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Physico-Chemical properties and antioxidant activity of rice cake with *Codonopsis lanceolata* powder

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Abstract

To increase the utilization of rice cake as a traditional food and encourage healthy consumption of rice cakes, rice cakes with greater functionality are required. This can be achieved by adding medicinal plants to rice cakes. This study aimed to improve the functionality of rice cake by addition of the medicinal plant *Codonopsis lanceolata*. An experiment was conducted on the quality, functionality, and physiochemical properties of rice cake containing *C. lanceolata* powder. We confirmed the antioxidant activity of rice cake containing *C. lanceolata* powder by evaluating the DPPH(1-diphenyl-2-picrylhydrazyl) radical scavenging activity. We also confirmed the physiochemical properties of rice cake in a basic analysis experiment to determine the pH, glucose amount, and total flavonoid content. This experiment showed that rice cake with *C. lanceolata* powder contains higher values in all five (pH, moisture, crude protein, crude fat, and total flavonoids) physical and chemical characteristics tested, compared to the control group. When measuring 100 mg/ml, the antioxidant activity in the control group was only IC_{50} = 844.26 mg/ml, whereas rice cake with *C. lanceolata* powder had activity of IC_{50} = 119.01 mg/ml. In this study, we obtained meaningful results confirming that rice cake containing *C. lanceolata* powder has high marketability and functionality. Consequently, we expect to increase the demand for traditional rice cakes, to enhance the value of rice cake as a healthy food and to contribute to the production of various rice cakes containing a medicinal plant.

Introduction

Our lives are enriched by the development of the economy. However, lifestyle diseases such as chronic addictive disease are rapidly increasing due to a lack of exercise, daily stress, environmental pollution, and nutritional imbalances caused by dietary changes (Ruiz-Núñez *et al*., 2013). These symptoms cause oxidative stress in body-modified DNA, cell membrane injury, protein degradations such as lipid oxidation, cancer, cerebrovascular diseases, autoimmune diseases, heart diseases, and atherosclerosis. They also increase the probability of chronic diseases such as digestive disorders (Jeon *et al*., 2013). One way to eliminate the risks of these diseases is to consume natural antioxidants that effectively eliminate free radicals (Joshi *et al*., 2007). Due to the increase in the standard of living and the improved quality of life, a large number of studies have been conducted with natural plant substances to aid health, aging, and other areas of interest to people's health (Devasagayam *et al*., 2004).

Codonopsis lanceolate (C. lanceolate), bellflower dicotyledonous plants and climbing vines that grow in mountainous areas in the South Korea, Japan, and China, are also called codonopsishaengyeop, white ginseng, gadeokmyeon, and jichwi (Kim *et al*., 2009). With its unique taste and aroma, *C. lanceolata* is used for various medicinal and edible purposes (Choi *et al*., 2014). The active ingredients and components in *C. lanceolata* are triterpenoid-type saponin, polyphenols, carbohydrates, saponins,

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fiber, tannin, essential oils, alkaloids, and steroids (He *et al.*, 2009). The skin is made of protein and carbohydrates, with a surplus of fat, vitamins and minerals such as calcium, phosphorus, iron, vitamin B_{12}, and vitamin B_2 (Kim *et al*., 2010). *C. lanceolata* is used as a traditional medicine in South Korea, China, and Japan. It has been used as an herbal remedy to prevent inflammation related to the lungs such as coughs (Lee *et al*., 2007) and bronchitis, as well as being established as an anti-cancer, anti-diabetic, anti-obesity, and an anti-lipogenic substance that effectively protects the liver (Lee *et al*., 2013). Studies conducted on this medicinal plant and its product development include the quality and antioxidant properties of cookies containing *C. lanceolata* powder (Song and Lee, 2014), antioxidative activity and quality characteristics of Kochujang amended with different ratios of *C. lanceolata* root powder (Kim *et al*., 2012), a study of the quality characteristics of Yanggaeng supplemented with *C. lanceolata* Traut (Benth et Hook) (Kim and Chae, 2011), and changes of physicochemical, sensory and antioxidant activity characteristics in rice wine, Yakju added with different ratios of *C. lanceolata* (Jin *et al*., 2008). However, no studies on *C. lanceolata* and its use in traditional Korean rice cakes were conducted until recently. The expansion of *C. lanceolata* powder usage in health enhancing foods and other types of foods for the purpose of diversifying its use was the main goal of these new studies. Rice cake with *C. lanceolata* powder comes from traditional agriculture, settlement and development within the South Korea, whose culture is deeply rooted in this dish (Shin *et al*., 2004). Also, by adding different ingredients to the traditional rice cake, one can increase the nutritional value, color, and texture of the dish (Park, 2014). The physiological function of other materials in the rice cake with *C. lanceolata* powder can also add value in the argument for the sale of healthy food compared to standard traditional food (Lee *et al*., 2002). In this study, rice cake with *C. lanceolata* powder was tested to investigate the possibility of rice cake with *C. lanceolata* powder aligning with previously proven antioxidant activity when prepared with the powder of medicinal plants. This was accomplished by measuring the quality characteristics and antioxidant activity as part of the development of functional foods.

