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Shelf-Life Extension of Rice Cake by the Addition of Persimmon Leaf Tea Powder

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Abstract Antimicrobial activity of persimmon leaf tea methanol extracts on *Klebsiella pneumoniae* and *Staphylococcus aureus* was examined through paper disk diffusion bioassay. *K. pneumoniae* and *S. aureus* showed 87.64 and 99.35% growth inhibitions by addition of steamed tea methanol extracts (10 mg/ml), respectively. The number of microorganisms in rice cakes was significantly reduced when leaf tea powder was added during production of rice cakes. Addition of 1% steamed leaf teas to rice cakes decreased the number of microorganisms by over 50%, and over 70% reduction was observed when final 2% (dried weight w/w) steamed leaf tea was added, whereas no significant effect was observed over 2%. These results indicate that different microorganisms were detected from persimmon leaf and rice cake, and persimmon leaf can inhibit normal microbial growth in rice cake.

Keywords: Persimmon leaf tea, rice cake, shelf-life extension

Introduction

Persimmon (*Diospyros kaki* Thunb.) grows in East Asian countries, such as Japan, China, and Korea. The fruit of persimmon is eaten fresh or dry, and the leaves are infused with hot (rather than boiling) water and drunk as kakinoha-cha in the same way as green tea. Kakinoha-cha has traditionally been drunk in localities with many persimmon trees, in the mountainous areas of Japan. Leaf of persimmon is also considered to have persimmon tannin, although there are still some unclear points with regard to the content and physiological effects of persimmon leaf tannin.

In Korea persimmon has been traditionally used for many medicinal purposes such as paralysis, frostbite, burns, and to stop bleeding (1). In particular leaf of persimmon contains flavonoid oligomers, tannins, phenols, organic acids, chlorophyll, and other compounds, among which tannin is the major component (4). Tannin and tannin with gallate group have various physiological functions such as antibacterial (5, 20), anti-allergic (6), scavenging of free radicals (7), and lowering of blood pressure (3).

Attempts are ongoing to develop processed rice products appropriate to the Korean diet pattern and taste preference. Recently, interest in functional foods has increased, and many applications to produce functional rice cakes, which contained functional ingredient such as mugwort and pine leaves (8), surichwi (9), mulberry leaves powder (10), and green tea powder (11), have been reported. However, no report has yet been made on the development of a functional rice cake containing persimmon leaf or teas that have various physiological actions. Therefore, the objective of the current study was to evaluate the effect of the addition of persimmon leaf tea powder on the shelf-life extension of rice cake.

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Material and Methods

Preparation of persimmon leaf tea Persimmon (cultivar: Dungsi) leaves were sampled at Sangju, Korea at the end of July, 2000 and used to make persimmon leaf tea. They were washed three times with fresh water and cut into 1-cm wide pieces. For the preparation of steamed tea, the leaves were treated for 3 min at 100°C using a steamer (DP-9804, Daepoong Electric Co., Korea) and dehydrated for 1 hr at 80°C in a dry oven. For the preparation of a roasted tea, the leaves were treated for 10 min at 220°C and dried as described above. To make a fermented tea, the leaves were fermented for 12 hr at 25°C and over 90% humidity using a fermenter (VS-1203PE-LN Multi Room Incubator, Vision Co., Korea), and dried as described above. Shade-dried tea was made by air-drying the leaves for 7 days under the shade followed by drying at 30°C for 1 hr in a dry oven. Freeze-dried tea was made by drying the leave for 48 hr in a freeze-drier (SFDSM24, Samwon Co., Korea).

Methanol extraction of persimmon leaf The persimmon leaves were dried in an oven at 40°C for 2 days and finely powdered. The powdered sample (100 g) was then extracted twice with 500 mL methanol at room temperature for 2 days and filtered. The combined filtrate was concentrated to dryness by rotary evaporation at 40°C.

