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Quality Characteristics and Content of Polysaccharides in Green Tea Fermented by *Monascus pilosus*

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Abstract

In this study, we designed a method to manufacture elevated fermented green tea by using *Monascus pilosus*, which is known as a functional microbe, and observe its antioxidant abilities and quality characteristics. The water-soluble substance (WSS) content of the fermented tea by *M. pilosus* (FTM) was lower than that of the non-fermented tea (NFT), although the alcohol-insoluble substance (AIS) content of the FTM was higher than that of NTM. On the other hand, the fractionated distilled water-soluble polysaccharide (DWSP), CDTA-soluble polysaccharides (CDSP), sodium carbonate-soluble polysaccharide (SCSP) and KOH soluble hemicellulose (HC) obtained from the AIS of the FTM was markedly higher than that of NFT. In the antioxidant parameters, the electron donating ability of all fractions, except HC, extracted from FTM was higher than that of NFT, and iron chelating ability of all fractions, except CDSP, extracted from FTM was higher than that of NFT. Whereas the DWSP and SCSP obtained from the FTM were higher than that of NFT, the activity of the HC fraction from both NFT and the FTM could not be detected. In addition, the xanthin oxidase (XO) inhibitory activities of the DWSP, CDSP and the SCSP obtained from the NFT were significantly higher than that of FTM, the aldehyde oxidase (AO) inhibitory activities of the DWSP and SCSP extracted from the FTM were markedly higher than that of the NFT. Meanwhile, the acceptance of NFT and FTM had no significant difference, while the quality of aroma, taste and mouthfeel of the FTM was higher than that of NFT. These results suggest that the post-fermented tea by *Monascus* microorganisms may be responsible for functional components as well as contribute to the improvement of the tea quality.

Key words: post-fermented tea, *Monascus pilosus*, tea polysaccharides, sensory evaluation, color

INTRODUCTION

Tea is the most widely consumed beverage in the world since tea was planted during the ancient three Kingdom period in Korea, its also been used during palace feasts and Buddhist events (1). Currently, 80% of the cultivated tea in the world is made primarily into fermented tea products (2). Depending on production methods, tea products are generally divided into non-fermented green tea, semi-fermented tea (oolong tea), fermented tea (black tea) and post-fermented tea (*Pu-erh* tea), and depending on the color, white tea (degree of fermentation 10~20%), yellow tea (lightly fermented), green tea (non-fermented), blue tea (semi-fermented, degree of fermentation 30~60%), red tea (fully fermented tea) and black tea (post-fermented) (3). In addition, the polyphenols in the post-fermented tea, which is not oxidized by tea phytoenzymes, is fermented by microorganisms and oxidized polyphenols, including new products such as theaflavins and thearubigins, as well as microbial ingredients such as statins (4,5).

Chinese *Pu-erh* tea, which is a famous post-fermented tea, is divided into dry-stored and wet-stored tea depending on the production method. Dry-stored tea is produced by long-term fermentation under low humidity, and wet-stored tea is relatively quickly fermented under moderate temperatures and high humidity. Microorganisms such as *Aspergillus niger*, *Aspergillus gloucus*, *Penicillium*, *Rhizopus*, *Saccharomyces* and *Bacterium* grow during fermentation (6). On the other hand, microorganisms such as *Monascus* produce statin-related components including monacolin K, which is an inhibitor of cholesterol biosynthesis in the body (7), as well as producing color ingredients such as rubropuntain and monascin, which exhibit anti-cancer activitites (8). As previously reported, *Monascus* extracts have an anti-obesity effect (9) as well as a hepatoprotective effect on obese rats induced with a high-fat diet (10). Functional studies and experimentation of tea focuses mainly on polyphenol antioxidant activity. However, a lack of functional studies on tea polysaccharides exists (11).

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In this study, we produced high quality fermented tea using *Monascus pilosus*, and determined the antioxidant quality on the tea extracts of hot water or ethanol from different fermentation periods (12). Also, with each fraction of tea polysaccharides derived from various fermented teas, we determined the antioxidant activity and compared the activity of each fraction.

