Antioxidant Activity of Korean Rice Cake Added
*Cicer arietinum* for Post-Menopausal Women

Gun Hee Park¹, Hyun-Mok Ju²,³, Yoon Jung Park⁴, Eun Hye Lee⁵, Han Sol Kim⁶, Ri Na Ma⁷, Hyun Hee Kim⁸, Hyo Bin Kim⁹, Dong Jun Seo⁵, Hae Rin Min⁹, Gug In Kang⁴, Da Young Kim⁸, Soohyeon Park⁵, Ju Hyeon Kim⁵, Gayoung Kii⁷, Jina Kim⁷, Geumbi Baek⁴,⁷ and Ki Han Kwon¹,²

¹Department of Food Science and Nutrition, College of Health, Welfare and Education, Gwangju University, Gwangju, South Korea
²Division of Food Science and Nutrition, Graduate School of Biohealth Science, Gwangju University, Gwangju, South Korea
³Seongun Biotech and Consultation Co., Gwangju, South Korea
⁴Gwangju Jeolla Food Culture Institute, Gwangju, South Korea
⁵Myoung In Co., Gwangju, South Korea
⁶Dong-gu Branch, JS Cuisine Academy, Gwangju, South Korea
⁷Department of Cooking Science and Tourism, Graduate School of Industry, Sunchon National University, Sunchon, South Korea

**Abstract:** The symptoms caused by a decrease in hormone levels in women are referred to as menopause. These affect loss of femininity, cause estrogen-dependent disease and often develop into a personal as well as social issue. Menopausal women try to alleviate their symptoms by hormone injections and intake of functional health foods; however, many homemakers do not respond positively. Many soybean products include phytoestrogen similar to estrogen and *Cicer arietinum* also include phytoestrogen. For this reason, this study examined antioxidant activity of rice cakes supplemented with *C. arietinum*. The results were confirmed that antioxidant activity increased rice cakes supplemented with *C. arietinum* than normal rice cake. Above all, antioxidant activity was not decreased in cooking process. In these results mean that antioxidant activity of lignin as phytoestrogen in rice cake will not decrease. We introduce the potential of functional Korean rice cake that effective for ameliorating the symptoms of menopause.

