Evaluation of Physical, Health Promoting Properties and Storage Stability of Ready-to-Drink Mixed Tea from Riceberry Rice and Mulberry Leaf

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Abstract

This study aimed to present the development of healthy ready-to-drink (RTD) tea from Riceberry rice tea infusion (RBTI) and mulberry leaf green tea infusion (MGTI). The color of RBTI was red with a positive a* value (8.1) due to the anthocyanins which were found only in RBTI. The MGTI color was pale green with a positive b* value (12.9). Four formulas of the RTD mixed teas were prepared by varying the ratio of RBTI:MGTI at 50:50, 60:40, 70:30, and 80:20 v/v. The color of the RTD mixed teas changed toward red pertaining to the content of RBTI. As the ratio of RBTI increased, the anthocyanin content in the RTD mixed teas increased. As a result, the 80:20 formula had a higher anthocyanin content (14.4 mg/L). However, total phenolic content (14.3 - 15.6 mg GAE/L) and DPPH antioxidant activity (49.2 - 52.9 %) of all four formulas changed insignificantly. The sensory test results indicated that 70:30 and 80:20 formulas received high liking scores on bitterness, overall flavor, and overall liking attributes. The 80:20 formula was selected as the most preferable formula in this study because of its high anthocyanin content. The evaluation of the storage stability of the 80:20 formula revealed that the color, total soluble solid content, anthocyanin, phenolic content, and DPPH antioxidant activity of the tea changed significantly on day 15. These results indicated that the RTD mixed tea from Riceberry rice and mulberry leaf was a healthy tea with consumer acceptance and adequate storage stability.

Keywords: Rice Tea; Riceberry; Mulberry Leaf Green Tea; Ready-to-Drink; Antioxidant

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Introduction

In Thailand, ready-to-drink tea drinks are wildly accepted due to the belief that they possess health-promoting properties. Recently, a limited type of ready-to-drink tea product is available in the market: green tea, black tea, flower tea, and rice tea. According to Thai community product standards, teas are defined as the product produced from the plant *Camellia sinensis* [1]. However, 201 plants are considered tea according to the Ministry of Public Health Notification [2]. The plants listed by this notification include fruits (e.g., Bael fruit, lime, bitter orange, etc.), flowers (e.g., Rose mallow, Torch ginger, Reishi, etc.), stems (e.g., Jaiogulan, wild leek, Welsh onion, etc.), seeds (spiny amaranth, Ethiopia oat, rice, etc.), leaves (coffee, buko, mulberry, etc.), roots (carrot, beetroot, chicory, etc.) [2]. Among these plants, mulberry and rice teas are wildly available in Thailand.

Mulberry (*Morus alba* L.) leaves have been used to treat human illnesses such as colds, diabetes, and other diseases [3]. In recent years, mulberry leaves are commercially available as tea and drink products in China, Japan, Thailand, and other Asian countries [3]. The health benefits of mulberry leaf reported relate to the high content of bioactive compounds, including phenolic acids and flavonoids. However, Angki, T. A., Saikim, F. H., Dawood, M. M., Ahmad, A. H., Godoong, E., Mahyudin, A., Bagul, A. H. B. P., Matanjun, P. C., Noor, N. Q. I. M., Janaun, J. A., Neoh, Y. Y., Sirinus, E., and Gibin, G. [4] reported that pure mulberry leaf tea drink received only slightly like level of taste and aroma attributes. This could indicate the unpleasant sensory characteristics of the mulberry leaf tea. Thus, diluting or mixing mulberry leaf tea with other tea could elevate consumer acceptance.

Rice tea is a common beverage in Japan and Korea. Hyeonmi-cha is a Korean rice tea made from roasted brown rice [5]. Brown rice is a common raw material for producing rice tea. Recently, the health benefit of black rice (or purple rice) has been reported. Black rice contains anthocyanins that show multiple inhibitory effects against chronic diseases: cardiovascular diseases, cancers, diabetes, visual health, obesity, and antimicrobial effects [6]. For this reason, the development of and studies on black rice tea have been performed. Wu, L., Zhai, M., Yao, Y., Dong, C., Shuang, S., and Ren, G. [7] prepared black rice tea and studied the effect of tea processing on the nutritional compositions and anthocyanin content. Germinated black rice tea was developed by Noosing, S., Mundee, P., and Leelawat, B. [8]. Riceberry rice, a non-glutinous black rice, has been widely cultivated throughout Thailand as it has superior health benefits to common brown rice [9].

