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Quantitative analysis of rutin content using silkworm genetic resources

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Abstract

Rutin is an important bioflavonoid that is consumed in the daily diet. This study compared the functional components of rutin from various silkworm species using a gene database with those of rutin produced by silkworms selectively bred through cross-combinations. We made comparisons between the geographical origin and species of silkworm using a gene database and discovered that rutin activity was ranked in the following order by species, Chinese (C5)> miscellaneous varieties (Jamsaeng 1 Ho) >Japanese (Jam 115) > European (E58) >Korean (Sun 3 ho). However, rutin levels with respect to various genetic traits (blood color, silk color, and egg color) were consistent. In order to study rutin changes that occurred during the cross breeding of the silkworm gene, we bred cross-combinations utilizing Jam 115 and the 4051 silkworms. In conclusion, in order to provide information about the constituents of functional materials contained in silkworm powder, it is imperative that silkworm cross breeding occurs so that the database of functional materials extracted from silkworms will expand.

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Introduction

Flavonoids exhibit a wide variety of biological activities, including antiviral, antibacterial, anti-inflammatory, and antioxidant actions (Javed *et al*., 2012; Richetti *et al*., 2011; Nassiri-Asl *et al*., 2010). Among flavonoids, rutin (2-phenyl-3,5,7,3',4'-pentahydroxybenzopyrone), is a non-toxic flavonoid glycoside with P vitamin activity and a bioflavonoid and antioxidant (Schwedhelm *et al*., 2003; Janbaz *et al*., 2002). Rutin can be broadly extracted from vegetables, fruits, herbs, leaves, seeds, red wine and several plantssuch as buckwheat, passion flower, apple and tea (Harborne, 1986; Havsteen, 1983). It has been demonstrated that rutin scavenges superoxide radicals and can chelate metal ions such as ferrous cations. More importantly, many studies have been conducted to prove the efficiency of rutin's pharmacological functions as an antioxidant (Gao *et al*., 2002; Nagasawa *et al*., 2003; Kamalakkannan and Prince, 2006).

The silkworm, *Bombyx mori* L. has long been used in China and Korea as a folk remedy for the treatment of diabetes and is an economically important insect that converts mulberry leaf (*Morus* spp.) protein into silk. Mulberry leaves have been used to feed silkworms (*Bombyx mori*). These leaves are rich in flavonoids, alkaloids, and polysaccharides components that have been identified as some of the most potent major active compounds by chemical constituent research. The flavonoids in mulberry leaves were rutin, quercetin, isoquercitrin,

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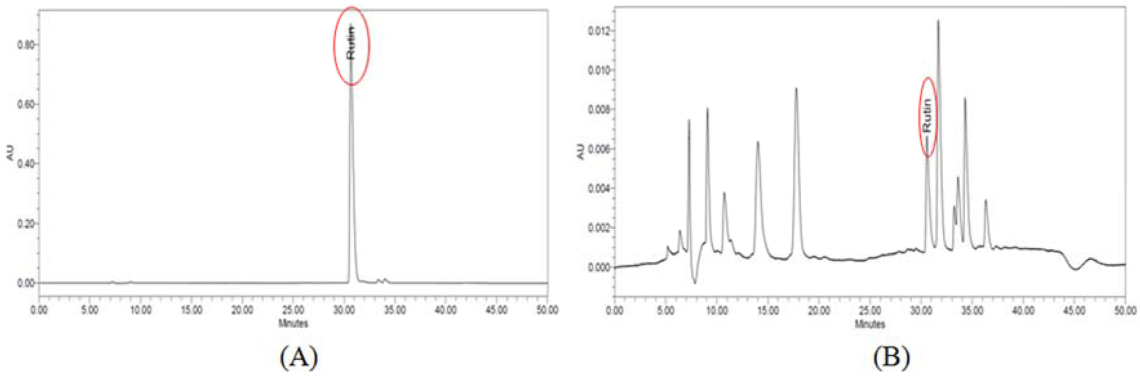

Fig. 1. Analysis chromatogram of rutin standard (A) and silkworm variety C5 containing the best rutin content (B).

