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Development of powder with increased rutin content from mulberry leaves for the application of food materials

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Abstract

We studied on improvement method of rutin content using mulberry leaf powder. Mulberry leaves were collected and then hot-air dried and powdered for experiment. As a result, we have developed a pre-treatment method that extracts mulberry leaf powder with water or fermented alcohol with reflux extractor and then increases the rutin content by improving the process. Citric acid (0.1 ~ 1%) and 1000 ml fermented alcohol (50 ~ 95%) or water (10 ~ 50 times) was extracted with 100 g of mulberry leaf powder using a reflux extraction device (80~90°C, 1 hour, twice). The extracts were collected, filtered and concentrated. For the recrystallization, the concentrate was dissolved by adding distilled water and allowed to stand at a low temperature. Then, the supernatant was discarded by centrifugation, and only the residue was lyophilized to prepare a final powder. As a result, regardless of the concentration of citric acid added, the content of rutin was higher in 90% fermented alcohol extract. Whereas, in the case of extracting with water, citric acid 0.5% was added to water 25 times as much as the weight of mulberry leaf powder, and 2274.4 (mg / 100g) of rutin content was highest in the case of refluxing twice at 80 ° C for 1 hour. The powder with increased rutin content is expected to be applicable to various foods as a food additive. In addition, it can contribute to the improvement of the farm income by promoting consumption of mulberry leaf while satisfying the consumers' desire for functional food intake.

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Introduction

The mulberry leaves, root bark, and twigs have long been used in Chinese medicine to treat fever, protect the liver, improve eyesight, strengthen joints, facilitate discharge of urine, and lower blood pressure(Kim and Seok, 2013; Lee *et al*., 2013). Different parts of the mulberry, from the root bark to the leaves, have been extensively investigated for their various health benefits, including antioxidative (Kim *et al*., 2007; Kim, 2005; Nam *et al*., 2004), hypolipidemic (Dietschy and Wilson 1970; Mahley *et al*., 1974; Kim *et al*., 1998; Kim *et al*., 1999), and antihyperglycemic (Kim *et al*., 1999; Kimura *et al*., 1995; Khaw and Barret, 1987) effects. These studies have indicated that mulberry fruits and leaves exhibited significant scavenging effects on free

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radicals and protected low-density lipoprotein against oxidative damage. The reports suggested that polyphenols and flavonoids present in mulberry contribute to these health. Based on these effects, mulberry leaves are most often used as tea, and they are used in foods such as noodles, rice cakes, and cakes in powder form.

However, since the consumption market of mulberry leaves is very small compared with the consumption market of mulberry fruit, it is required to develop mulberry leaf food as a means for promoting the consumption of mulberry leaves and improving the income of farmers. Consumers also have a strong need for functional substance intake. Therefore, we have conducted studies to improve the content of functional ingredients to meet these needs. As a solution to this problem, we have developed a functional additive by preparing a powder with increased 'rutin' from mulberry leaves.

The first rutin ($C_{27}H_{20}O_{16}$) isolated from buckwheat is a vitamin P complex and quercetin-3-O-rutinoside, which is basically soluble in water, soluble in alcohol, acetone and alkaline solutions and not soluble in chloroform and ether (Kim *et al*., 1998; Park, 2003). The exact compound name for rutin is 2- (phenyl) -3,5,7,3 ', 4'-pentahydroxy benzopyrone, a compound of flavonoid that is widely distributed in plants. In addition to buckwheat, it is also abundant in painting trees, magnolias, pansy, marronier flowers, tobacco, sycamore leaves, rhubarb, tea leaves, persimmon leaves and kidney beans. Rutin exhibits capillary strengthening and capillary vasoconstriction and is used as a major component in the treatment of circulatory diseases, hypertension, and cofactors (Markham, 1989; Maeng *et al*., 1990; Choi *et al*., 1994; Kim *et al.,* 1994).

In this regard, it has been reported that mulberry leaves contain a large amount of rutin in mulberry leaves (Yun and Lee, 1995; Kim *et al*., 2014a; Kim *et al*., 2014b) and mulberry fruit (Lee *et al*., 1998; Kim and Kim, 2004).

As the function of mulberry leaves became known, many foods using mulberry leaves such as tea, noodles, kimchi, and ice cream have been developed, but the amount consumed is not so large.

Therefore, it is necessary to develop technology that can increase the income of the farm household by promoting the consumption of mulberry leaves and increasing the added value. To cope with this, rutin powder used as a functional food additive was prepared from mulberry leaves.