Antibacterial assay The antibacterial activity of the persimmon tea against two bacteria strains, *Staphylococcus aureus* (KCTC 1621) and *Klebsiella pneumoniae* (KCTC 2142), frequently reported food spoilage bacteria, was evaluated *in vitro* by the paper-disk diffusion method. The strains, supplied by Korean Collection for Type Cultures

(KCTC) (Korea Research Institute of Bioscience and Biotechnology, Dageon, Korea), were maintained on Nutrient Agar (NA; Merck, Darmstadt, Germany),. Aliquots of the samples were dissolved in DMSO, and a 20-μL portion of each solution was soaked on an 8-mm Whatman paper disk to give concentrations of 2, 4, 6, 8, and 10 mg/mL. The disks were then placed on agar plates, which had been separately seeded with each bacterium. After incubating for 24 hr, the zone of inhibition was measured.

Production of persimmon leaf tea-added rice cake The rice (cultivar, Illmi) was purchased from a local supermarket in Sangju, Korea. Persimmon leaf tea was homogenized, and the resulting powder kept at a -20°C freezer until use. Other materials used including salt (Hanju salt, Seoul, Korea) and sugar (CJ Co., Seoul, Korea) were purchased from a department store and kept in a 4°C refrigerator until use. Rice cakes, *Sulgiduck, Julpyun,* and *Injeulmi*, were manufactured according to the method of Kang *et al.* (12). The composition ratio of each ingredient is shown in Table 1. Persimmon leaf rice cake was prepared by adding persimmon leaf tea at 0, 1, 2, 4, and 8% of rice flour content.

Microorganisms assay in rice cake and persimmon leaf The sample cake (1 g) was placed in a sterile Stomacher bag (Seward, London, England) containing 10 mL distilled water. Samples were further diluted in 0.85% saline buffer as need. To determine the total cell count, the following methods were used. For the enumeration of the diluted samples, 100 μL each sample was spread on Luria broth agar (LBA), NA or Tryptic soya agar (TSA) for bacteria isolation and incubated at 37°C for 2 days. For the isolation of fungi, Potato dextrose agar (PDA) was used (how?)and (what was?) incubated at 28°C for 7 days. Total cell count was determined using Petrifilm™ plate kit according to the protocols of the manufacturer (Microbiology Products 3M Health Care, St. Paul, MN, USA), and total cells were counted with a hemacytometer using a optical microscope. The data are the means of five experiments. Total cell units for bacterial and fungi were designated as cfu/g and mycelia/g, respectively.

Table 1. Formulas for the preparation of three rice cake with the addition of the persimmon leaf tea powder

Items	Ratio of leaf (%)	Rice powder (g)	Persimmon leaf tea powder (g)	Water (g)	Salt (g)
Sulgiduck	0	400	0	100	2.4
	1	396	4	100	2.4
	2	392	8	100	2.4
	4	384	16	100	2.4
	8	368	32	100	2.4
Julpyun	0	300	0	60	2.4
	1	297	3	60	2.4
	2	294	6	60	2.4
	4	288	12	60	2.4
	8	276	24	60	2.4
Injeulmi	0	300*	0	30	2.4
	1	297*	3	30	2.4
	2	294*	6	30	2.4
	4	288*	12	30	2.4
	8	276*	24	30	2.4

*Glutinous rice powder.

Results and Discussion

Antimicrobial activity by the methanol extracts of persimmon leaf Persimmon leaf extracts exhibited different inhibition levels against *S. aureus* (KCTC 1621) and *K. pneumoniae* (KCTC 2142) (Table 2). The inhibition zone increased with increasing concentration of extracts. *S. aureus* was more sensitive than *K. pneumoniae* to the extracts.

Persimmon leaf extract strongly inhibited the activity of DNA polymerase alpha in eukaryotic cells (13), and had cytotoxic, multidrug resistance reversal, anti-human inmmunodeficiency virus, and anti-*Helcibacter pylori* activities (15). In addition, the growth of human lyphoid leukemia cells was inhibited by the polyphenol compounds of persimmon such as catechin, epicatechin, and epicatechingallate (14).