and quercetin 3-(6-malonylglucoside) (Lee *et al*., 2007). A previous study indicated that mulberry leaf extracts could significantly reduce blood glucose, high blood pressure, high cholesterol, neutral fat and prevent thrombus formation and ageing. Mulberry is one of the plants that contain the highest levels of flavonoids in their dried leaves with levels of 1-3% (Chen *et al*., 2008). These flavonoids have found applications in food and pharmaceutical industries for their valuable properties, and adsorbent resins have been used to separate and concentrate these products from the natural matrixes (Fu *et al*., 2005; Qi *et al*., 2007). In particular, 1-deoxynojirimycin (DNJ), an intestinal α-glucosidase inhibitor, lowers blood glucose levels and is present in mulberry leaves and sericulture products such as silkworm powder (Asano *et al*., 2001; Asano, 2003). A previous study showed that in silkworms that eat only mulberry leaves, DNJ concentrations in the blood of the silkworms are 3 times higher than that in the mulberry leaves.

Similarly, silkworm powder is also rich in useful components and has long been favored as an antidiabetic agent, and for its various amino acids and flavonoids. However, the flavonoid contents of silkworm powder have not been tested using scientific methods. To date, to our knowledge, no studies have shown the extraction of bioactive compounds (including rutin) from silkworm powder except for DNJ. The aim of this study is to determine the main flavonoid (rutin) using high performance liquid chromatography (HPLC). In addition, we wanted to provide a basis for understanding rutin content in various silkworm species. Furthermore, our goal was to create a database for silkworm genetic resources utilizing morphological and, genetic characteristics data.

Materials and Methods

Materials

Silkworm resources were collected from the Sericulture and Apiculture Division for the Department of Agricultural Biology, RDA in Suwon, Republic of Korea. Both spring and autumn reared silkworms of 168 varieties were used for the rutin analysis (Japanese 64; Chinese 69; European 28; miscellaneous species 5; Korean 2). The freeze-dried 5^{th} instar, 3-day-old silkworms (Ilshin Lab Co., Ltd) were ground and used within 48 h.

Sample preparation procedures

About 1 g of larva powder was mixed with 20 mL methanol, followed by vigorous shaking at 80°C for 1 h, and then treated with sonication for 30 min. These extracts were cooled to 4°C and subjected to preliminary filtration. The pellet was treated again by repeating the above steps, and the extracts were diluted to 50 mL methanol. The extracts were filtered using a 0.45 μm Millipore filter and used for HPLC analysis.

Rutin measurement

In order to measure rutin within the analytes, HPLC (Waters e2695), Waters 486 Tunable Absorbance UV/visible detector (Em 254, EX 322), and Waters Nova-Pak C_{18} column were used. The absorbance of the effluent was monitored at 355 nm and flow rate was 1 mL/min. The mobile phase used was on 2.5% acetic acid : methanol : acetonitrile = 70:10:20 (V/V/V). The flow rate was 0.6 mL/min. The standard material was rutin (SIGMA), and the rutin content was calculated (Fig. 1.).

Results and discussion

Rutin contents of 168 silkworm varieties

There are many reports on rutin in *Morus*, and it was found that rutin contents in mulberry leaves were affected by many factors, such as varying *Morus* species and seasons. However, almost no studies were performed on the silkworm, and rutin accumulation and excretion in the larva are still not clear.

Table 1. Rutin contents for Japanese silkworm varieties

No.	Strain (Variety)	Content (%)	No.	Strain (Variety)	Content (%)
1	Jam 115	0.446	33	N12	0.215
2	N15	0.399	34	N64	0.199
3	J95	0.376	35	Suwon 10	0.199
4	Myeon 49	0.369	36	W-IL-2	0.197
5	N27	0.369	37	IL-111	0.197
6	Chuhwa	0.355	38	34	0.194
7	JIN	0.352	39	Jam 107	0.188
8	N13	0.352	40	Gwaechuk	0.185
9	Seolak	0.350	41	Bukak	0.176
10	N80	0.344	42	Baekdoo	0.173
11	N76	0.331	43	N32	0.169
12	N6	0.318	44	N59	0.168
13	MooDeung	0.315	45	N30	0.163
14	N74	0.314	46	N39	0.159
15	Hwangyu	0.307	47	Baekan EB	0.159
16	JF	0.295	48	Jam 109	0.159
17	N44	0.295	49	N29	0.157
18	N65	0.289	50	Hinode	0.157
19	Myohyang	0.281	51	N19	0.156
20	N43	0.275	52	Wooseongrokeu	0.154
21	N69	0.268	53	N71	0.153
22	N28	0.267	54	Bibaekjam	0.147
23	Jam 103	0.255	55	SK-1	0.134
24	Wooseok	0.254	56	N-Heebaeklan	0.124
25	Heukjam	0.252	57	IL-9	0.124
26	11	0.250	58	RHS	0.123
27	N26	0.247	59	BN	0.122
28	N9	0.245	60	W109	0.121
29	N63	0.225	61	WooseongJeokeu	0.119
30	N18	0.224	62	N50	0.115
31	Moran	0.219	63	IL-83	0.088
32	N24	0.215	64	4051	0.069