Materials and methods

Sample treatment

In the spring of 2016 (May), mulberry leaves, which are managed by standard cultivation method, were collected at the National Academy of Agricultural Science, Wanju, Korea. After washing 3 times in running water, it was spread thinly in the shade, and the water remaining in the mulberry leaves was removed by using a fan afterward. Then, the mulberry leaves were dried in a hot air circulating drier (Hanbaek Scientific, HB-503LF, Korea) until the mulberry leaves were completely dried (moisture content 10~13%) at 60~65° C, and they were made into powders using a household blender. Mulberry leaf powder was stored in the refrigerator until used for the experiment to improve the rutin content.

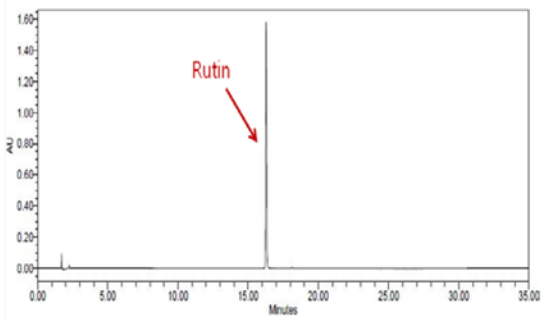
Rutin powder manufacturing process

Citric acid (0.1 ~ 1%) and 1000 ml fermented alcohol (50 ~ 95%) or water (10 ~ 50 times) was extracted with 100 g of mulberry leaf powder using a reflux extraction device (80~90° C, 1 hour, twice). The extracts were collected, filtered and concentrated. For the recrystallization, the concentrate was dissolved by adding distilled water and allowed to stand at a low temperature. Then, the supernatant was discarded by centrifugation, and only the residue was lyophilized to prepare a final powder.

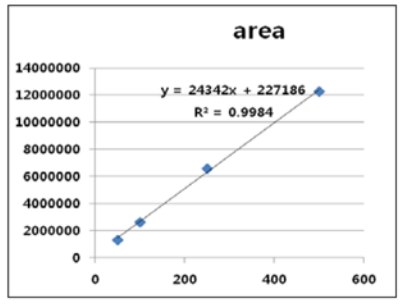
Rutin analysis

The rutin content of each sample prepared by pretreatment to increase the content of rutin, a functional material of mulberry leaves, was analyzed by HPLC and LC-MASS using a Waters Nova-Pak C18 column (300 × 3.9 mm). The detector was a Waters 486 Tunable Absorbance Detector and the wavelength was 355 nm. As a mobile phase, a mixed solvent of 2.5% acetic acid: methanol: acetonitrile = 70: 10: 20 (V: V: V) was used and the flow rate was 0.6 ml / min. The injection amount of the sample for HPLC analysis was adjusted to 20 μl, and the standard sample was the one of Sigma.

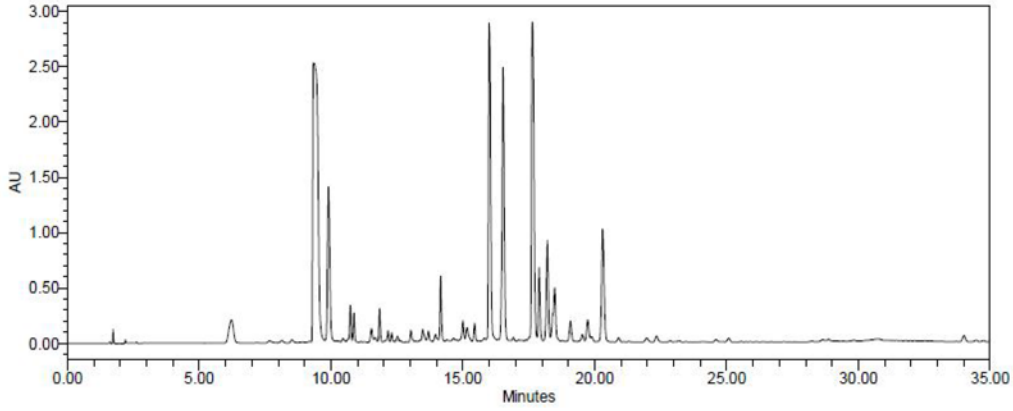
The standard curve was prepared at the concentrations of 50, 100, 250, 500 ppm. Retention time of rutin was about