MATERIALS AND METHODS

Microorganism, medium and tea fermentation

Green tea (*Camellia sinensis*) leaves from Hadong-gun in Gyeongsangnam-do, Korea were harvested and sun dried in July, 2011. *Monascus pilosus* IFO4480 were obtained from the Korean Culture Center of Microorganisms (Seoul, Korea) for fermentation. *M. pilosus* seed culture was prepared, as described previously by Lee et al. (9), and used as a starter. The fermented tea by *Monascus* (FTM) and the non-fermented tea (NFT) were prepared according to Fig. 1.

Color evaluation of tea infusion

Two grams of dried NFT and FTM samples were infused with 200 mL of boiled distilled water for 3 min using a Tea Extractor (Damian Tea Co., Gyeonggi, Korea). The color of the tea infusion was measured for L^* (lightness), a^* (redness), b^* (yellowness) and H^o (hue angle) using a Chromameter (CR-200, Minolta, Tokyo, Japan).

Content of WSS, AIS, and measurement of brix degree

The content of the water soluble substance (WSS) in tea infusions was measured by weight of the freeze dried tea infusion. Dried tea powder (10 g) was added to 200 mL of 80% ethanol solution and boiled to prepare the alcohol insoluble substance (AIS). The extracted solution and precipitate were filtered and washed three times using a Whatman No 1 filter paper (Whatman International Ltd., Maidstone, England) and the residual AIS was freeze-dried and weighed according to Yamaki et al. (13). The brix degree of the tea infusion samples was measured using a Hand Refractometer (Atago, Tokyo, Japan).

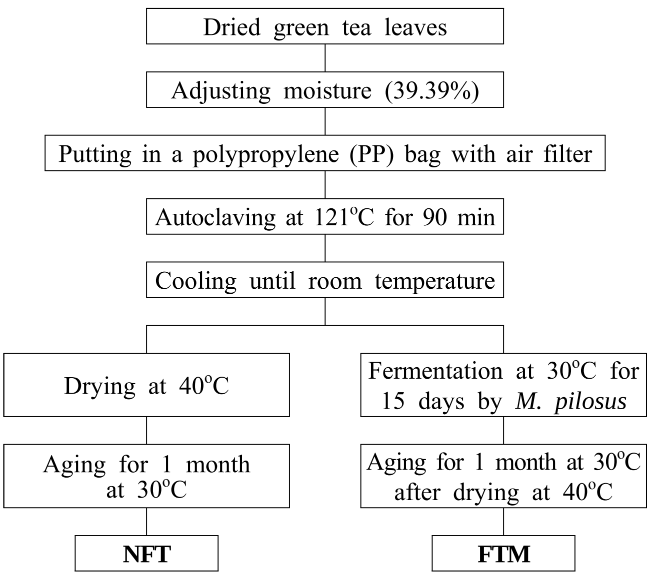

Fig. 1. Preparation procedure of non-fermented tea (NFT) and fermented tea by *M. pilosus* (FTM).

Fractionation of tea polysaccharides and sample preparation

Tea polysaccharides (TPs) were fractionated using distilled water (distilled water soluble polysaccharide; DWSP), 0.05 M 1,2-diaminocyclohexane tetra acetate (CDTA) soluble polysaccharide (CDSP), 0.05 M Na_2CO_3 (sodium carbonate soluble polysaccharide; SCSP), or 8 M KOH soluble hemicellulose (HC) by the method of Redgwell et al. (14).

Measurements of antioxidant activities

The electron donating ability was measured as described by Blois (15). Iron chelating and superoxide dismutase-like (SOD) activities were measured using methods by Dinis et al. (16) and Martin et al. (17), respectively. The xanthine oxidase (XO) inhibitory activity was measured by the use of partially purified milk containing XO (18,19), whereas the aldehyde oxidase (AO) inhibitory activity was evaluated using partially purified rabbit hepatic enzyme (20).

