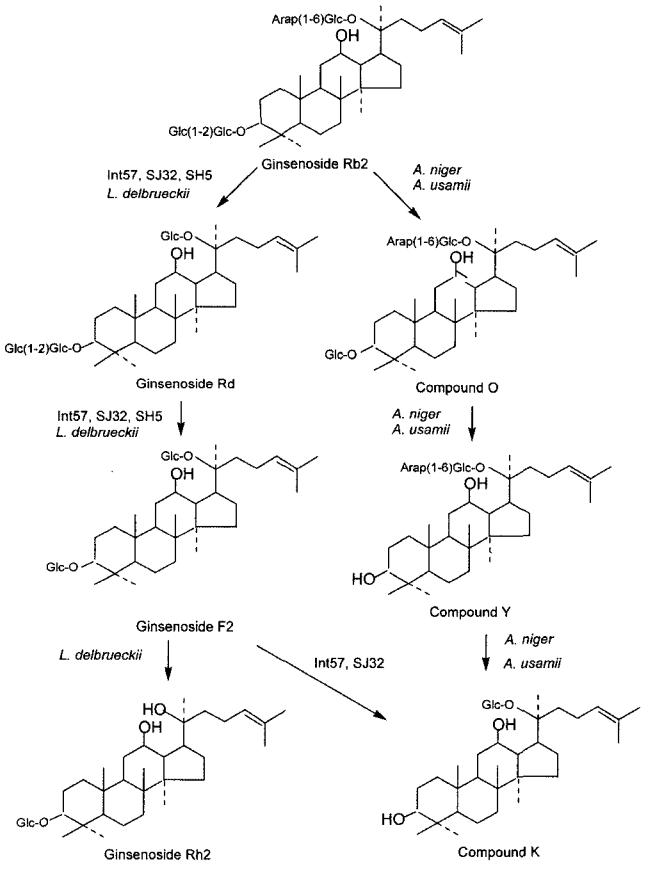

Fig. 2. Proposed transformation pathways of Rb2 by cell extracts from various food microorganisms [10].

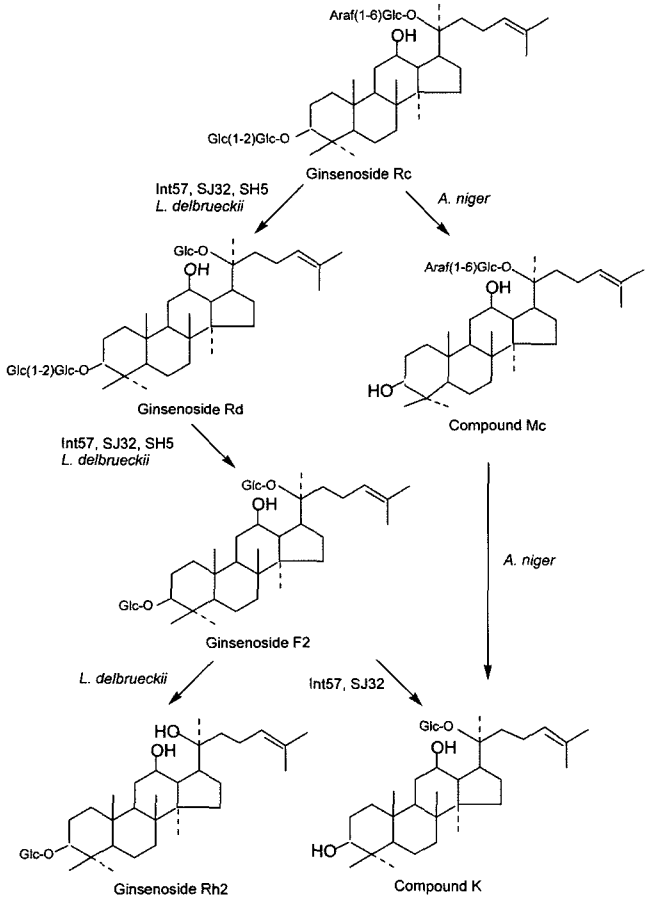

Fig. 3. Proposed transformation pathways of Rc by cell extracts from various food microorganisms [10].