Materials and Methods

Material

C. lanceolata powder used in this experiment was purchased online (Baekjangsaeng Co., South Korea). Rice flour (Daedusicpoom Co., South Korea), Sugar (CJ Co. Ltd., South Korea), Salt (Haepou Co., South Korea), and water (Gwangdongjaeyac Co. Ltd., South Korea) were used in this experiment.

Making rice cake with C. lanceolata *powder*

Several preliminary tests were performed to achieve the right ratio between rice cake and *C. lanceolata* powder. For this experiment, 60 g of water, 20 g of *C. lanceolata* powder, 200 g of rice flour, 20 g of sugar, and 2 g of salt were used. The first step was mixing all the ingredients thoroughly. The mixture was strained twice through 20-mesh standard sifters (850 µm, Chung Gye Industrial MFG. Co., Seoul, South Korea). In preparation for steaming, the mixture was poured into a wooden steamer and care was taken to keep the top even. The steamer was then covered with a cotton cloth, and the product steamed for 25 minutes in a clay stew pot. The product was left inside the stew pot for an additional five minutes without heat. The steamed mixture was then cooled for 10 minutes at room temperature. Finally, the rice cake with *C. lanceolata* powder was ready for the experiment. To verify the effects of antioxidants and the physicochemical characteristics of *C. lanceolata* powder, rice cake prepared without *C. lanceolata* powder was used as the control group.

Physiochemical properties of rice cake with C. lanceolata *powder*

To test the general components, the pH (hydrogen exponent), amount of moisture, crude protein, crude fat, and flavonoids were measured. The pH of the homogenized mixture (homogenous stomacher, Mayo Co., Italy) was measured with a pH meter after adding 9 mL of distilled water to the sample (Model 420 Mayo Co., Italy). The pH measurements were repeated several times to determine the mean value. The amount of moisture was determined using moisture measuring instruments (MB45 Moisture Analyzer, Ohaus Corporation, Switzerland), the crude protein amount was measured using a Micro Kjelahl, and quantitative analysis of nitrogen was performed with automatic nitrogen distillation equipment. Also, an automated crude fat extractor was used to measure the amount of crude fat using Soxhlet's sampling process, and the total flavonoids amount was measured using the Nieva Moreno method (Isla *et al*., 2001).

Antioxidant activity in rice cake with C. lanceolata *powder*

To measure 1-diphenyl-2-picrylhydrazyl (DPPH) radical scavenging activity, 9mL methanol (100 mg/

mL) was added to 1g of rice cake and left for 16 hours at room temperature. Then, 1 mL of solvent was created by mixing 1 mL of the rice cake hydrogen exponent with 0.2 mM DPPH. After 30 minutes of reaction at room temperature, the absorbance was measured at 517 nm. The DPPH radical activity was compared against the sample group and the percentage was calculated using [1-(Absorbance of the sample/Absorbance of the control)] × 100.

Antifungal activity in rice cake with C. lanceolata *powder*

The antifungal effect of rice cake with *C. lanceolata* powder was tested by observing the difference in the amount of fungi in a potato agar plate culture of the mixture made with PBS 90 mL and 10 g of rice cake with *C. lanceolata* powder over five days.

Antistaling activity in rice cake with C. lanceolata *powder*

To test the age delaying effect of rice cake with *C. lanceolata* powder, the amount of moisture in rice cake with *C. lanceolata* powder was measured and compared against the control rice cake over five days.

Statistical analysis

All of the results in this study were repeated three times to obtain the mean and standard deviations. The significance of verification was calculated with version 12 SPSS (Statistical Package for Social Sciences, SPSS Inc., Chicago, IL, USA) software package program using a variance analysis tool (ANOVA). Duncan's multiple range test was done to minimize the error to $p<0.05$.