Isolation and identification of microorganisms from the persimmon leaf and rice cake Addition of persimmon leaf to rice cakes was expected to inhibit the growth of microorganisms. However, treatment of persimmon leaf was also expected to cause microbial contamination in samples. Therefore, microorganisms were isolated from rice cakes treated with or without persimmon leaf, and a total of 24 strains were collected from the persimmon and rice cakes (Table 3). In the untreated *Sulgiduck*, the number of microorganisms was 8.65×10^6 cfu/g, and four kinds of bacteria and two of mold were detected. In *Sulgiduck* added with 1% steamed leaf, the number of microorganisms was 4.33×10^6 cfu/g, and four kinds of bacteria and one of

Identification of microorganisms The isolates selected for further research were identified according to their phenotypic characteristics. Microorganisms were identified based on their morphological characteristics and by analysis of their fatty acid profiles using the Sherlock system according to the recommendations of the manufacturer (MIDI Co., Newark, DE, USA). The fungus isolated from the mixed culture was grown on PDA for 48 hr and was observed under the microscope. Its colony morphology was also examined.

Table 2. Antibacterial activity of the methanolic extracts of persimmon leaf tea (the steamed tea) on *Klebsiella pneumoniae* and *Staphylococcus aureus* after 48 hr incubation

Conc. (mg/mL)	Inhibition of growth (%)	
	K. pneumoniae	*S. aureus*
2	12.46	36.42
4	36.70	50.83
6	57.32	84.21
8	73.95	98.94
10	87.64	99.35

Table 3. Number of microorganism in the rice cakes and persimmon leaf tea (the steamed tea) after the storage at room temperature for 5 days

Items		*E. coli*/colony form (cfu/g)	Aerobic bacteria (cfu/g)	Mold (mycelia/g)
Persimmon leaf		$2.60\pm0.30\times10^2$	$9.53\pm0.13\times10^7$	$5.72\pm0.30\times10^4$
Sulgiduck in markets		0	$9.82\pm0.14\times10^6$	$2.00\pm0.10\times10^2$
Sulgiduck	-	0	$8.65\pm0.21\times10^6$	$3.23\pm0.10\times10^2$
	+	0	$4.33\pm0.36\times10^6$	$1.74\pm0.20\times10^2$
Julpyun	-	0	$7.39\pm0.28\times10^6$	$2.18\pm0.10\times10^2$
	+	0	$3.77\pm0.13\times10^6$	$1.04\pm0.20\times10^2$
Injeulmi	-	0	$6.43\pm0.23\times10^6$	$1.17\pm0.10\times10^2$
	+	0	$3.66\pm0.36\times10^6$	$0.83\pm0.20\times10^2$

+: Added 1% persimmon leaf tea.

mold were observed. Untreated *Julpyun* showed 7.39×10^6 cfu/g microorganisms, and three kinds of bacteria and two of molds were observed (data not shown). In *Julpyun* with 1% steamed leaf, the number of microorganisms was 3.77×10^6 cfu/g, and three kinds of bacteria and one of mold were observed (data not shown). In *Injeulmi*, the number of microorganisms decreased with the addition of steamed leaf. The number of microorganisms (9.82×10^6 cfu/g) and isolated bacterial strains (five bacterial strains and two molds; data not shown) of commercial *Sulgiduck* were higher than those of our rice cakes.

The above results showed that the addition of persimmon leaf in rice cakes resulted in over 50% decrease of microorganisms. In addition, an ingredient or an extract of persimmon leaf was presumed to be involve in the growth retardation of microorganisms. Therefore, for further study, we chose nine kinds of bacteria and one mold from our rice cake samples, as well as three kinds of bacteria and one mold from commercial *Sulgiduck*, based on their dominancy and colony morphologies such as shape and color.

