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Brief Communication

Quantitative Analysis of Chemical Components of Hydrolysate from Silkworm Fed with *Cudrania tricuspidata* Leaves

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The purpose of this study was to develop hydrolysate from silkworm (Cudrania Silkworm Fibroin Peptide; CSFP), a food containing components for improving blood vessel health. In general, *Cudrania tricuspidata* leaves contain about 5 times more rutin and 25 times more dihydroquercetin than mulberry leaves. 1-Deoxynojirimycine (1-DNJ), dihydroquercetin and rutin inhibit the activity of carbohydrate-digesting enzymes, inhibit blood lipid peroxidation, and regulate insulin secretion, which helps blood vessels to be healthy. When the diet-controlled silkworm was enzymatically hydrolyzed, it was confirmed that rutin content was about 8 times higher than that of the in general silkworm as a control. In the silkworm hydrolysate, CSFP, developed as a final food material, the active ingredients were 65 mg/kg for rutin, 3,328 mg/kg for DNJ, 0.43 mg/kg for dihydroquercetin, and 82,624 mg/kg for total polyphenol, which was confirmed through LC-MS/MS analysis. In conclusion, it was found that silkworms fed with *C. tricuspidata* leaves as a diet had more active components that can help control blood sugar and improve blood vessel health than silkworms fed with mulberry leaves.

Key Words: Silkworm, *Cudrania tricuspidata*, CSFP, Cudrania Silkworm Fibroin Peptide, Vascular health

Recently, in the sericulture industry, functional sericulture is actively being developed as a health functional food development for lowering blood sugar using in general dried silkworms (*Bombyx mori* L.) for the purpose of increasing farm household income, that is, a study from "wearing sericulture" to "eating sericulture", but industrial use is still limited.

Common silkworm powder is used as a food material for blood sugar control, energy recovery, male health promotion, skin beauty improvement, and pharmaceuticals (Choi et al., 2000; Jang and Rhee, 2004; Kim et al., 2005).

Silkworms fed with mulberry leaves have different components and functions depending on the leaves they feed. Compared to in general mulberry leaves, *Cudrania tricuspidata* leaves contain about 5 times more GABA (gamma-aminobutyric acid), which helps brain activity and promotes the metabolic function of brain cells. It also contains about 18 times more rutin, which is effective for blood vessel health. However, studies and industrialization on the functionality of silkworms fed with *C. tricuspidata* leaves and products using silkworms are insufficient.

The results of studies on the rutin content of each type of mulberry leaf are also well known (Kim et al., 2014). There is a study reporting the contents of DNJ by extracting *Cudrania tricuspidata* leaves with water and ethanol. At this time, the contents of DNJ and rutin were higher in

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leaves than in stems. Also, the DNJ content of the alcohol extract was higher than that of the water extract. The physiological activities of extracts from the leaf, stem, and fruit of *Cudrania tricuspidata* were investigated. The total polyphenol contents were the highest in the leaf extracts under all extraction conditions (Do et al., 2011; Lee et al., 2011).

Silkworm cocoon extract was certified as a memory enhancing food for improving memory by the Food and Drug Administration in 2005.

In the case of large-scale farms, silkworms that are difficult to consume as food are mainly collected because it is difficult to input intensive labor force when collecting silkworms at the appropriate harvest time. In order to increase farm household income and increase the value of silkworm products, processing technology that can develop edible ingredients from silkworms that cannot be used as food raw materials is required.

From the food industry point of view, silkworms raised by feeding *C. tricuspidata* leaves have a wide range of application to the health functional food field, and are highly likely to be excellent high-functional, high-protein food materials when mass-produced and industrialized. For the efficient production of *C. tricuspidata* leaves fed silkworms containing various active ingredients, it is essential to carry out various researches such as stable supply of *C. tricuspidata* leaves, optimization of production efficiency of *C. tricuspidata* leaves fed silkworms, powderization of *C. tricuspidata* leaves fed silkworms, and establishment of an optimal mass production process. In addition, it is possible to commercialize and develop a sales network as an elderly-friendly product by verifying the health promotion effect related to metabolic syndrome and memory improvement of *C. tricuspidata* leaves fed silkworm.