**Key words:** Rice cakes, *Cicer arietinum*, antioxidant activity, menopause, phytoestrogen

**INTRODUCTION**

Menopause is caused by a dysfunction of the ovaries and involves a large number of physical and mental changes in middle-aged women (Nelson, 2008). Symptoms of menopause include night sweating, flushing, headache, joint pain, anxiety, vaginal dryness, mood swings, insomnia irritability, anxiety, depression, heart palpitations and memory decline (Bungay et al., 1980); (Eorré and Ernst, 2010). Also menopause is often the cause of obesity at middle aged women (Ali-Safi and Polotsky, 2015) and extremely is often the cause of Alzheimer’s disease (Henderson, 2014). As such, menopause will bring a lot of middle-aged disease in postmenopausal women (Blake, 2006). Studies have shown that decreasing ovarian function results in decreased production of estrogen (Kemper et al., 2013). Lack of estrogen leads to bone turnover, activity of osteoclasts and absorption of bone material resulting in osteoporosis in postmenopausal women (Borjesson et al., 2013). Moreover, decreased estrogen levels induce estrogen-dependent cancers, such as breast and ovarian cancer (Strauss et al., 2014). These diseases cause mental and physical distress in middle-aged women and may lead to suicide associated with depression (Kornstein et al., 2010). Problems in postmenopausal women are no longer extended to social issues, not personal issues (Kornstein et al., 2010) Classically, hormone replacement therapy for treatment of menopause has used a combination of estrogens with synthetic progesterone and recently phytoestrogens are a popular alternative estrogens/progesterone therapy (Moreira et al., 2014). Historically, carbohydrates have been the primary energy source for humans (Song et al., 2014). Carbohydrate intake is supplemented by rice in Eastern countries and wheat in Western countries (Painter and Burkitt, 1971). The discovery of carbonized rice in Stone Age ruins in South Korea (Kim, 2007) indicates that Koreans have a long history of rice supplementation and that Koreans have developed a culture of rice-oriented food (Kim and Choi, 2008). Koreans often make rice cakes out of steamed crushed rice and rough grains (Song and Park,
Rice cakes were a food of the holidays and rituals, because rice was expensive and difficult to obtain. Since South Korea has become a developed country, the lifestyle and eating habits of Koreans have been changing rapidly. The westernization of food culture and the increase of instant food intake have led to an increased consumption of wheat and a decreased consumption of rice. The annual wheat consumption (2,000 years) has shown a trend to increase by 34 kg/year (Lee et al., 2012). (Metcalfe, 1984) and rice consumption has declined to below 70 kg/year in 2010 (Metcalfe, 1984). Gluten, a structural protein of wheat, may contribute to conditions such as rhinitis, asthma and inflammation. In contrast, rice contains various essential nutrients and does not cause allergies (Bartłomiej et al., 2012). Recently, the interest of Koreans in a "healthy lifestyle" (or well-being) has increased and so has an interest in functional foods (Borneo and Leon, 2012). Functional foods are defined as foods supplemented with bioactive substances (Borneo and Leon, 2012). These foods are thought to protect against obesity, diabetes, cancer and etc. Functional foods containing raw materials are becoming increasingly popular (Ahmed et al., 2011). These foods can be made and consumed at home. Noodles, rice wine, jam, cake and other products have been made of such functional raw materials (e.g., blueberries, purple sweet potatoes (Zeng et al., 2013), cabbage, carrots (Abdel-Aal et al., 2013) and chickpeas (Licznarska et al., 2013)). Functional rice cakes contain indole-3-carbinol from cabbage powder (Sunwoo et al., 2013), ginsenosides from ginseng powder (Sunwoo et al., 2013) and anthocyanin from purple sweet potatoes (Zeng et al., 2013). These materials have been shown to have anti-cancer and anti-obesity effects and to protect against various diseases (Abdel-Aal et al., 2013). Significant attention has been paid to the super-foods chickpeas (Cicer arietinum, also called Egyptian beans or chikupi), lentils and quinoa (Hithamani and Srinivasan, 2014). C. arietinum is a low-calorie food and is high in protein, calcium and vitamin C and is low in fat content (Hithamani and Srinivasan, 2014). Above all, C. arietinum is known as a powerful antioxidant and contains high levels of flavonoids and polyphenol (Miquel et al., 2003). Intake of food including antioxidants may help to protect women against oxidative stress (Sierens et al., 2001). As well, phytoestrogens are one of the important antioxidant in food (Sierens et al., 2001).

Phytoestrogens are plant-derived hormone analogues that their structural is similar to mammalian estrogens and they play both estrogen effects and anti-estrogen effects (Borrelli and Ernst, 2010). Phytoestrogens are classified with isoflavones, coumestans and lignans (Sierens et al., 2001). Isoflavones present a lot of soybeans, soybean product and red clover (Soni et al., 2014). Lignans are found in flaxseed which present at considerable concentrations in fibre-rich foods (Fritz et al., 2013) and coumestans are existed clover and soybean sprouts have a large number of coumestans (coumestrol and 4-methoxycoumestrol) predominantly coumestrol and 4-methoxy coumestrol (Landete, 2012). Isoflavones are a class of phytoestrogens (which are a type of polyphenols) that stimulate estrogen receptors and hence mimic physiological estrogen in women (Cornwell et al., 2004). Isoflavone-rich foods are thought to help overcome menopause (HaiRong et al., 2013). Complementary and alternative medicine (CAM) to alleviate menopausal symptoms used many prescription include phytoestrogen (Borrelli and Ernst, 2010). Phytoestrogen had the function of scavenging harmful free radicals (Sierens et al., 2001). The daidzein (one of phytoestrogen) had function of decreased ROS-induced toxicity by antioxidant and estrogenic action (Brandao et al., 2009) and isoflavones prevent various diseases caused by oxidative stress (Liu et al., 2006). For this reason, the confirm of antioxidative effect will be an index to determine the content of the phytoestrogen. In this study, we report the intake frequency of legumes containing isoflavones (in the form of rice cakes) in middle-aged South Korean women with the aim to alleviate symptoms of menopause through phytoestrogen intake and we suggest alleviating menopause through rice cake that have antioxidant in C. arietinum.