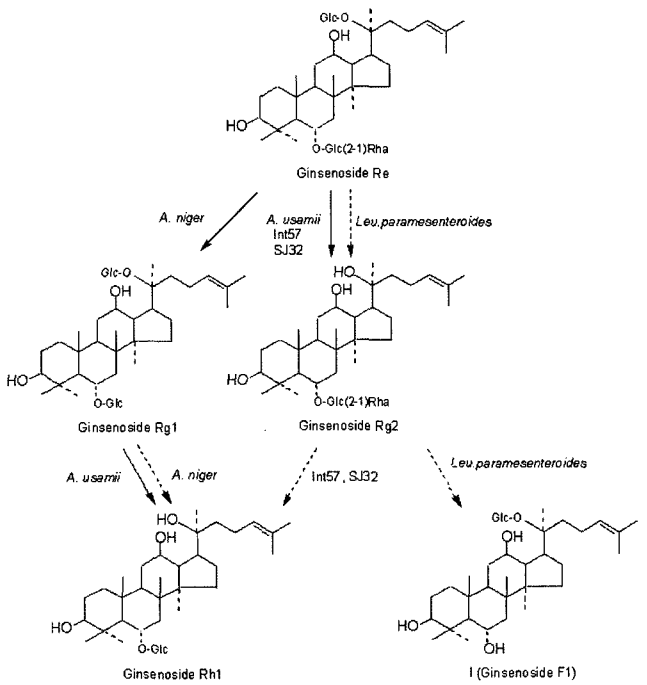

Fig. 4. Proposed transformation pathways of Re by cell extracts from various food microorganisms [11].

produced Rh2 from both Rb2 and Rc, unlike other experimental microorganisms. Biotransformation of Rb1 into Rh2 via Rd and Rg3 by the fungus *Rhizopus stolonifer* has been reported [2].

In the transformation of Re, *Bifidobacterium* sp. Int57, *Bifidobacterium* sp. SJ32, and *A. niger* produced Rh1 as a final product, although the intermediately produced compounds differ between *Bifidobacterium* and *A. niger*. *A. usamii* produced Rg2 but did not convert Rg2 thereafter (Fig. 4). Even though *A. usamii* could not transform Re into Rg1 it was able transform Rg1 into Rh1.

Upon hydrolysis of the total ginsenoside extracts, *A. niger* and *A. usamii* not only showed high transforming activities of ginsenosides to compound K but also produced various transformed ginsenosides such as compound Y and compound Mc, and Rh1. This suggests that these molds possess more diverse types of glycosidase than the bacteria used in this study to attack sugars attached to ginsenosides. Interestingly, the ginsenosides with only one sugar residue linked to the saponin backbone were resilient to further cleavage by the microbial enzymes. For instance, β-glucose of the C-20 residue of compound K produced by Int57, SJ32, *A. niger*, and *A. usamii*, and β-glucose of C-6 of Rh1 produced by Int57, SJ32, and *A. niger* were resilient to the cleavage. Therefore, the important step for the determination of the final transformed products might be the stereospecific affinity of the enzyme for the intermediate substrates.

The functions of Rh1, Rh2, compound K, Rg2, and F2, which are the specific final products for the respective enzymes in this study, have been reported. The suggested activities for Rh2 include inhibition of the initiation and progression of tumor cells [27], secretion of insulin [35], and reduction of the plasma glucose in STZ-diabetic rats based on an increase in beta-endorphin secretion that activates opioid mu-receptors [32]. Rh1 possesses anti-allergic and anti-inflammatory activities [45]. The production of nitric oxide by IFN-gamma plus LPS-treated macrophages is markedly reduced by Rh1 or Rh2 (in a dose-dependent manner), but is not inhibited by Rb1, Rc, or Re [48]. Kudo *et al.* [29] suggested that Rg2 reduced the acetylcholine-evoked secretion of catecholamines from cultured bovine adrenal chromaffin cells. F2 also has been shown to be a potent inhibitor of acetylcholine-evoked secretion of catecholamines [54]. Compound K is known to induce an antimetastatic or anticarcinogenic effect by blocking tumor invasion or preventing chromosomal activation and tumorigenesis [37]. Compound K protected HepG2 cell cytotoxicity induced by tert-butyl hydroperoxide (t-BHP) and significantly inhibited the increment of serum alanine aminotransferase and aspartate aminotransferase as markers

of hepatoprotective activity induced by t-BHP in mice, when it was orally administered [36]. Rf, another transformed metabolite of ginsenoside, showed a regulation on the lipoprotein, apo A-I and C-III, mRNA metabolism by altering peroxisome proliferator-activated receptor alpha (PPARα)-mediated pathways [35].