Table 2. Rutin contents for Chinese silkworm varieties

No.	Strain (Variety)	Content (%)	No.	Strain (Variety)	Content (%)
1	C5	0.662	36	Hooksilkworm	0.217
2	Shinjoong102	0.504	37	ZO	0.211
3	C66	0.421	38	C70	0.211
4	C16	0.394	39	C44	0.201
5	C46	0.389	40	C7	0.198
6	KH	0.381	41	C53	0.198
7	Gwaseolpyung	0.381	42	LY	0.195
8	C12	0.378	43	Joong 116	0.186
9	C14	0.356	44	C11	0.186
10	Daedong	0.349	45	C57	0.186
11	C18	0.339	46	C48	0.172
12	Nakdong	0.334	47	Ungjinhee	0.158
13	C68	0.333	48	Hanseongbanmoon	0.157
14	C45	0.320	49	C76	0.154
15	C27	0.320	50	C78	0.151
16	C10	0.309	51	C60	0.149
17	Wooseongrokgyeon	0.309	52	Geumgang	0.145
18	R-Hwang	0.298	53	C51	0.144
19	C25	0.297	54	141	0.143
20	4056	0.294	55	Jam 104	0.141
21	C79	0.289	56	Woorokbaeklan	0.137
22	Galwon	0.269	57	Cheongcheon	0.134
23	C26	0.267	58	Hansaeng 2	0.133
24	C17	0.259	59	Joong 17	0.130
25	UR	0.259	60	Jam 116	0.130
26	Gal H	0.255	61	Sammyeonhong	0.128
27	Hanseonghueklan	0.252	62	C31	0.124
28	Joongjong	0.241	63	Cheonmoon	0.117
29	Q-Heebaeklan	0.237	64	Hangang	0.116
30	C-Heebaeklan	0.234	65	TeukC60	0.102
31	SC	0.231	66	Y4	0.095
32	C3	0.231	67	CRIMSON	0.087
33	Soyang	0.229	68	Joong 14	0.080
34	C42	0.227	69	C61	0.056
35	Yonggakjam	0.220			

Table 3. Rutin contents for various of silkworm varieties

Species	No.	Strain (Variety)	Content(%)	No.	Strain (Variety)	Content(%)
Europe (28 types)	1	E58	0.403	15	Z3	0.198
	2	Hojam	0.372	16	NTIOPE	0.194
	3	LT	0.308	17	Goo 27	0.188
	4	IJPE	0.308	18	ROMOGUA	0.188
	5	E56	0.274	19	B-1	0.181
	6	Goo 17	0.268	20	Woobaek	0.180
	7	BAGDAD	0.255	21	AKT	0.149
	8	PEDTS	0.221	22	Q	0.135
	9	LEMON	0.219	23	Hueka	0.125
	10	Hanseonghojam	0.219	24	SK-2	0.098
	11	IHENALIG	0.216	25	Gyeonsaekjeok	0.097
	12	PK	0.208	26	ROK191	0.073
	13	Yulkookjam	0.200	27	AP	0.062
	14	Syansuerian	0.198	28	ROK1042	0.056
Miscellaneous species (5 types)	1	Jamsaeng 1 Ho	0.613	4	Baeklan	0.213
	2	Jamsaeng 2 Ho	0.276	5	PR	0.155
	3	TBO	0.228			
Korean (2 types)	1	Sun 3 ho	0.319	2	Sammyeonhong-hoibaek	0.209

By using data acquired from this study, we built a database of silkworm and mulberry resources with morphological and genetic characteristics data. Moreover, certain bioactive compounds have been receiving increasing attention. We analyzed rutin content in addition to the base genetic information by using 168 varieties of silkworms. All silkworms were freeze-dried 5^{th} instar species that were 3 d old.

Results showed that overall, Jam 115 had the highest rutin content with 0.446% (Table 1). In contrast, the 4051 had the lowest rutin content with 0.069%. Of the Chinese silkworm types, C5 had the highest rutin content (0.662%) while C61 had the lowest of all varieties with 0.056% (Table 2).