The selected strains isolated from our *Sulgiduck* were identified as S1-1, S4-1, S8-1, and S22-1, showing high similarity to *Pantoea ananas* (0.835 similarity), *Morganella morganii* (0.683), *Erwinia rhapontici* (0.775), and *Klebsiella pneumoniae* (0.753), respectively (Table 4). J40-1, J40-2, and J50-1 from *Julpyun* were identified as *Kluyvera ascorbata* (0.781), *Citrobacter freundii* (0.680), and *E. rhapontici* (0.809), respectively. I52-1 and I72-1 from *Injeulmi* were identified as *K. pneumoniae* (0.765) and *K. ascorbata* (0.841), respectively. G1-1, G2-2, and G4-1 from commercial *Sulgiduck* were identified as *K. pneumoniae* (0.776), *K. ascorbata* (0.802), and *E. rhapontici* (0.664), respectively. In persimmon leaf, AL-1, AL-5, and AL-6 were dominant strains and tentatively identified as *Bacillus subtilis* (0.754), *Pseudomonas chlororaphis* (0.635), and *B. pumilus* (0.610), respectively. Among the strains examined, S8-1 and S22-1 from our *Sulgiduck*, I52-1 and I72-1 from *Injeulmi*, and J40-1 and J50-1 from *Julpyun* were identified as the same strain, suggesting this to be a common bacterial strain in rice cakes. All strains identified were found to be not bio-hazardous. In the present study, food-poisoning bacteria such as *Staphylococcus aureus* and *Salmonella* sp. or sanitary indicative bacteria such as *Eschericha coli* were not identified. The isolated molds

Table 4. Identification of isolated strains by Sherlock system

Items	Strains	Identification name	Similarity
Sulgiduck	S1-1	*Pantoea ananas*	0.835
	S4-1	*Morganella morganii*	0.683
	S8-1	*Erwinia rhapontici*	0.775
	S22-1	*Klebsiella pneumoniae*	0.753
Julpyun	J40-1	*Kluyvera ascorbata*	0.781
	J40-2	*Citrobacter freundii*	0.680
	J50-1	*Erwinia rhapontici*	0.809
Injeulmi	I52-1	*Klebsiella pneumoniae*	0.765
	I72-1	*Kluyvera ascorbata*	0.841
Sulgiduck in markets	G1-1	*Klebsiella pneumoniae*	0.776
	G2-2	*Kluyvera ascorbata*	0.802
	G4-1	*Erwinia rhapontici*	0.664
Leaf	AL-1	*Bacillus subtilis*	0.754
	AL-5	*Pseudomonas chlororaphis*	0.635
	AL-6	*Bacillus pumilus*	0.610

were identified based on the shapes of hyphae and sporangium. A-1 isolated from tea leaf-added rice cakes and T-1 from commercial *Sulgiduck* were identified as *Penicillum* sp. and *Aspergillus* sp., respectively. P-2, P-4, and P-12 from persimmon leaf were identified as *Aspergillus* sp., *Fusarium* sp., and *Alternaria* sp., respectively. Results showed that the dominant microorganisms present in rice cakes and persimmon leaf differed, thus suggesting the microorganisms present in rice cakes did not originate from the persimmon leaf.

Growth of microorganisms in persimmon leaf tea-added *Sulgiduck*, *Julpyun*, and *Injeulmi* Changes in the number of microorganisms in persimmon leaf tea-added *Sulgiduck* after storage at room temperature for 96 hr are shown in Fig. 1. In untreated *Sulgiduck*, the number of microorganisms was 8.65×10^6 cfu/g, and decreased gradually with the addition of leaf tea. Addition of 1% steamed leaf tea resulted in over 50% inhibition of the microorganisms, and over 70% inhibition was achieved when 2% steamed leaf tea was added. However, no significant effect was observed with over 2% addition of steamed leaf (Fig. 1). In addition, the growth-retarding activities of the steamed, roasted, fermented, and shade-dried teas were not significantly different (Fig. 1). Among all teas, 2% addition was the most effective on the growth retardation of microorganisms. Changes in the number of microorganisms in persimmon leaf tea-added *Julpyun* and *Injeulmi* after storage at room temperature for 96 hr are shown in Figs. 2 and 3, respectively. As observed in Fig. 1. the number of microorganisms was also reduced by the addition of persimmon leaf tea. In all tested teas, 2% addition was the most effective on the growth retardation of microorganisms, and no significant effect was observed with over 2% addition of all treated teas, presumably because the inhibition effect on the microbial growth reached maximum in all rice cakes with the addition of 2 % leaf tea powder. These results showed that shelf-life of rice cakes could be extended by the addition of persimmon leaf tea powder. We also supposed that the growth retardant activity of microorganism originates from an antioxidant