MATERIALS AND METHODS

Study participants: Of the 25,534 individuals that participated in the 5th Korean National Health and Nutrition Examination Survey (KNHANES, 2011), 1,992 middle-aged women (≥ 40 years old) were enrolled in this study. KNHANES data collection was approved by the Korea Centers for Disease Control Institutional Review Board (2011-02CON-06-C).

Materials: Commercially available rice was purchased and C. arietinum was harvested in Australia. We purchased 1,1-diphenyl-2-picrylhydrazyl (DPPH) from Sigma Chemical Co. (St. Louis, MO).

Survey: The dietary intake frequencies of 1,992 middle-aged women (≥ 40 years old) were derived from the 5th KNHANES (2011). We conducted a cross-sectional analysis of the intake frequency of rice, beans, tofu and bean sprouts in these women. It was confirmed ratio in the comparison rice is the staple food of Korea.

Preparation of C. arietinum flour: C. arietinum was washed with running water and then immersed in water for 24 h. The soaked C. arietinum was boiled for 50 minutes and subsequently dried. The dried C. arietinum was grinded with a mixer (Hanil electricity Co. Ltd., South
Korea) and a thin powder was produced by sieving multiple times with a 30-mesh sieve (Joungwoo Industry, South Korea).

**Preparation Rice Cake added C. arietinum flour:** Prepared rice flour (500 g) and C. arietinum flour (50 g) was immersed in water (150 ml) for 5 h. After adding an appropriate amount of salt and to the flour, the rice and C. arietinum powder and all supplements (salt; 5 g, sugar; 50 g) were mixed well. The mixture was turned into a thin powder mix using a sieve. The rice flour was steamed in a bamboo steamer for 25 min and the boiled rice was allowed to settle for 5 min.

**Analysis of the antioxidant activity of rice cakes supplemented with C. arietinum:** To analyze the antioxidant activity of rice cakes, 1 g of the rice cake and 9 mL methanol were mixed and shaken at room temperature for 16 h. The supernatant was centrifuged for 1 min at 13,000 rpm and mixed with methanol to achieve various concentrations (C, 20, 40, 60, 80 and 100 mg/ml). We added 1 mL DPPH (0.2 mM) to the supernatant and the mixture was incubated at room temperature for 30 min. Absorbance was analyzed at 517 nm.

**Statistical analysis:** Data of at least three different experiments were collected and are presented as mean±standard deviation. All data were analyzed using SPSS (version 12.0, Raleigh, NC, USA). The statistical significance of differences was evaluated by one-way analysis of variance and a least significant difference test at a 95% confidence level (p<0.05).

**RESULTS**

**Intake frequency of bean-derived products:** A total of 1,992 middle-aged women (>45 years; 23.39% of the total respondents in the 5th KNHANES (2011) answered the questionnaire. Response options included 'no intake', 'once/week' and 'three times/day'. Among the women reporting an intake of bean-derived products of 3 times/day, 1,327 (66.62%) and 457 (22.94%) reported rice and bean intake, respectively and no women reported tofu or bean sprout intake. Among those reporting an intake of 'once/week', 6 (0.30%), 465 (23.34%), 116 (5.82%) and 539 (27.06%) reported rice, tofu, bean and bean sprout intake, respectively. Finally, 1 (0.05%), 156 (7.83%), 263 (13.20%) and 114 (5.72%) women who answered 'no intake' reported rice, tofu, bean and bean sprout intake, respectively (Table 1).