Sensory evaluation

The brewed tea (1%) was prepared by boiling NFT and FTM in water for 3 minutes. Sensory evaluation of tea infusion was evaluated by the well-trained 25 members of the Woori-tea Culture Association (Daegu, Korea) for aroma, taste, color, brightness and mouth feel by a 5 point method as follows: very poor = 1, poor = 2, moderate = 3, good = 4 and very good = 5.

Statistical analysis

Values for analysis were represented as mean ± standard deviations of triplicate determinations. Sensory values were represented as mean ± standard deviation of 25 panels. Significance tests were carried out by performing a two-way analysis of variance (ANOVA) test followed by Duncan's multiple range test and t-test using SPSS statistical software (version 12.0, SPSS Inc., Chicago, IL, USA). A p value <0.05 denotes statistically significant differences.

RESULTS AND DISCUSSION

Color of tea infusion

Hunter color values and photographs of 1% tea (NFT

Table 1. Color of tea infusions with and without fermentation by *M. pilosus*

Plots	Hunter values and hue angle			
	L*	a*	b*	H°
NFT[1)]	59.03±2.34[a3)]	8.42±1.30[b]	25.71±1.41[a]	68.93±2.45[a]
FTM[2)]	40.25±2.21[b]	26.17±2.28[a]	15.17±1.22[b]	39.23±2.32[b]

[1,2)]See Fig. 1.
[3)]Values are mean±SD of triplicate determinations, and different superscripts within a column (a,b) indicate significantly different at $p<0.05$.

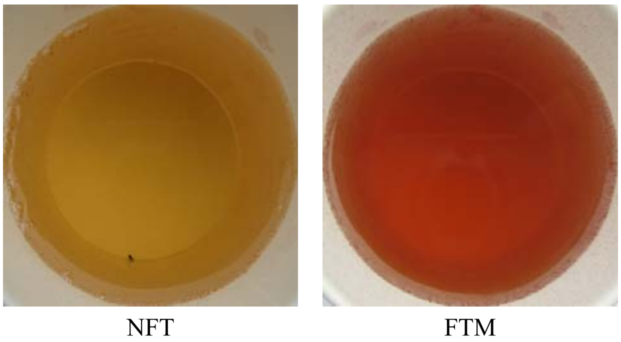

Fig. 2. Photographs of 1% tea fusion. Abbreviations: See Fig. 1.

and FTM) infusions are showed in Table 1 and Fig. 2. Whereas the L* (lightness) value of the NFT infusion (59.03) was higher than that of FTM (40.25), the a* (redness) value of the FTM infusion (26.17) was markedly higher than that of NFT (8.42). On the other hand, the b* (yellowness) value of FTM infusion (15.17) was lower than that of NFT (25.71). In addition, while the hue angle in FTM infusion was 39.23 (reddish orange), the NFT infusion value was 68.92 (yellowish red). These results indicated that color of FTM infusion may be due to theaflavins and thearubigins derived from polyphenols, including catechins, via oxidation and polymerization, which also produce pigments such as rubropuntain and monascin during fermentation by *M. pilosus* (21). The color of the NFT infusion may be due to the production of relatively lower amounts of theaflavins and thearubigins. Therefore, the above results suggest that a considerable part of polyphenols could convert to flavonoids as thearubigins during fermentation by *M. pilosus*.

Content of WSS and brix degree of tea infusion

The WSS content and the brix degree of 1% tea infusions are shown in Fig. 3. WSS content of FTM (299.30 mg/g, dry basis) was 17.60% lower than that of NFT (363.20 mg/g, dry basis). Furthermore, brix degree of the FTM infusion (0.84) was also lower than that of the NFT (0.98). This result may be caused by a high reduction of water-soluble components, such as carbohydrates, despite newly formed water-soluble color components during tea fermentation (Fig. 2). Zuo et al. (22) reported that catechins make up 30～42% of tea leaves; furthermore, 25% of catechins are oxidized and 6～14% are converted to water-soluble flavonoids in the post-fermented tea manufactured by microorganisms. Also, carbohydrates within raw tea leaves are being used as microorganism nutrients. Therefore, these results suggest that water-soluble components are consumed by the growth of microorganisms during fermentation.