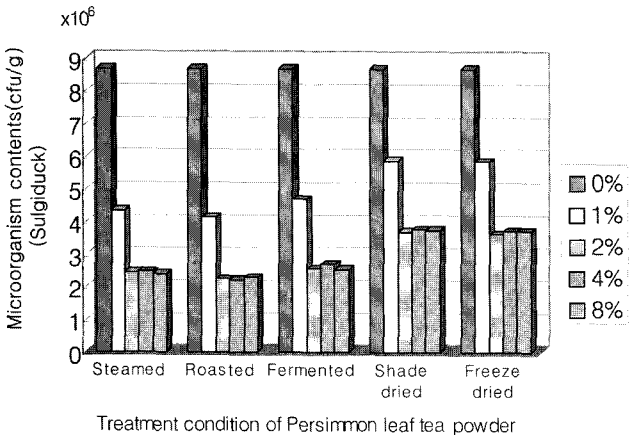

Fig. 1. Inhibitory effect of addition of persimmon leaf tea powder treated differently on microbial growth of *Sulgiduck* during the storage at room temperature for 96 hr.

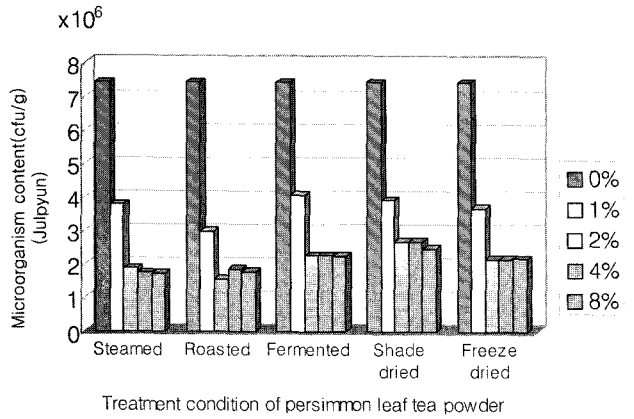

Fig. 2. Inhibitory effect of addition of persimmon leaf tea powder treated differently to *julpyun* on the microbial growth during the storage at room temperature for 96 hr.

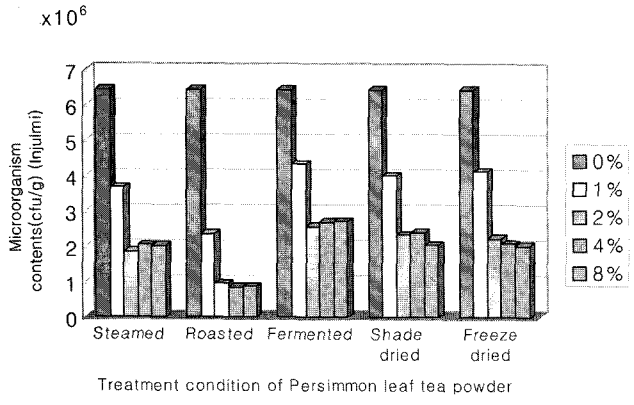

Fig. 3. Inhibitory effect of addition of persimmon leaf tea powder treated differently to *Injeulmi* on the microbial growth during the storage at room temperature for 96 hr.

component of persimmon leaf. To address this issue, further study will be necessary to isolate the active ingredient from persimmon leaf and confirm its structure. Kim *et al.* (16) reported that 3% addition of mugwort extended the shelf-life of rice cakes for 2 days. Other studies also reported on the extension of self-life of rice cake by mugwort, plantain (17), peony (*Paeonia japonia* Miyabe et Takeda; 18), and dandelion (19).