Results

Physico-chemical properties

The results of analyzing pH, moisture, crude protein, crude fat, and total flavonoids of rice cake showed that rice cake containing *C. lanceolata* powder at pH 5.52 is 0.21 higher than rice cake with nothing added. Rice cake with *C. lanceolata* powder had a higher amount of moisture (44.54 g/100g), which was 0.21 g/100g higher than rice cake by itself. Rice cake with *C. lanceolata* powder contained 0.53 g/100g more crude protein, which was 4.28 g/100g higher than Rice cake with nothing added. Rice cake with *C. lanceolata* powder contained more crude fat (0.39 g/100g) than the control group, which had 0.38 g/100g of crude fat, and rice cake with *C. lanceolata* powder had higher flavonoid content (0.0082 mg/g) than the control group (0.0079 mg/g). This experiment showed that rice cake with *C. lanceolata* powder contains higher values in all five physical and chemical characteristics tested, compared to the control group (Table 1).

Table 1. Physicochemical characterization of normal rice cake and rice cake with *C. lanceolata* powder

	Control	Rice cake with *C. lanceolata*
pH	5.73	5.52
Moisture	44.33 g/100g	44.53 g/100g
Crude Protein	3.75 g/100g	4.28 g/100g
Crude Fat	0.01 g/100g	0.39 g/100g
Total Flavonoid	0.0003 mg/g	0.0082 mg/g

Antioxidant activity

Rice cake with *C. lanceolata* powder showed significant antioxidant activity compared to the control group. When measuring 100 mg/ml, the antioxidant activity in the control group was only IC_{50} = 844.26 mg/ml, whereas rice cake with *C. lanceolata* powder had activity of IC_{50} = 119.01 mg/ml. Also, as the amount of rice cake with C. lanceolata powder increased by 0, 20, 40, 60, 80 and 100 mg/mL, the antioxidant effect also increased (Figure 1, Figure 2).

Antifungal activity

To determine the antifungal effect of *C. lanceolata* powder, 90 mL of PBS was added to 10 g samples of rice cake with C. lanceolata powder and regular rice cake with. The amount of bacteria and fungi on the samples was then compared. On the fifth day, the number of bacteria was $2.35\times10^7 \pm 5.00\times10^5$ for the control group and $4.48\times10^9 \pm 3.62\times10^8$ for the rice cake with *C. lanceolata* powder. The number of fungi on day five was $6.85\times10^4 \pm 5.00\times10^2$ for the control group and $3.00\times10^6 \pm 1.55\times10^5$ for rice cake with *C. lanceolata* powder. No antifungal activity was found on the first day, but 50 % more antifungal activity was found in rice cake with *C. lanceolata* powder than in regular rice cake with by the fifth day (Figure 3).

Antistaling activity

Analysis of delayed aging in rice cake with *C. lanceolata* powder and the control group showed insignificant differences. The moisture content in rice cake with *C. lanceolata* rice cake with was 44.54 ± 1.29 g/100g, with 44.33 ± 0.97 g/100g for the control group on the first day. On the fifth day, the control group had 42.44 ± 0.14 g/100g of moisture, while the rice cake with with C. lanceolata had 44.70 + 0.37 g/100g. Thus, the effect was not visually obvious (Figure 4).

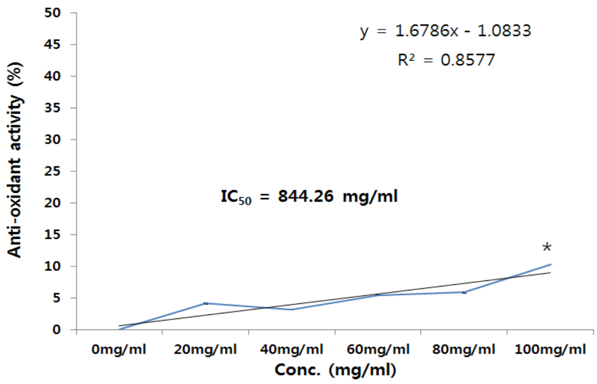

Figure 1. Anti-oxidant activity of rice cake. Anti-oxidant activities were analyzed by DPPH radical scavenging activity. The IC_{50} calculated by the trend line was 844.26 mg/ml. Values are shown as means ± standard deviation, and data are from three independent experiments