Content of tea polysaccharides

Tea polysaccharides (TPs) content fermented by *M. pilosus* are shown in Fig. 4. AIS content in FTM (910.11 mg/g, dry basis) is significantly higher than that of NFT (830.03 mg/g). Also, fractionated DWSP, CDSP, SCSP and HC from AIS in FTM is 2.15, 2.40, 2.68 and 1.57 folds higher than that of NFT, respectively. AIS in plants are well-known as cell wall constituents (23) and consist of polysaccharides such as cellulose, hemicellulose and

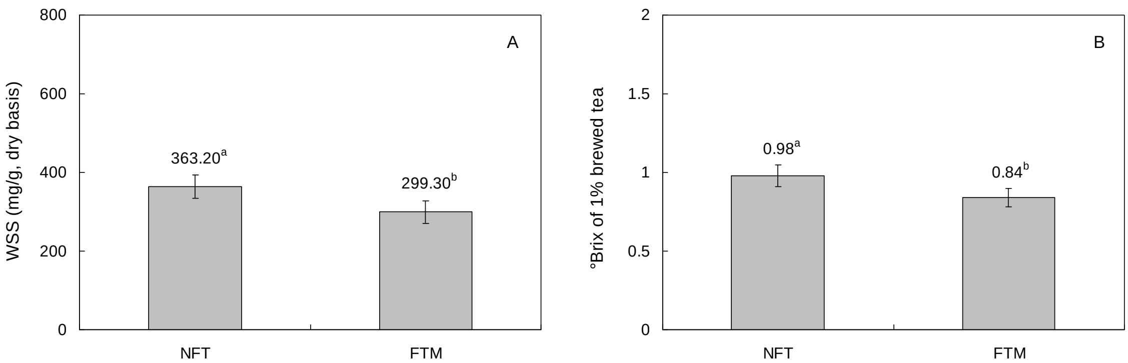

Fig. 3. Content of water soluble substance (WSS) (A) and brix degree of tea infusion (B). Abbreviations: See Fig. 1. Values are mean±SD of triplicate determinations, and different superscripts on the bars (a,b) indicate significant differences at $p<0.05$.

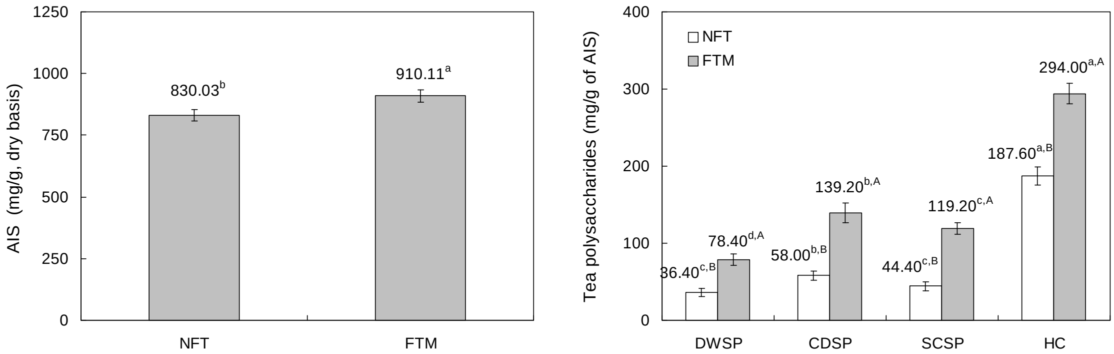

Fig. 4. Content of alcohol insoluble solids (AIS) and tea polysaccharides in NFT and FTM. NFT and FTM: See Fig. 1. DWSP: water soluble polysaccharide, CDSP: 0.05 M CDTA-soluble polysaccharides, SCSP: 0.05 M sodium carbonate soluble polysaccharide, HC: 8 M KOH soluble hemicellulose. Values are mean±SD of triplicate determinations, and different superscripts on the same bars (a-d) and different bars (A,B) indicates significant differences at $p<0.05$.