Of the European breeds, E58 had the highest rutin content (Table 3). The rutin content of the Korean varieties was relatively 0.319% (Sun 3 ho) and 0.209% (Sammyeonhonghoibaek) (Table 3). On the other hand, the highest rutin levels of the miscellaneous varieties were found in Jamsaeng 1 Ho (0.613%). For comparison of geographical origins of the silkworm gene resource, rutin concentration ranking was as follows: Chinese (C5) > miscellaneous varieties (Jamsaeng 1 Ho) >Japanese (Jam 115) > European (E58) > Korean (Sun 3 ho) (Table 3). In addition, no discernable difference in rutin content was noted with regard to blood, cocoon, or egg color of the silkworm.

Many flavonoids have been identified from the root bark of the mulberry tree. The leaves of *M. alba* L. contain quercetin derivatives such as isoquercitrin and rutin (Naito, 1968). Fujimoto and Hayashiya (1972) reported that the flavonoids in the cocoon shell of *B. mori* were also made from an artificial diet containing quercetin, rutin or isoquercitrin. Rutin was also found to be similarly distributed within caper plant, common buckwheat (Sofic *et al*., 2010), and amaranth plants, where the highest amount of rutin was found in the leaves (Kalinova and Dadakova, 2009). This was established from levels of 4% to 9% of rutin per dry weight depending upon the stage of development of the plant (Kalinova *et al*., 2006). In addition, Atanassova and Bagdassarian (2009) found that in the case of dry fruits and vegetables, the rutin content show little variation in range (from 0.15% to 0.18%), with the exception of the red hot chili pepper (0.22%) and aronia (0.34%).

Table 4. Rutin contents by silkworm cross-breeding

Cross combination		Content (%)	
		F0	F1
Japanese	Jam 115 (♀)	0.446	0.297(↓)
	Jam 115 x 4051		
	4051 (♂)	0.069	
Chinese	C5 (♀)	0.662	0.383(↓)
	C5 x C61		
	C61 (♂)	0.056	

Rutin contents produced by crossbreeding

The purpose of the cross-breeding experiment was to optimize hybrid combinations that yielded rutin concentrations higher than that by the basic silkworm larvae. The rutin contents of various silkworm varieties were investigated with results indicating a difference among the various silkworm varieties. When the varieties Jam 115 and 4051 were hybridized, the rutin content of the offspring was lower than the parent Jam 115. Likewise, in a cross between the Chinese breeds C5 and C61, the rutin content was lower in the hybrid than in the C5 parent (Table 4). These results show that the crossbreeding tests were not effective in the production of offspring with sustained or improved rutin concentrations. The breeding objectives employed in different countries where sericulture is practiced remained quite different for many of the traits and also to provide productive hybrid strains specifically for commercial exploitation (Nagaraju, 2002). In addition, hybrid silkworm strains are recommended for large scale laboratory and intensive, commercially viable sericulture operations. Regarding the concentration of DNJ in silkworm powder, the DNJ contents produced by crossbreeding silkworm were higher than those in non-crossbreeding silkworm (Ju *et al*., 2014). However, these results indicate that the rutin content in silkworm powder was affected by the variety of silkworm. We conclude that it is the most effective and economic strategy to use non-hybrid silkworms by crossing the 5th instar parent variety larvae of 3 d of age.

In conclusion, obtaining information about the concentration of functional materials in silkworm powder could contribute to the development and promotion of processed, functional products derived from silkworm.

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References

Asano N, Yamashita T, Yasuda K, Ikeda K, Kizu H, Kameda Y, Kato A, Nash RJ, Lee HS, Ryu KS (2001) Polyhydroxylated alkaloids isolated from mulberry trees (*Morus alba* L.) and silkworms (*Bombyx mori* L.). J. Agric. Food Chem. 49, 4208-4213.

Asano N (2003) Glycosidase inhibitors: update and perspectives on practical use. Glycobiol., 13 93R-104R.

Atanassova M, Bagdassarian V (2009) Rutin content in plant products. J. Chem. Technol. Metall. 44(2), 201-203.

Chen Y, Wang J, Jiang H (2008) Optimization of extraction technology of total flavonoids from mulberry leaves by orthogonal design. J. Food. Drug 10(3), 17-20.