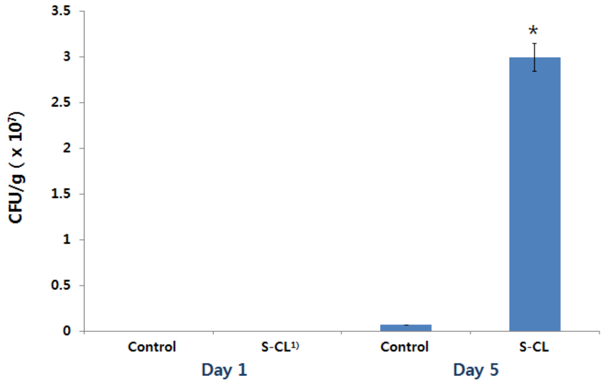

Figure 3. Anti-fungal activity of normal rice cake and rice cake prepared with *C. lanceolata* powder. Anti-fungal activities were analyzed by serial dilution in potato agar plate. No antifungal activity was found on the first day and fifth day. Values are shown as means ± standard deviation, and data are from three independent experiments

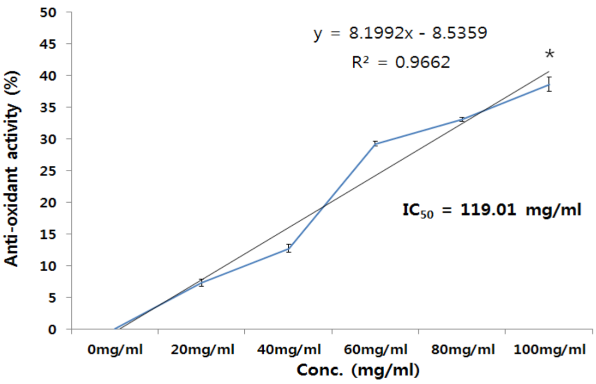

Figure 2. Anti-oxidant activity of rice cake prepared with *C. lanceolata* powder. Anti-oxidant activities were analyzed by DPPH radical scavenging activity. The IC_{50} calculated by the trend line was 119.01 mg/ml. Values are shown as means ± standard deviation, and data are from three independent experiments

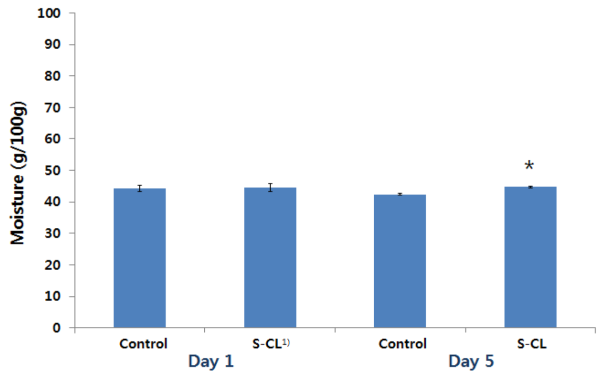

Figure 4. Anti-staling activity of normal rice cake and rice cake prepared with *C. lanceolata* powder. Anti-staling activities were analyzed by moisture content. The effect was not visually obvious. Values are shown as means ± standard deviation, and data are from three independent experiments

Discussion

The aim of this study was to provide basic research data in the hopes of utilizing a common but essential traditional medicinal herb, *C. lanceolata*, as an ingredient in a variety of processed foods. The study analyzes and compares chemical characteristics, antioxidant effects, antifungal effects, and delayed aging between rice cake with *C. lanceolata* power and regular rice cake.

First, the pH of the control rice cake with was 5.73, while rice cake with *C. lanceolata* powder had a lower pH level of 5.52. The rich organic acids and amino acids containing alcohol in rice cake with *C. lanceolata* with created fewer ester ingredients, likely causing the lower pH level. The moisture content in the control group was 44.33 g/100g while rice cake with *C. lanceolata* powder had slightly more moisture (0.21 g/100g) at 44.54 g/100g. The crude protein content in rice cake was 4.28 g/100g, which was higher than the control group's 3.75 g (g/100g), confirming the higher nutrition in rice cake with *C. lanceolata* powder. The crude fat content in rice cake with *C. lanceolata* powder was 0.39 g/100g, which was higher than the control group's 0.01 g (g/100g). Similar to the crude protein content, adding *C. lanceolata* powder to rice cake was found to increase crude fat content and thus increase nutritional value as well. Flavonoids, which are generally found in plants, is a generic term for all particle groups made with two phenyls or pyrenes (Mpalantinos *et al.*, 1998). They are usually either colorless or yellow and in aglycone form. Their biological activities take various forms in plants, but when they are absorbed into the human body, they are known as remarkably functional substances (Kim *et al.*, 1998). The amount of flavonoids in rice cake with *C. lanceolata* powder was 0.0082 mg/g, which was higher than the control group that had 0.0003 mg/g. Previous physiochemical property studies on