In order to contribute to generating farm household income by diversifying sales channels of sericulture farms and fostering the future agricultural industry, we intend to produce hydrolysate from *C. tricuspidata* leaves fed silkworm. In addition, through product development using this food material, we will improve the usability of *C. tricuspidata* leaves fed silkworm and develop materials to commercialize multifunctional food materials with excellent price competitiveness.

Silkworms (*Bombyx mori* L.) are raised up to 7 days, 5 aged in a farmhouse located in Wonju, Korea. The seed silkworms were initially fed mulberry leaves until the 6th day of the 5 aged, and after fasting for 1 day, they were fed *C. tricuspidata* leaves again until the 7th day of the 5 aged and steamed. Steam runs at 100℃ for 120 minutes. Mulberry (*Morus alba*) leaves and *Cudrania tricuspidata* leaves were collected from a mountain located in Jeongeup, Korea.

Rutin and dihydroquercetin (Sigma) were used as a standard. Initially, the optimization of the LC-MS/MS program was made. For which different energies were used to generate a qualifier ion and a quantifier ion for each standard. Serial dilutions of the standards (1-2-5-10-20-50-100-200 μg/kg) were used to obtain a linear standard curve. A C18 column was used, and methanol was used as the mobile phase solution.

The sample preparation was extract 1 g of sample with 10 mL of deionized water for 10 minutes. And extracted with 10 mL of methanol for 10 minutes. Diluted by step dilution with methanol. And finally filtered with a syringe filter of 0.2 μm and use it.

1-Deoxynojirimycin (Sigma) was used as a standard. Serial dilutions of the standards (1-2-5-10-20-50-100-200 μg/kg) were used to obtain a linear standard curve. A C18 column was used, and 50% acetonitrile was used as the mobile phase solution.

The sample preparation was extracted with 1 g of sample with 10 mL of deionized water for 10 minutes. And extracted with 10 mL of ethanol for 10 minutes. Diluted it with 50% acetonitrile by step dilution. And finally filtered with a syringe filter of 0.2 μm and use it.

The aqueous solution for enzyme treatment of silkworm was produced by adding 5 times (w/w) distilled water to the silkworm powder ground at 60 mesh, vortexing it for 1 minute and adjusting the appropriate pH of the enzyme to 5.5 with 0.5 N HCl. The enzyme diluted at 1:9 was pre-activated at 50℃ for 2 hours, and then added to 0.5%, 1%, 1.5%, and 2% of the weight of the silkworms fed *C. tricuspidata* leaves. Experiments were conducted using Viscozyme and Celluclast (NOVOZYMES) as candidates for selecting an appropriate enzyme. After the enzyme was added, the mixture was stirred at 170 rpm in a constant temperature water bath at 50℃ and reacted for up to 12 hours. After the decomposition reaction, the enzyme was inactivated and then centrifuged (4,000 rpm, 20 minutes) to separate the solution and measure the decomposition.

Mulberry leaves of *Morus alba*, a deciduous broad-leaved tree, have been used as a food source for silkworms for a long time. There are various varieties, and white mulberry is the most cultivated in Korea, and it has been used for sericulture, food, medicine, and industrial purposes. Mulberry leaves contain amino acids, vitamins, minerals, dietary fiber, and physiologically active substances such as flavone, steroids, and triterpenes, and contain rutin, quercetin, quercitrin, iso-quercitrin, and 1-deoxynojirimycin having α-glucosidase inhibitory activity (Yoshikuni et al., 1988; Kim et al., 1998; Doi et al., 2000; Park et al., 2012; Kim et al., 2018).