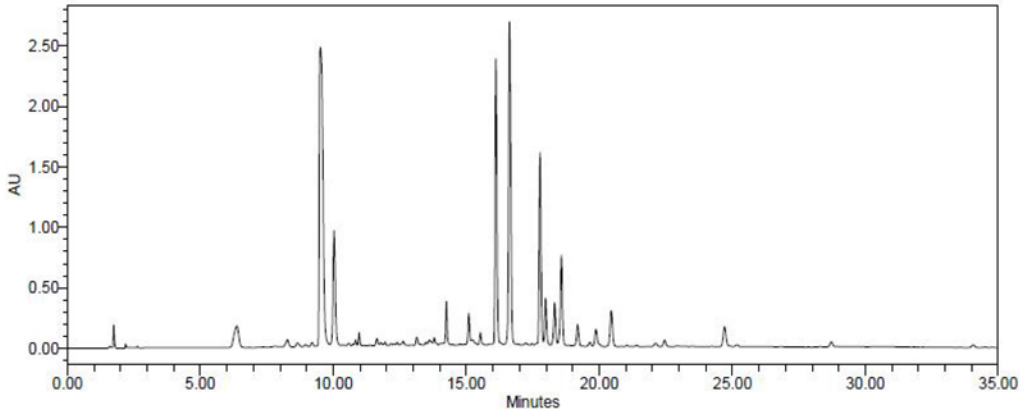
Standard 250 ppm



Standard curve(50, 100, 250, 500 ppm)



Mulberry rutin powder (Fermented alcohol extraction, 90% EtOH-0.5% citric acid)



Mulberry rutin powder (Water extraction, 25times diluton-0.5% citric acid)

Fig. 1. HPLC chromatogram of rutin in the mulberry

16minutes. The rutin content of each treatment was analyzed once, and its content was calculated by area ratio based on the standard concentration (Fig. 1). Also, the absorption spectrum and molecular weight of the mulberry rutin powders finally obtained by each treatment were confirmed using LC-MASS (Fig. 2).

Results and discussion

Mulberry leaves are originally feedstuffs of silkworms. Since 1995, however, Korea has been trying to use it as a food product as part of its 'functional sericulture'. As a result, in the early stage of research and development, mulberry leaf

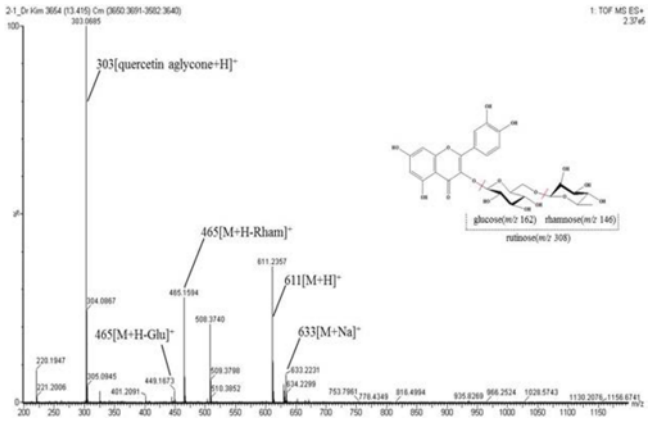


Fig. 2. LC-MASS spectra (positive ion mode) of rutin isolated in the mulberry leaf powder

noodle, tea, herb were developed (Kim, 2013). Especially mulberry leaves products such as powder, tablet and drink are continuously distributed in the market. Currently, mulberry leaves are sold at local festivals and experiential events centered on mulberry leaves, and the response from consumers is very good. However, these results are merely using the mulberry leaves themselves, and there are limitations in meeting the needs of modern people seeking for health except mulberry ice cream (Kim *et al*., 1999) and mulberry beer (Kim, 2012). The ice cream made with mulberry leaf powder, and the beer manufacturing method with mulberry leaf extract are considered to be technologies that enhance the added value of mulberry leaves.

Now, it is urgent to develop technology to meet the needs of consumers while improving income of mulberry leaf farmers.

We focused on the 'rutin' used as food additive among various functional ingredients contained in mulberry leaves and started research on 'making high functional food materials' using mulberry leaves.

To develop functional additives from mulberry leaves, a method to increase the rutin content was developed. By extracting, using a reflux extractor, filtering, concentrating, dissolving and low temperature curing (recrystallization), centrifugation and freeze drying, mulberry rutin powder was prepared.

Generally, methanol has been used to extract mulberry leaf rutin (Yun and Lee, 1995; Kim *et al*., 2014a; Kim *et al*., 2014b). However, in order to use mulberry leaves as a food additives, we decided to use fermented alcohol, water, and alkali solution that can be uses as an extraction solvent with references to the patent information for extracting the rutin from buckwheat (Ryu, 2002; Lee, 2006; Kim, 2007).