pectin (24). Furthermore, these polysaccharides exist as binding forms with color components and polyphenolic antioxidant materials in plants (25); therefore, according to well documented research, these TPs have various functions such as antioxidant, antidiabetic, hypolipidemic, immunomodulatory and anticancer activities (26-28). Generally, water-insoluble components in the cell wall are greater than water-soluble components; however, the function of water-insoluble TPs in related studies have not been established. On the other hand, water-soluble or CDTA-soluble TPs are mainly components of middle lamellae, and sodium carbonate-soluble fractions are of primary cell walls (29). In addition, most components of AIS are also cell wall materials (23); distilled water or CDTA-soluble polysaccharides are components of middle lamellae, and sodium carbonate-soluble fractions are of primary cell walls (29). Potassium hydroxide-soluble fractions, known as hemicellulose, are bridged between the middle and primary cell walls (30). Wang et al. (28) reported that the contents of water-soluble polysaccharides in fermented tea are increased according to the degree of fermentation. Therefore, the above results indicate that increased extractable polysaccharides in FTM may be due to changes of cell wall structure by the fermentation in this study.

Antioxidant activities of tea polysaccharides

Antioxidant activities of fractions of TPs separated by various solvents are shown in Table 2. Whereas, the electron donating ability of DWSP, CDSP and SCSP extracted from FTM fermented by *M. pilosus* was 15～58% higher than that of NFT, the HC fraction obtained from NFT was higher (43.89%) than that of FTM (9.94%). Although, iron-chelating ability of the CDSP extracted from NFT was higher (86.80%) than that of FTM (28.90%), DWSP and HC from FTM was significantly higher than that of NFT. On the other hand, SOD-like activity of the CDSP extracted from both NFT and FTM was similar, although DWSP and SCSP obtained from FTM was 27～82% higher than that of NFT. The activity of HC fraction from both NFT and FTM

Table 2. Antioxidant related activities of tea polysaccharides

	Teas[1)]	DWSP[2)]	CDSP[3)]	SCSP[4)]	HC[5)]
Electron donating ability (%)	NFT	$43.47 \pm 1.65^{aB6)}$	36.51 ± 2.14^{bB}	30.82 ± 2.25^{cB}	43.89 ± 2.87^{aA}
	FTM	49.86 ± 2.08^{bA}	57.53 ± 3.00^{aA}	48.58 ± 3.01^{bA}	9.94 ± 2.40^{cB}
Iron chelating activity (%)	NFT	12.36 ± 2.66^{bB}	86.80 ± 4.71^{aA}	9.11 ± 2.85^{NS}	3.44 ± 1.04^{cB}
	FTM	21.10 ± 1.95^{bA}	28.90 ± 2.45^{aB}	9.85 ± 1.74	24.72 ± 2.38^{aA}
SOD-like activity (U)	NFT	0.69 ± 0.03^{aB}	$0.22 \pm 0.02^{NS7)}$	0.39 ± 0.03^{bB}	0.00 ± 0.00^{NS}
	FTM	0.88 ± 0.07^{aA}	0.23 ± 0.02	0.71 ± 0.04^{bA}	0.00 ± 0.00
XO inhibitory activity (%)	NFT	12.09 ± 2.97^{bA}	2.46 ± 1.88^{cA}	49.18 ± 2.32^{aA}	0.00 ± 0.00^{dB}
	FTM	0.00 ± 0.00^{bB}	0.00 ± 0.00^{bB}	0.00 ± 0.00^{bB}	19.06 ± 3.12^{aA}
AO inhibitory activity (%)	NFT	10.41 ± 1.98^{bB}	0.00 ± 0.00^{cB}	7.51 ± 2.58^{bB}	99.42 ± 4.09^{aA}
	FTM	33.53 ± 2.23^{aA}	19.65 ± 1.45^{cA}	28.90 ± 1.82^{bA}	0.00 ± 0.00^{dB}

[1)]NFT and FTM: See Fig. 1.
[2-5)]DWSP, CDSP, SCSP and HC: See Fig. 4.
[6)]Values are mean±SD of triplicate determinations, and different superscripts within a rows (a-d), and a column (A,B) indicates significant differences at $p<0.05$.
[7)]Not significant.