C. tricuspidata stem contains a large amount of xanthones (polyphenols) and flavonoids, so it is effective in antioxidant and cytotoxicity (Byun et al., 2019; Shi et al., 2014), and the fruit is effective in antipyretic antibacterial activity, nerve cell protection, and muscle relaxation (Jung et al., 2013; Kim et al., 2018). It has been reported that the *C. tricuspidata* tree is rich in antioxidants and can be sufficiently obtained by hot water extraction (Cha et al., 1999).

Table 1. Difference in functional components of mulberry leaves & *Cudrania tricuspidata* leaves

Functional substance	Mulberry leaf	Cudrania Mulberry leaf
Rutin (mg/kg)	1,302.2	5,198.7
Total polyphenol (mg/kg)	36,972.8	52,676.8
Dihydroquercetin (mg/kg)	3.8	75.9
1-Deoxynojirimycin (mg/kg)	650.8	0

C. tricuspidata leaves are about four times higher in rutin and 25 times higher in dihydroquercetin than mulberry leaves (Table 1). It was confirmed that rutin and total polyphenol content were higher in *C. tricuspidata* leaves than in general mulberry leaves, and it was confirmed that the content of polyphenols and dihydroquercetin related to antioxidant activity was relatively high.

Silkworms eat mulberry leaves well and consume less *C. tricuspidata* leaves, so the rutin content is not high in general silkworms. The reason for the high level of rutin in the silkworms used in this study seems to be that the breeding conditions were well controlled to ensure good intake of *C. tricuspidata* leaves and that they consumed a lot of *C. tricuspidata* leaves.

Enzymatic treatment was performed to maximize the extraction of active ingredients from mulberry leaves and *C. tricuspidata* leaves. The enzymes used at this time were

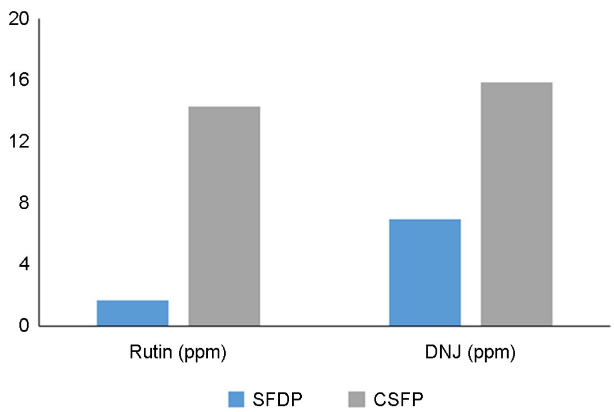

Fig. 1. Comparison of rutin and 1-deoxynojirimycin of steaming silkworm FD powder (SFDP[1]) and steaming silkworm concentrate (semi-purified CSFP[2]) with 2% Viscozyme treatment conditions with the same dry basis evaluation as a raw material. SFDP[1]: Steaming silkworm Freeze Drying Powder, CSFP[2]: Cudrania Silkworm Fibroin Peptide concentrate.

Table 2. Change in hydrolysis degree of mulberry leaves and *Cudrania tricuspidata* leaves dried powder during enzyme hydrolysis condition

Enzyme DH	Enzyme concentration							
	Mulberry leaves				*Cudrania tricuspidata* leaves			
	0.5%	1.0%	1.5%	2.0%	0.5%	1.0%	1.5%	2.0%
Viscozyme	38.52	46.60	48.48	49.77	29.62	58.15	58.42	59.51
Celluclast	38.17	44.96	46.84	48.83	18.21	18.48	29.62	19.02