Mixed green tea and rice tea is common in Japan. Genmaicha is a Japanese style rice tea in which roasted brown rice is mixed with green tea [10]. Ready-to-drink mixed tea products of rice tea and other tea leaves are currently available in the market in Thailand. However, the optimum ratio between rice tea and leaf tea needs to be studied as it affects consumer preferences [11] and also alters the profile of the bioactive compounds in the mixed tea.

This study involved the preparation of mulberry leaf green tea and Riceberry rice tea and their physical and chemical properties analysis. These teas were used to prepare ready-to-drink mixed teas by varying the ratio of the Riceberry rice tea infusion to the mulberry leaf tea infusion. The changes in the physical, chemical, and antioxidant properties of the mixed

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teas was studied. The sensory study on these mixed teas was also performed to evaluate consumer preference. The storage stability of the selected final tea product was evaluated.

Materials and Methods

1. Preparation of the Riceberry rice tea

Organic Riceberry rice sample was obtained from a small local rice mill in Kok-Charoen village, Kok-Charoen Sub-district, Lopburi Province, Thailand. The rice grains (200 g) were roasted in a heated (170 - 210 ^{o}C) iron pan until they were partially popped and produced an aroma [7]. The Riceberry rice tea (RBT) was vacuum stored in a plastic laminated bag.

2. Preparation of mulberry leaf green tea

Mulberry leaves of the Sakon-Nakorn cultivar were obtained from the Organic Farmer Group of Tambon Nong-Pak-Wanne of Thaluang Sub-district, Lopburi Province, Thailand. The preparation of the mulberry leaf green tea (MGT) was conducted using the method described by Wongcharoenkit, S., Nawanukor, P., Kaewrueng, W., and Weerasopon, P. [12]. Briefly, mulberry leaves were cleansed with tap water and drained. The leaves were cut across by a stainless-steel knife yielding leaf strips with a width of approximately 0.5 cm. These leaves were blanched in boiling water for 30 seconds and let to cool down to room temperature. They were kneaded and curled in a heated pan (60 - 70 ^{o}C) for 20 min yielding bruised and slightly dried leaves. The leaves were then dried using a tray-dry at 80 ^{o}C until the moisture content was below 8 %w/w. The obtained mulberry leaf green tea was vacuum stored in a plastic laminated bag.

3. Development of ready-to-drink mixed tea from RBT and MGT

The mixed tea drinks were developed by increasing the ratio of the RBT to the MGT, 50:50, 60:40, 70:30, and 80:20 v/v, to promote the health benefit of the anthocyanin from RBT. Both tea stocks were prepared using the same procedure. To prepare 1 L of the tea infusion stocks, 50 g of MGT was used to produce MGT infusion (MGTI) while 250 g of RBT was used to produce RBT infusion (RBTI). The tea was extracted by infusing the tea in 90 - 95 ^{o}C water for 5 min and then filtering the residue. The tea stocks were kept in glass bottles with screw-capped and let to cool down to room temperature before being stored in a refrigerator at 4 ^{o}C.

4. Physical property analysis

Color parameters in the CIE L*a*b* system of the raw materials, the tea samples, and the tea drinks were measured using a colorimeter (MiniScan XE Plus, Hunter Associates Laboratory, Inc., Reston, VA, USA). At least three replicate measurements were performed.

The moisture content was analyzed using the gravimetric method AOAC 934.01 [13]. The sample (2 g) was weighed in an aluminum moisture can and dried in an electric hot air oven (ShelLab 1375 FX Forced Air Oven, Sheldon Manufacturing, Inc.) at 105±2 ^{o}C until the weight of the solids changed insignificantly. The experiment was performed with three replications and the moisture was presented as the percentage of the sample weight loss after the drying.

The water activity (a_w) value was analyzed using the water activity meter (Aqua Lab 4TE, USA). The samples were crushed using a porcelain mortar before the analysis.