**Antioxidant activity of normal rice cake and rice cake supplemented with C. arietinum:** Normal rice cake and rice cake supplemented with C. arietinum had IC50 values of 510.57 and 41.51 mg/ml, respectively. Rice cake supplemented with C. arietinum thus had an antioxidant activity that was 10 times higher than that of normal rice cake.
DISCUSSION

Menopausal women suffer from melancholia associated with the loss of femininity and hormone-related diseases caused by decreasing hormone levels (Dixit et al., 2012). Recently, there is an attempt to treat menopause by CAM (Borrelli and Ernst, 2010). The Complementary and alternative therapies for the menopause have many treatment that are acupuncture (Freeman et al., 2014), calcium (Lee et al., 2009), homeopathy (Lanham-New, 2008), phytoestrogens (Ernst, 2002) and vitamins (Jacobs et al., 2009). In addition, many herbal plants were used to treat menopause of CAM. Black cohosh (Cimicifuga racemosa L.) (Papadimitriou et al., 2002), Dong quai (Angelica sinensis Oliv.) (Heyerick et al., 2006), Hops (Humulus lupulus L.) (Papadimitriou et al., 2002), Wild yam (Dioscorea villosa L.) (Hirata et al., 1997), Ginseng (Panax ginseng C.A. Mey) (Komesaroff et al., 2001) are mainly herbs of CAM. Many plant extracts are known to exert beneficial effects on postmenopausal women as they contain phytoestrogens (also referred to as natural estrogen-mimicking compounds) (Wiklund et al., 1998). Phytoestrogens are found in many plant-derived human foods (e.g., soy beans and legumes) (Landete, 2012) and have been reported to prevent cancer (Jungbauer and Medjakovic, 2014). Considering this point, intake of soybean with middle-aged women are respected (Soni et al., 2014).

We analyzed the legume intake frequency in middle-aged women using data from the 5th National Health and Nutrition Examination Survey (KNHANES, 2011). Legume consumption of middle-aged women (>40 years) was analyzed to low level compared to the consumption of rice. A total of 13% women reported no legume intake. Based on these finding, opportunities were able to compensate for female hormone decreased by low consumption of legumes. Some women can afford to replenish their hormone levels through the intake of functional foods or hormone injections; however, the majority of homemakers are not able to do so due to financial constraints. Therefore, the replenishment of hormone substitutes through food intake should be recommended. However, middle-aged women often prioritize their families first and not themselves. This leads to a loss in confidence and susceptibility to depression in these women. To overcome menopausal symptoms, the activity for only themselves needed for them.

Functional food and drugs must be demonstrated to have a pharmacological effect. This procedure is very costly and preclinical and clinical tests are time-consuming and expensive. In reality, functional foods are able to prevent the disease that can be easily accessed. The goal of this study was to provide information on the intake of functional rice cakes in middle-aged women with the aim of disease prevention.

Fig. 1: Antioxidant Effect of Rice cake without Cicer arietinum. Antioxidant activities were analyzed by DPPH radical scavenging activity. The IC_{50} calculated by the trend line was 510.57 mg/ml. Values are shown as mean±standard deviation and data are from three independent experiments. DPPH, 1,1-diphenyl-2-picrylhydrazyl

\[
y = 1.1992x + 0.2165 \\
R^2 = 0.433
\]

Fig. 2: Antioxidant Effects of Rice cake Supplemented with Cicer arietinum. Antioxidant activities were analyzed by DPPH radical scavenging activity. The IC_{50} calculated by the trend line was 41.51 mg/ml. Values are shown as mean±standard deviation and data are from three independent experiments. DPPH, 1,1-diphenyl-2-picrylhydrazyl

\[
y = 20.425x + 22.341 \\
R^2 = 0.9953
\]