TRANSFORMATION OF ISOFLAVONES FROM SOYBEANS AND *PUERARIAE RADIX* (PR)

Soybeans and PR are known to contain abundant glycoside conjugates of isoflavones. Studies performed *in vitro* and in animal models have shown that these substances have a phytoestrogenic activity [31]. Phytoestrogens are estrogen-like compounds found in a wide variety of plants. Epidemiological studies provide evidence for a protective role of isoflavones against the development of numerous chronic diseases, including several cancers, cardiovascular disease, and osteoporosis [3]. Studies performed *in vitro* and in animal models have shown that these substances have an affinity to the estrogen receptor and exert hormonal and antihormonal effects [31]. Phytoestrogens may present an alternative to postmenopausal hormone therapy because of concerns about side effects and long-term health consequences of the hormone therapy [30].

The soybean glycosides were demonstrated to be hydrolyzed into aglycosides, daidzein and genistein, from the corresponding glycosides, daidzin and genistin, respectively, by bacterial beta-glucosidases [19, 52]. Bioavailability of soy isoflavones depended upon gut microflora in woman [64]. Additionally, it was suggested that soy isoflavone aglycone-rich products are more effective than glucoside-rich products in preventing chronic disease such as coronary heart disease [20, 64]. Izumi *et al.* [20] found that the plasma concentrations after both low and high doses of aglycone (0.11 mmol and 1.7 mmol) were more than two and five times higher than that after glucoside intake, respectively.

The isoflavone glycosides, daidzin and genistin, in soymilks were completely hydrolyzed by *Bifidobacterium* sp. Int-57 in 18 h [21]. This study demonstrated that daidzin and genistin can be efficiently converted into daidzein and genistein, respectively, by proper combination of probiotic strains and fermentation conditions. The transformed aglycoside genistein was further degraded into dihydrogenistein and 6'-hydroxy-O-desmethylangolensin, and then further to yield the end-products 4-hydroxyphenyl-2-propionic acid and 1,3,5-trihydroxybenzene [13]. Daidzein, another transformed aglycoside of soy isoflavone, can be transformed into equol and O-desmethylangolensin (O-DMA) by the intestinal bacteria in human. It was suggested that the ability to produce equol and O-DMA may be associated with reduced risk of certain diseases including breast and prostate cancers in the observational and intervention studies in humans [4]. Humans have acquired an ability to exclusively synthesize S-equol from the precursor soy isoflavone daidzein, and it is significant that, unlike R-equol, this enantiomer has a relatively high affinity for estrogen receptor beta [51]. The particular probiotic supplement did not generally affect plasma isoflavones, although the large differences between plasma and urinary equol in some subjects suggested that equol producer status might be modifiable in some individuals [44]. Consequently, the administration of the already transformed equol might be advantageous compared with that of daidzin or daidzein. The reported activity of certain strains of *Bifidobacterium* producing equol from daidzin would provide a potential industrial process for the equol production [57].

Puerariae radix (PR), the root of *Pueraria labata* (Willd) Ohwi, a wild creeper leguminous plant, is one of the earliest and most important crude herbs used in Chinese medicines. Intake of diet with a high dose of PR increased trabecular bone volume and trabecular thickness and decreased trabecular separation in ovariectomized mice compared with that in the sham-operated mice, which suggests that PR may represent a potential alternative medicine for hormone replacement therapy in the prevention of osteoporosis in postmenopausal women [61].

The isoflavone contents of PR are about tenfold those of soybean (amounting to 2 g/100 g dry weight). The main isoflavones of PR are purarin and daidzin. Kim *et al.* [26] reported that puerarin and daidzin are metabolized to daidzein by human intestinal microflora and the metabolite daidzein exhibited the more potent antioxidant and antitumor cytotoxic effects.