5. Determination of total anthocyanin content (TAC)

The anthocyanin in the Riceberry rice and RBT was extracted by using methanol: HCl (85:15 v/v) as a solvent [9]. The anthocyanin of RBTI was measured directly. The determination of TAC was performed using the pH differential method [14]. The major anthocyanin in black rice, cyanidin-3-*O*-glucoside, showed red color in KCl-HCl buffer solution pH 1.0 while showing colorless in the acetate buffer solution pH 4.5. This color change was measured for the absorbance at 510 nm using a spectrophotometer (Hitachi UH5300, Japan). The TAC was then calculated using Equation (1).

$$TAC = \frac{(A \times MW \times DF \times 1000)}{\varepsilon \times L} \quad (1)$$

Where $A = (A_{510} - A_{700})$ pH 1.0 - $(A_{510} - A_{700})$ pH 4.5), *MW* of cyanidin-3-glucoside (C3G) (449.2 g/mol), *DF* = dilution factor, ε = molar absorptivity (26,900 L/mol•cm) and *L* = path length (1.0 cm).

6. Determination of total phenolic content (TPC)

The bioactive compounds from the raw materials and the teas were extracted by following the procedure of Jiamyangyuen, S., Nuengchamnong, N., and Ngamdee, P. [9], in which the acidified methanol (methanol:HCl = 85:15 v/v) was used as a solvent. The TPC was determined using the Folin-Ciocalteau reagent [9]. The methanolic extracts or tea infusions (0.1 mL) were mixed with the Folin-Ciocalteau reagent (0.5 mL) and left to react in the dark or 5 min. A 1.5 mL of 7.5 % sodium carbonate solution was added and mixed well before 5 mL of distilled water was added. The mixture was measured at 765 nm using a spectrophotometer (Hitachi UH5300, Japan). The calibration curve was plotted with a series of gallic acid dilutions. The TPC was reported as milligram gallic acid equivalent (GAE) per 100 g sample weight or mg GAE/L.

7. Determination of DPPH antioxidant activity

For the determination of DPPH antioxidant activity, the methanolic extracts of the raw materials and the teas were used while the tea drinks were used directly without treatment. The DPPH antioxidant activity was determined by following the method described by Jiamyangyuen, S., Nuengchamnong, N., and Ngamdee, P. [9]. The sample solution (50 µL) was mixed with 100 µM 2,2-diphenyl-1-1-picrylhydrazyl (DPPH) solution (2 mL) and left to react for 30 min in the dark. The absorbance of the solution was measured at 515 nm using a spectrophotometer (Hitachi, UH5300, Japan). The antioxidant capacity was calculated as the percentage scavenging of DPPH against the blank (DPPH reagent without sample).

8. Preparation of ready-to-drink (RTD) mixed tea

The preparation of the RTD mixed tea was performed by following the description of Baek, N., Kim, Y., Duncan, S., Leitch, K., and O’Keefe, S. [15]. For each formula of the

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mixed tea, fifteen clean glass bottles (280 mL each) were washed and sanitized by immersing into boiling water for 10 min and then air-dried. The previously prepared mixed tea (5 L) was heated to 90 ^{o}C for 5 min. The hot RTD mixed tea was filled hot into the bottles and capped before they were placed upside down for 2 min. The filled bottles were subsequently immersed in an ice-water bath.

9. Sensory evaluation

The sensory characteristics of the RTD mixed teas were evaluated using the 9-point hedonic scale method. Fifty panelists (18 - 35 years of age) who drink a type of ready-to-drink tea at least once in a week were selected. Six sensory attributes, color, clarity, bitterness, overall flavor, aftertaste, and overall liking, were evaluated as described by Vittayaporn, V., Chompreeda, P., Haruthaithanasan, V., and Rimkeeree, H. [11]. The RTD mixed teas at 4 ^{o}C was served (100 mL) in white plastic cups along with drinking water (room temperature) for mouth rising. The tea was served to the panelists within 1 min after the preparation. Each panelist evaluated 4 samples marked with a 3-digit random number.

10. Storage stability analysis

The RTD mixed teas were stored in a refrigerator at 4 ^{o}C for 20 days. The properties including color, total soluble solid content (TSS) (Atago PAL-3, Atago, Japan), TAC, TPC, and DPPH, of the RTD mixed teas, were analyzed every 5 days.

11. Statistical analysis

The data were expressed as means±standard deviation (SD). Statistical analysis was performed using a statistical analysis software and the analysis of variance was assessed using One-Way ANOVA with completely randomized design (CRD). The differences of the mean values were established by Duncan's new multiple range test at 95 % confidence level ($p \leq 0.05$).