References

1. Matsuo T, Ito S. The chemical structure of kaki-tannin from immature fruit of the persimmon (*Diospyros kaki*). Agri. Biol. Chem. 42: 1637-1643 (1978)
2. Kameda K, Takaku T, Okuda H, Kimura Y, Okuda T, Hatano T, Agata I, Arichi S. Inhibitory effects of various flavonoids isolated from leaves of persimmon on angiotensin-converting enzyme activity. J. Nat. Prod. 50: 680-683 (1987)
3. Funayama S, Hikino H. Hypotensive principles of *Diospyros kaki* leaves. Chem. Pharm. Bull. 27: 2865-2868 (1979)
4. Bae DK, Choi HJ, Son JH, Park MH, Bae JH, An BJ, Bae MJ, Choi C. The study of developing and stability of functional beverage from korean persimmon (*Diospyros kaki* L. folium) leaf. Korean J. Food Sci. Technol. 32: 860-866 (2000)
5. Choi C. Studies on investigation into biologically activated substances from korean persimmon leaves and developing high function beverages. pp. 149-150 Report of Ministry of Agriculture and Forestry. (2000)
6. Kotani M, Matsumoto M, Fujita A, Higa S, Wang W, Suemura M, Kishimoto T, Tanaka T. Persimmon leaf extract and astragalin inhibit development of dermatitis and IgE elevation in NC/Nga mice. J. Allergy Clin. Immunol. 106: 159-166 (2000)
7. Uchida S, Edamatsu R, Hiramatsu M, Mori A, Nonaka GY, Nishioka I, Niwa M, Ozaki M. Condensed tannins scavenge active oxygen free radicals. Med. Sci. Res. 15: 831-834 (1985)
8. Kim JG. Nutritional properties of chol-pyon preparation by adding mugwort and pine leaves. Korean J. Soc. Food Sci. 11: 446-455 (1995)
9. Kim MH, Park MW, Park YK, Jang MS. Effect of the addition of surichwi on quality characteristics of surichwijulpyun. Korean J. Soc. Food Sci. 10: 94-98 (1994)
10. Kim AJ, Lim LH, Kim MW, Kim MH, Woo KJ. Mineral contents and properties of pongihp julpyun preparation by adding mulberry leaves powder. Korean J. Soc. Food Sci. 16: 311-315 (2000)
11. Kim HH, Park GS. The sensory and texture characteristics of julpyun and sulgidduk in according to concentrations of greentea powder. J. East Asian Dietary Life 8: 454-461 (1998)
12. Kang IH. Korea rice cake and a fried honey-cake. Daehan Printing & Publishing Co., Seoul. Korea (1997)
13. Umekawa H, Takada Y, Furuichi Y, Takahashi T, Achiwa Y, Komiya T, Yoshida S. Inhibition of eukaryotic dna polymerase alpha by persimmon (*Diospyros kaki*) extract and related polyphenols. Biochem. Mol. Biol Int. 47: 795-801 (1999)
14. Achiwa Y, Hibasami H, Katsuzaki H, Imai K, Komiya T. Inhibitory effects of persimmon (*Diospyros kaki*) extract and related polyphenol compounds on growth of human lymphoid leukemia cells. Biosci. Biotechnol. Biochem. 61: 1099-1101 (1997)
15. Kawase M, Motohashi N, Satoh K, Sakagami H, Nakashima H, Tani S, Shirataki Y, Kurihara T, Spengler G, Wolfard K, Molnar J. Biological activity of persimmon (*Diospyros kaki*) peel extracts. Phytother Res. 17: 495-500 (2003)
16. Kim SI, Kim KJ, Jung HO, Han YS. Effect of mugwort on the extention of shelf-life of bread and rice cake. Korean J. Soc. Food Sci. 14: 106-113 (1998)
17. Kim KH, Oh ST, Jung HO, Han YS. Shelf-life of noodle and rice cake by the addition of plantain. Korean J. Soc. Food Sci. 15: 68-72 (1999)
18. Sung JM, Han YS. Effect of bakjakyak (*Paeonia japonica*) addition on the shelf-life and characteristics of rice cake and noodle. Korean J. Food. Culture 18: 311-319 (2003)
19. Kim KH, Chun HJ, Han YS. Effect of dandelion on the extention of shelf-life of noodle and rice cake. Korean J. Soc. Food Sci. 15: 121-126 (1999)
20. Park JS, Baek HH, Bai DH, Oh TK, Lee CH. Antibacterial activity of fennel (*Foeniculum vulgare* Mill.) seed essential oil against the growth of *Streptococcus mutans*. Food Sci. Biotechnol. 13: 581-585 (2004)