Puerarin, a C-glucoside of daidzein in PR, was hydrolyzed into daidzein to a lesser degree than daidzin, the O-glucoside of daidzein. In rats, C-glycoside puerarin was partially hydrolyzed to aglycone in the body, but mainly excreted in the urine as unchanged puerarin [58]. Among the various microbial strains tested, *Bifidobacterium* sp. Int-57 showed the greatest daidzin hydrolysis activity. When yeast extract was added to the PR medium during fermentation with *Bifidobacterium* sp. Int-57, nearly all of the daidzin was hydrolyzed to near completion. The addition of skim milk and whole milk also improved the conversion of daidzin into daidzein [12].

The transformation of PR by probiotic bacteria might be a suitable process for improving the level of bioactive structures of PR isoflavones.

TRANSFORMATION OF SAPONINS FROM *PLATYCODON GRANDIFLORUM* AND *TRIGNOELLA FOENUM-GRAECUM*

Platycodon grandiflorum A. DC (Campanulaceae) has been used for food material and treatment of inflammatory

disease, phlegm, cough, and asthma in the oriental medicines for a long time [34]. *Platycodi radix*, the root of *Platycodon grandiflorum* A. DC commonly known as Doraji (Chinese drug, "Jiegeng", and Japanese name, "Kikyo') contains more than 20 saponins of oleanane type. Among them platycodin D has been known to be responsible for the anti-inflammatory activities of this plant and is linked with 3-O-glucose and 28-O-apiose-xylose-rhamnose-arabinose.

Various microorganisms such as bifidobacteria, lactobacilli, leuconostocs, yeasts, and aspergilli showed various degree of hydrolysis of platycodin glycosides during fermentation. The cell extracts of *Bifidobacterium* sp. Int57 showed the greatest hydrolysis activity of platycodin glycosides among the cell extracts of various probiotic bacteria. *Aspergillus niger* KCTC 6906 showed enhanced hydrolysis when the organism was incubated in the presence of rhamnose and platycodins. Liquid chromatography coupled with electrospray ionization tandem mass spectrometry (LC-MS) analysis of the hydrolysis patterns of the platycodins with *Aspergillus niger* KCTC 6906 showed that the main site of partial hydrolysis of 28-sugar chains in platycodin D lay between rhamnose and xylose. The cytotoxicity on V79-4 (Chinese hamster lung fibroblasts, normal cell) of platycodin was significantly decreased after hydrolysis (90% cell viability). The hemolytic toxicity was significantly decreased after transformation. In addition, the sensory scores responsible for the pungency, bitterness, and after-tastes were all significantly reduced by the hydrolysis of platycodin glycosides. Interestingly, the foaming property of platycodin saponin was maintained after hydrolysis. These results suggested that the proper combination of food microorganisms and fermentation conditions might provide a transformed platycodin product, revealing reduced cytotoxicity and enhanced sensory value.

Trignoella foenum-graecum (TFG) (fenugreek), a widely used medicinal and dietary herb, is abundant in saponins, such as diosgenin [43]. Its seeds have been used as herbal medicine for antidiabetic and hypocholesterolemic agents and also employed as a condiment and for improvement of appetite [8]. In mild to moderate type 2 diabetes mellitus patients enrolled in a double-blind placebo-controlled study, fenugreek seeds improved glycemic control and decreased insulin resistance [14]. On a first transformation study of the fenugreek glycosides, TFG saponins were transformed by extracted crude microbial enzymes, but weakly by live microorganisms in culture media. Among those microbes tested, *Bifidobacterium* sp. Int57 showed the greatest enzyme activities and effectively transformed TFG saponins. Antidiabetic activity was measured by assessing the inhibition of α-glucosidase and α-amylase. Deglycosylated fenugreek saponins inhibited α-glucosidase and pancreatic α-amylase activity higher than glycoside saponins at the concentration of 0.1 mg/ml. Overall, these findings suggested that transformed TFG saponins using microbe enzymes might contribute to hypoglycemic effect.

The reviewed demonstrations that various edible microorganisms produce specific forms of transformed compounds may indicate that it is feasible to develop a specific bioconversion process to obtain specifically designed functional products by the appropriate combination of glycosides substrates and specific microbial enzymes.

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