In preliminary experiments using these solvents, the rutin content was increased by 2~4 times as compared with methanol using fermented alcohol or water. Therefore, the alkali solution was excluded in this experiment.

Table 1. Rutin contents according to citric acid and alcohol extraction

Citric acid (%)	EtOH (%)	Powder yield (%)	Rutin content (mg/100g)
0.1	50	5.8	1161.4
	60	11.3	1217.5
	70	6.0	1153.3
	80	7.4	1250.0
	90	7.3	1318.0
	95	6.6	1261.7
0.3	50	5.7	1053.6
	60	7.6	860.6
	70	7.5	950.0
	80	6.2	953.8
	90	8.8	1250.6
	95	8.0	1200.1
0.5	50	6.9	1108.6
	60	8.9	967.1
	70	9.0	1076.6
	80	7.2	901.6
	90	8.8	1351.0
	95	8.4	1215.5
0.7	50	5.8	986.4
	60	6.5	1057.8
	70	8.3	805.9
	80	9.0	1139.0
	90	9.6	1181.7
	95	8.9	801.1
1.0	50	5.6	1011.5
	60	9.3	1005.1
	70	9.3	913.0
	80	8.3	788.0
	90	9.9	1062.1
	95	8.2	921.2

In the first fermented alcohol extraction experiment, the yield of mulberry rutin powder was 5.6 ~ 11.3%. In the case of 50% fermented alcohol extraction, the yield of the rutin powder was as low as 5.6 ~ 6.9%. On the other hand, in the case of 90% fermented alcohol extraction, the yield of rutin powder was 7.3 ~ 9.9%. As a result of analyzing the rutin contents for each treatment, regardless of the concentration of citric acid added, the content of rutin was higher in 90% fermented alcohol extract, especially when 0.5% citric acid was added (Table 1).

Therefore, in the next water extraction experiment, the treatment was simplified by reflecting this result. So, when the extract was extracted with water, citric acid 0.5% was added to water 25 times as much as mulberry leaf powder at the reflux extraction twice at 80 ° C for 1 hour, rutin content was the highest of 2274.4 (mg / 100g), and the yield was 6.5% (Table 2).

This method is highly likely to be used because it can produce rutin powder without special facilities or equipments in the farmhouse.

It is expected that the rutin powder developed from mulberry leaves will be applied to food such as noodles and confectionery as a functional additive, and the consumption market of mulberry leaves will be expanded in the future.

Table 2. Rutin contents according to dilution of water and citric acid extraction

Water dilution (times)	Citric acid (%)	Powder yield (%)	Rutin content (mg/100g)
×10	0.1	4.0	-
	0.5	4.7	126.6
	1.0	7.7	557.9
×25	0.1	5.2	1835.4
	0.5	6.5	2274.4
	1.0	6.0	1596.6
×35	0.1	5.0	1289.6
	0.5	6.3	1763.9
	1.0	8.3	1784.9
×50	0	7.5	1485.1
	0.1	8.0	2075.2
	0.5	6.5	858.8
	1.0	12.0	2110.3

Conclusion

Rutin is commonly known as a functional substance with antihypertensive effect. Furthermore, in the field of food industry, routines are used for beverages, confectionery, noodles, frozen foods, and retort foods as functional additives for preventing maintenance oxidation or maintaining flavor of foods. Therefore, modern people are seeking a healthy life through eating foods known to have a high content of roux such as buckwheat.

In this regard, we paid attention to mulberry leaves as a new material. Mulberry leaves are not only rich in rutin, but also contain various functional substances such as 1-deoxynojirimycin, and have been reported to have effects such as hypoglycemic action, hypertension inhibiting action, and cholesterol lowering. A variety of foods using mulberry leaves such as tea, noodles, kimchi, and ice cream have been developed, but the amount consumed is not so large.

Therefore, it is necessary to develop technology that can increase the income of the farm household by promoting the consumption of mulberry leaves and increasing the added value. To cope with this, rutin powder used as a functional food additive was prepared from mulberry leaves.

The mulberry leaf powder was prepared by extracting, using a reflux extractor, filtering, concentrating, dissolving and low temperature curing (recrystallization), centrifugation and freeze drying.

It is expected that the rutin content increase powder obtained from mulberry leaves can be used for various products as food additives and can contribute to the improvement of the farm income if the consumption is promoted.

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