Fu B, Liu J, Li H, Li L, Lee FSC, Wang X (2005) The application of macroporous resins in the separation of licorice flavonoids and glycyrrhizic acid. J. Chromatogr. A 1089 (1-2), 18-24.

Fujimoto N, Hayashiya K (1972) Studies on the pigments of cocoon. (IX) The precursor of the pigments of green cocoon in the silkworm, *Bombyx mori*. J. Seric. Sci. Jpn 41, 383-386.

Gao Z, Xu H, Huang K (2002) Effects of rutin supplementation on antioxidant status and iron, copper, and zinc contents in mouse liver and brain. Biol. Trace. Elem. Res. 88, 271-279.

Harborne JB (1986) Nature distribution and function of plant flavonoids. Prog. Clin. Biol. Res. 213, 15-24.

Havsteen B (1983) Flavonoids, a class of natural products of high pharmacological potency. Biochem Pharmacol. 32, 1141-1148.

Janbaz KH, Saeed SA, Gilani AH (2002) Protective effect of rutin on paracetamol and CCl4-induced hepatotoxicity in rodents. Fitoterapia 73, 557-563.

Javed H, Khan MM, Ahmad A, Vaibhav K, Ahmad ME, Khan A, Ashafaq M, Islam F, Siddiqui MS, Safhi MM, Islam F (2012) Rutin prevents cognitive impairments by ameliorating oxidative stress and neuroinflammation in rat model of sporadic dementia of Alzheimer type. Neuroscience 17, 340-352.

Ju WT, Kim KY, Sung GB, KimYS (2014) Quantitative analysis of 1-Deoxynojirimycin content using silkworm genetic resources. Int. J. Indust. Entomol. 29(2), 162-168.

Kalinova J, Triska J, Vrchotova N (2006) Distribution of vitamin E, squalene, epicatechin, and rutin in common buckwheat plants (*Fagopyrum esculentum Moench*). J. Agric. Food. Chem. 54(15), 5330-5335.

Kalinova J, Dadakova E (2009) Rutin and total quercetin content in amaranth (*Amaranthus* spp.). Plant. Foods. Hum. Nutr. 64(1), 68-74.

Kamalakkannan N, Prince PS (2006) Antihyperglycaemic and antioxidant effect of rutin, a polyphenolic flavonoid, in streptozotocininduced diabetic wistar rats. Basic Clin. Pharmacol. Toxicol. 98, 97-103.

Lee CY, Sim SM, Cheng HM (2007) Systemic absorption of antioxidants from mulberry (*Morus alba* L) leaf extracts using an in situ rat intestinal preparation. Nutr. Res. 27(8), 492-497.

Nagaraju J (2002) Application of genetic principles for improving silk production. Curr. Sci. 83(4), 409-414.

Nagasawa T, Tabata N, Ito Y, Aiba Y, Nishizawa N, and Kitts DD (2003) Dietary G-rutin suppresses glycation in tissue proteins of streptozotocin-induced diabetic rats. Mol. Cell Biochem. 252, 141-147.

Naito K (1968) Studies on the micro constituent in mulberry leaves (VII). Nippon Nogeikagaku Kaishi (in Japanese) 42, 423–425.

Nassiri-Asl M, Mortazavi SR, Samiee-Rad F, Zangivand AA, Safdari F, Saroukhani S, Abbasi E (2010) The effects of rutin on the development of pentylenetetrazole kindling and memory retrieval in rats. Epilepsy Behav. 18, 50-53.

Qi Y, Sun A, Liu R, Meng Z, Xie H (2007) Isolation and purification of flavonoid and isoflavonoid compounds from the pericarp of *Sophora japonica* L. by adsorption chromatography on 12% cross-linked agarose gel media. J. Chromatogr. A 1140 (1-2), 219-224.

Richetti SK, Blank M, Capiotti KM, Piato AL, Bogo MR, Vianna MR, Bonan CD (2011) Quercetin and rutin prevent scopolamine-induced memory impairment in zebrafish. Behav Brain Res. 217, 10-15.

Schwedhelm E, Maas R, Troost R, Böger RH (2003) Clinical pharmacokinetics of antioxidants and their impact on systemic oxidative stress. Clin. Pharmacokinet. 42, 437-459.

Sofic E, Copra-Janicijevic A, Salihovic M, Tahirovic I, Kroyer G (2010) Screening of medicinal plant extracts for quercetin 3-rutinoside (rutin) in Bosnia and Herzegovina. Med. Plant. 2(2), 97-102.