C. lanceolata include evaluation of physicochemical properties and biological activities of steamed and fermented *C. lanceolata* by Jung *et al.* (2012), and changes of physicochemical, sensory and antioxidant activity characteristics in rice wine, Yakju added with different ratios of *C. lanceolata* by Jin *et al.* (2008). Jin *et al.* (2008) also reported changes of physicochemical and sensory characteristics in the *C. lanceolata* Saengsik, uncooked food by different drying methods (Jin *et al*., 2008).

Second, higher antioxidant activity was found in rice cake with *C. lanceolata* compared to the regular rice cake. Measuring the change in the color of DPPH can reveal antioxidant capacity. When testing the DPPH color, rice cake with *C. lanceolata* changed color from purple to yellow, while the control group had minimal difference in the color. This indicated that the antioxidant activity in rice cake with *C. lanceolata* was significantly higher than in regular rice cake. Other antioxidant activity studies on *C. lanceolata* include the quality and antioxidant properties of cookies containing *C. lanceolata* powder by Song *et al.* (Song and Lee, 2014), antioxidant activities of processed *C. lanceolata* extracts by Jeon *et al.* (2013) and quality characteristics and antioxidant activities of fermented *C. lanceolata* tea with *Pleurotus eryngii* mycelium by Lee *et al*. (2013). *Codonopsis lanceolate* have antioxidant activities due to the physiological activity of flavonoids including saponins, tannins, triterpene, alkaloids, and steroids (Hossen *et al.*, 2015). Also, Kandaswami *et al.* (1994) reported that flavonoid of plant have highly antioxidant activities.

Third, according to the antifungal effect test results, rice cake with *C. lanceolata* had approximately the same amount of antifungal activity as the control rice cake on the first day, but had 50 percent activity after five days. This suggests that *C. lanceolata* enhances the antifungal activities over time. Rice cake with *C. lanceolata* had higher amounts of moisture, crude protein, crude fat, and flavonoids than the regular rice cake, which indicates that it is more beneficial to health. To obtain higher nutritional value, it would be best to consume the snack immediately after preparation or heating. Kim *et al.* (2008) conducted a research study on the antifungal activity of *Zanthoxylium schinifolium* against *Fusarium graminearum*, a barley powdery mildew fungus and noted an increased number of fungus that is similar to this experiment' result.

Fourth, aging in various types of starch has a significant impact on the shrinking process, temperature, amlyiopectin content, water content, and pH over time. Rice cake with *C. lanceolata* powder was not affected by the aging process on the first day (Lund and Lorenz, 1984). Comparative analysis on the fifth day (44.70 ± 0.37 g/100g) showed that the water content did not change much from the initial value of 44.33 ± 0.97 g/100g. In contrast, the water content in the control rice cake dropped to 42.44 ± 0.14 g/100g on the fifth day from an initial value of 44.54 g/100g. Noticeable difference in the water content between the groups was a nice distinction empirically, but the effect on aging was not obvious upon visual observation. There are various ways to curb the effects of water content loss over time, including sugar or emulsifiers (Johansson and Bergenståhl, 1992). Adding sugar during a dehydration process achieves the same effect in less time than using an emulsifier (Neri *et al*., 2014). Increasing the stability of the colloidal solution of *C. lanceolata* may discourage aging and take rich nutrients in their diet (Meng *et al*., 2008). Notable aging process research includes Park's suppression effect of maltitol on retrogradation of Karedduk (the other type of Korean rice cake) (Park *et al*., 2003), as well as Jung's effects of oyster mushroom on quality of rice cake and Gyeongdan (Chung and An, 2012).

Conclusions

The results confirmed the physical and chemical characteristics of rice cake with *C. lanceolata* in terms of antioxidant activity, antifungal activity, and delayed aging effects. Based on the delayed aging process and other test results, rice cake with *C. lanceolata* powder has been shown to possess increased functionality, with high nutritional and antioxidant properties. Therefore, rice cake with *C. lanceolata* powder adds potential value to efforts to increase the availability and quality of healthy food options in the South Korea.

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