Results

1. Riceberry rice tea (RBT) and mulberry leaf green tea (MGT)

When roasted, RBT had the appearance of popped rice grains (Figure 1(a)). The MGT appeared dry and the color was dark green as shown in Figure 1(b). These teas were used to prepare the tea infusion stocks for developing ready-to-drink mixed tea of RBT and MGT.

(a) RBT (b) MGT

Figure 1 Physical appearance of Riceberry rice tea (RBT) and mulberry leaf green tea (MGT)

2. Physicochemical and chemical properties of RBT and MGT

Roasting significantly decreased the moisture of the unpolished Riceberry and the mulberry leaves by approximately 42 % and 89 %, respectively, resulting in the final moisture of the RBT and MGT being 7.1 % and 7.5 % (Table 1), respectively. The a_w value of RBT (0.42) changed insignificantly ($p > 0.05$) compared to that of the unpolished Riceberry (0.47). On the other hand, the a_w value of mulberry leaves (0.74) largely decreased by approximately 58 % after the leaves were roasted to produce MGT.

Table 1 Physical and chemical properties of unpolished Riceberry, fresh mulberry leaves, RBT and MGT

Sample	Moisture (%)	a_w	Color			TAC (mg/100 g)	TPC (mg GAE /100 g)
			L*	a*	b*		
Unpolished Riceberry	12.3±0.2^a	0.47±0.12^a	50.6±2.4^a	8.3±1.0^a	14.4±1.0^a	34.0±2.1^a	175.1±4.4^a
RBT	7.1±0.2^b	0.42±0.03^a	33.3±1.1^b	5.1±0.3^b	5.8±0.9^b	5.4±0.9^b	7.4±0.7^b
Fresh mulberry leaves	67.1±1.7^a	0.74±0.18^a	38.1±1.8^a	-8.1±1.0^a	14.7±3.2^b	*nd*	2,443±27^a
MGT	7.5±0.4^b	0.31±0.08^b	21.1±2.2^b	-11.7±2.0^b	24.8±7.9^a	*nd*	1,297±46^b

nd = not detected, Means±sd in the same column of the same type of tea (rice or mulberry leave) with the same superscript letter are not significantly different (p>0.05)

The color parameters of the unpolished Riceberry and fresh mulberry leaves changed significantly after they were processed into the corresponding teas. The lightness (L*) of the unpolished Riceberry (50.6) and fresh mulberry leaves (38.1) significantly decreased ($p \leq 0.05$) as they were roasted to produce RBT (33.3) and MGT (21.1), respectively. The positive a* value (8.3) indicated the redness of the unpolished Riceberry. This redness significantly ($p \leq 0.05$) decreased to 5.1 in RBT. The a* value of the fresh mulberry leaves was negative (-8.1) indicating the green color of the material. When it was roasted to produce MGT, the a* value decreased ($p \leq 0.05$) to -11.7 indicating the dark green color of the tea. The b* values of the unpolished Riceberry, fresh mulberry leaves, and the teas were positive indicating the yellow color tone of the material. The b* value of the unpolished Riceberry (14.4) decreased significantly ($p \leq 0.05$) in the RBT (5.8). On the contrary, the b* value of the fresh mulberry leaves (14.7) significantly ($p \leq 0.05$) increased in MGT (24.8).

Total anthocyanin content was detected only in the Riceberry raw rice and RBT. The TAC value of the unpolished Riceberry (34.0 mg/100 g) was approximately 6.3 times higher than that of the RBT (5.4 mg/100 g). The anthocyanin content in black rice could vary depending on the cultivars. Some studies reported TAC values of black rice at 37 mg/100 g dw [9] and approximately 30 mg/100 g [16]. The TAC in the heat-treated black rice is c