Phytoestrogens (isoflavones, coumestans and lignans) are found in soybeans and other legumes (Soni et al., 2014). Isoflavones in soybeans include daidzein, genistein and glycitein (Dodge et al., 1996), (Csaky and Fekete, 2004). High contents of isoflavones are found in beans, rosaceae (Csaky and Fekete, 2004) and iridaceae (Daruhazi et al., 2013). Isoflavones are well known for their anti-oxidative (Lee et al., 2011), antibacterial (Xiao et al., 2011), cholesterol-lowering (Weber et al., 2013) and blood pressure-lowering effects (Lee et al., 2013). These phytoestrogen are effected lack of
hormones related dyspnea, flushing etc. (Lee et al., 2013). Moreover, phytoestrogens stimulate osteoblasts and inhibit osteophages to being densely density of bone cells (Borjesson et al., 2013). It has also been shown that lignin supplementation results in a reduction of oxidized low-density lipoprotein levels, decreases the incidence of cardiovascular disease (Liu et al., 2012) and is effective for the prevention of breast cancer and osteoporosis d4 (Borjesson et al., 2013; Strauss et al., 2014). Hydnocarpin (a lignin) is thought to be effective for colon cancer prevention (Hillman et al., 1985). The antioxidative and anti-cancer effects of coumestans have also been demonstrated (Brando et al., 2009).

HaiRong (HaiRong et al., 2013) reported high level of isoflavones in C. arietinum. The administration of isoflavone extracted from C. arietinum in mice with removed ovaries increased bone mineral density and bone and tissue volume and positively affected intrabecular thickness and trabecular separation. Isoflavones extracted from C. arietinum have also been reported to affect breast and prostate cancer. C. arietinum has been reported to contain 2,174±0.654 g/100 g lignin in its raw form, 0.59±0.756 g/100 g when immersed in water and 2.50±0.721 g/100 g when cooked. Lignin is a precursor of lignan, which is a phytoestrogen. C. arietinum, which contains phytoestrogens, has a high antioxidant effect. The hormonal alterations of menopause in middle-aged women is vulnerable to prevent oxidative stress (Cameron et al., 1997) and diet supplementation with antioxidants help to overcome menopause against oxidant stress in the female hormone deficiency (Sierens et al., 2001). In additionally, the anti-cancer and other pharmacological effects of phytoestrogens have been shown to be associated with their antioxidant activity. Hence, functional rice cakes made of C. arietinum are thought to be beneficial for menopausal women. Our findings confirmed the antioxidant activity of rice cake supplemented with C. arietinum. Normal rice cake had antioxidant effects with an IC50 of 510.57 mg/mL and rice cake supplemented with C. arietinum had antioxidant effects with an IC50 of 41.51 mg/mL. The antioxidant effect of C. arietinum extract was not analyzed in this study because functional cuisine is made of raw foods. Moreover, it has been shown that the antioxidant effect of C. arietinum is not reduced by the cooking process.

The lignan content of C. arietinum in meals was similar to that in raw C. arietinum and C. arietinum immersed in water. Lignan is a major factor of the antioxidant effect of rice cake supplemented with C. arietinum. Unchanged antioxidant effect during making rice cake supplemented with C. arietinum enables speculation that in no change in phytoestrogen levels. Premenopausal middle-aged women have decreased hormone levels. Phytoestrogens can compensate for these reduced levels.

Our study introduced a potential of rice cake supplemented with C. arietinum as a hormone replacement strategy for middle-aged women. These rice cakes can easily be made at home. The purpose of the female hormone replacement is clear as functional foods that will be helped recovery of menopausal by giving satisfaction.

Conclusions: Rice cakes can easily be made at home and C. arietinum, which has a significant antioxidant effect, can be added to rice cake. The antioxidant effect of C. arietinum is not reduced by cooking. Importantly, this attribute of C. arietinum can help ameliorate menopausal symptoms. Thus, the intake of functional foods should be recommended for middle-aged women.

ACKNOWLEDGMENTS
This research was supported by Basic Science Research Program through the National Research Foundation of Korea (NRF) funded by the Ministry of Education, Science and Technology (NRF-2013R1A1A4A01013550) and the Leaders in Industry-university Cooperation funded by the Ministry of Education. Moreover, this study was conducted in part by research funds from Gwangju University, South Korea in 2015.

Author disclosure statement: The authors declare that they have no conflict of interest.

REFERENCES


Gun Hee Park and Hyun-Mok Ju contributed equally to this work