could not be detected. Compared to the XO inhibitory activities of DWSP, CDSP and SCSP obtained from NFT (2.46～49.18%), the inhibitory activity of DWSP, CDSP and SCSP from FTM could not be detected. On the other hand, the inhibitory activity of HC fraction from NFT was undetectable, while that of FTM was 19.06%. The AO inhibitory activity of the DWSP and SCSP extracted from FTM was 3.22～3.85 fold strikingly higher than that of NFT; however, the inhibitory activity of CDSP from NFT did not appear, while that of FTM was 19.65%. Furthermore, the inhibitory activity of HC from NFT was 99.42%, although that of FTM could not be detected. Interestingly, we observed the XO inhibitory activities of all polysaccharide fractions, except HC, were decreased, whereas the AO inhibitory activities were increased due to the fermentation by *M. pilosus* in this study. These results indicated that newly formed components from fermentation may be responsible for this phenomenon, but further studies are needed.

Wang et al. (28) reported that oolong tea also has a higher content of DWSP, depending on the degree of fermentation, as well as higher antioxidant activities such as electron donating ability, ABTS radical scavenging and ferric iron reducing power, and higher α-glucosidase inhibitory activity. The above results suggest that markedly increased content of polysaccharides in FTM compared to NFT may be responsible for an overall increase in antioxidant activities.

Sensory evaluation

Evaluating sensory quality of infused tea by extraction from tea powder (1 g) with hot water (100 mL) for 3 min is shown in Table 3. Appearance for the acceptability of the NFT and FTM are 2.63 and 2.55 points, respectively, but not significantly different. Whereas, aroma and taste of NFT are 3.15 and 2.84 points, respectively, those of FTM are 4.11 and 3.95 points, respectively; the value of FTM is significantly higher than that of NFT. On the other hand, in the color of brewed tea, NFT is a high yellow color, while FTM as a post-fermented tea is red-brown due to theaflavins and thearubigins as maroon and pink color ingredients produced by *Monascus* microorganisms (Fig. 2). Preference for the brightness and mouth feel of the NFT are 2.85 and 2.88 points, respectively, although those of FTM are 4.26 and 4.25 points, respectively; the value of FTM is significantly higher than that of NFT.

Table 3. Sensory evaluation of tea infusions with and without fermentation by *M. pilosus*

Attributes[1)]	NFT[2)]	FTM[3)]
Appearance	2.63±0.31[NS4)]	2.55±0.37
Aroma	3.15±0.28[b5)]	4.11±0.34[a]
Taste	2.84±0.37[b]	3.95±0.36[a]
Color	2.78±0.32[b]	4.15±0.37[a]
Brightness	2.85±0.35[b]	4.26±0.41[a]
Mouth feel	2.88±0.45[b]	4.25±0.44[a]

[1)]All attributes were evaluated from very poor (1 point) to very good (5 points).
[2,3)]See Fig. 1.
[4)]Not significant.
[5)]Values are mean±SD of 25 panels, and different superscripts within a row (a,b) indicate significantly different at $p<0.05$.

The widely-accepted important attributes as quality determinant factors of post-fermented tea are appearance, aroma and taste as well as color, brightness and mouth feel in tea infusion (31,32). In general, low-quality tea is almost a bad color or appearance due to fermentation technology. In other words, the appearance of optimized fermented tea is greenish black due to the increased contents of theaflavin (golden yellow) thearubigins (orange-brown), undegradated chlorophyll, and pheophytin and pheophorbide derived from chlorophyll. As mentioned above, it is widely accepted that various important attributes of tea also affect flavor, taste and mouth feel. Therefore, these results indicate that the sensory evaluation of tea could be improved with fermentation by *M. pilosus*.

In summary, the present study provides evidence that post-fermented tea by *Monascus* microorgansims may be responsible for functional components as well as contributing to improvement of the tea quality, specifically the tea-manufacturing period, which could be shortened.

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