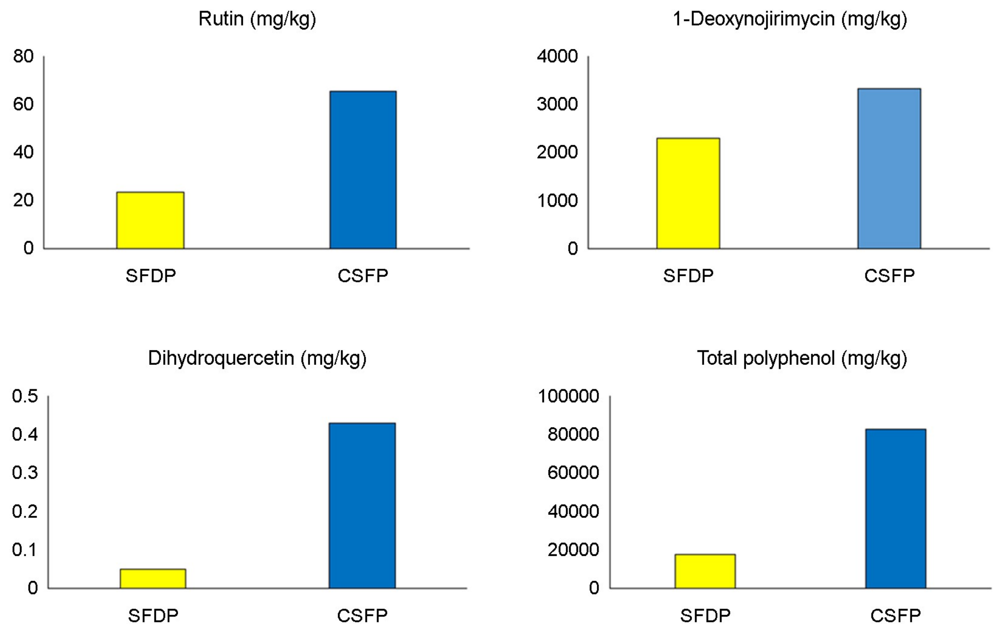

Fig. 2. Comparison of rutin, 1-deoxynojirimycin, dihydroquercetin and total polyphenol of steaming silkworm freezed dried powder (SFDP[1]) and powdered steaming silkworm hydrolysate using dextrin as an excipient maltodextrin (CSFP[2]). SFDP[1]: Steaming silkworm Freeze Drying Powder, CSFP[2]: Cudrania Silkworm Fibroin Peptide concentrate.

Viscozyme and Cellullast, which consist mainly of cellulase. As the concentration of each enzyme increased, the hydrolysis effect was proportional. Among them, the selected enzyme was Viscozyme, which was effective in hydrolysis of *C. tricuspidata* leaves. The hydrolysis of leaves was highest in Viscozyme with 2% of leaves content (Table 2). Viscozyme and Celluclast, which are polysaccharide degrading enzymes. Viscozyme is composed of arabinase, cellulase, β-glucanase, xylanase, and hemicellulase, and Celluclast is cellobiohydrolase, 1,4-β-D-glucisidase, 1,4-D-gluconase. It is a complex enzyme and its optimal temperature and optimum pH ranged from 40 to 60℃ and 3.5 to 5.5, respectively, and other reagents used special grade reagents.

When the Silkworm fed *C. tricuspidata* leaves was hydrolyzed, it was confirmed that it had 8 times rutin and 2 times 1-DNJ content compared to in general silkworm powder (Fig. 1). Compared to the freeze-dried silkworm powder obtained by steam treatment, the silkworm hydrolyzed powder ingested the *C. tricuspidata* leaves showed relatively high rutin and 1-DNJ content. Due to the relatively high rutin and 1-DNJ content, the silkworms that ate the leaves of *C. tricuspidata* leaves were shown to be endowed with sufficient functionality through the enzyme hydrolysis process.

As shown in Fig. 2, the hydrolyzed powder of silkworms fed with *C. tricuspidata* leaves generally has a relative content of rutin, 1-DNJ, dihydroquercetin, and total polyphenol known to help vascular health compared to the freeze-dried powder of silkworms fed with mulberry leaf.

Since research and product development related to *C. tricuspidata* leaves fed silkworm hydrolysate is the first field attempted in Korea and abroad, there is no standardization standard, and quality standards for materials and products developed through this study will be standardized.

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CONFLICT OF INTEREST

The authors declare that they have no conflict of interest.

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