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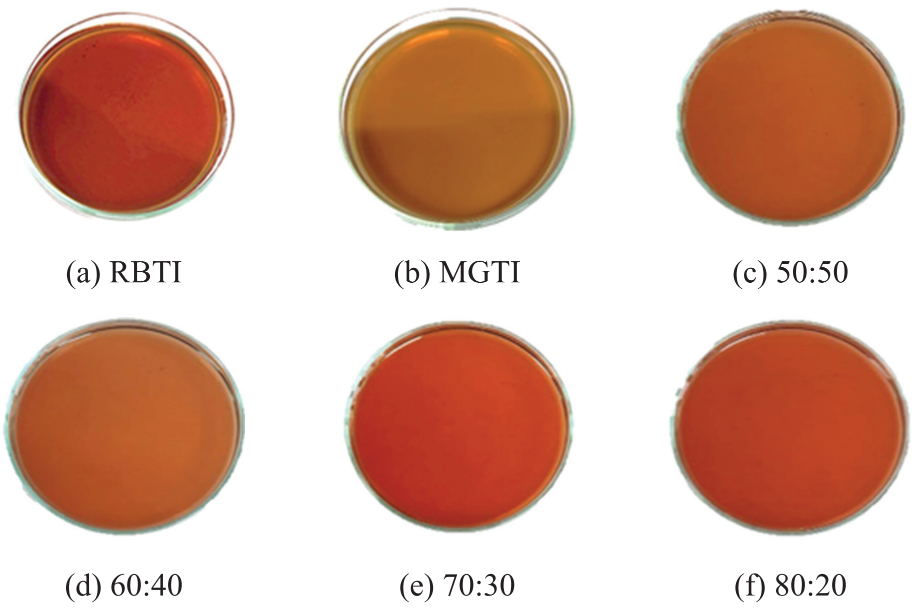
generally lower than that of the raw material. Arora, S., Virdi, I. K., Sharanagat, V. S., Kheto, A., Dhua, S., Suhag, R., Kumar, R., Kumar, Y., and Patel, A. [16] reported that the TAC values of black rice roasted using microwave powder ranged from approximately 3 to 9 mg/g.

Total phenolic content was found in both Riceberry and mulberry leaves and the corresponding teas. The TPC of the unpolished Riceberry was 175.1 mg GAE/100 g and reduced to 7.4 mg GAE/100 g in RBT. The TPC values in black rice were reported by Jiamyangyuen, S., Nuengchamnong, N., and Ngamdee, P. [9] (116 mg GAE/100 g) and Arora, S., Virdi, I. K., Sharanagat, V. S., Kheto, A., Dhua, S., Suhag, R., Kumar, R., Kumar, Y., and Patel, A. [16] (148 mg GAE/100 g). Arora, S., Virdi, I. K., Sharanagat, V. S., Kheto, A., Dhua, S., Suhag, R., Kumar, R., Kumar, Y., and Patel, A. [16] also reported TPC values of roasted black rice to be as low as 51.75 mg GAE/100 g. The TPC value of the fresh mulberry leaves (2,443 mg GAE/100 g) was higher than that of MGT (1,297 mg GAE/100 g). Yu, Y., Li, H., Zhang, B., Wang, J., Shi, X., Huang, J., Yang, J., Zhang, Y., and Deng, Z. [3] reported TPC content in 19 varieties of mulberry leaves which ranged from 887 to 2,026 mg/100 g dw. The tea processing significantly reduced the TPC value of the mulberry leaves. Wanyo, P., Siriamornpun, S., and Meeso, N. [17] reported the TPC value of a commercial mulberry leaf tea which was approximately 1,500 mg GAE/g dw.

3. The appearance of RBTI and MGTI and the ready-to-drink mixed teas

The stocks of the RBTI (Figure 2(a)) and MGTI (Figure 2(b)) were used to prepare the ready-to-drink mixed tea by varying the ratio of RBTI:MGTI (v/v) at 50:50, 60:40, 70:30, and 80:20. The appearance of these mixed teas is shown in Figures 2(c)-(f). As the ratio of RBTI was increased, the color of the mixed tea changed toward a red color which corresponded to the color of the RBTI.



(a) RBTI (b) MGTI (c) 50:50

(d) 60:40 (e) 70:30 (f) 80:20

Figure 2 Physical appearance of RBTI, MGTI, and the mixed tea of RBTI:MGTI (v/v) at 50:50, 60:40, 70:30, and 80:20

4. Physical and chemical properties

The color parameters of RBTI and MGTI were significantly different. For the RBTI, the a* and b* were both dominant while only b* was the major one for the MGTI. The positive a* (8.1) and b* (9.5) values (Table 2) indicated that the color of the RBTI was red which agreed with its appearance in Figure 1(a). The negative a* (-1.3) and positive b* (12.9) indicated that the color of MGTI was yellow. The color of the mixed tea changed with the ratio of RBTI and MGTI. The redness of the mixed tea increased significantly ($p \leq 0.05$) from 5.1 to 9.6 as the ratio of RBTI increased from 50 to 80. This increase in the redness of the mixed teas was contributed by the positive a* value of the RBTI. On the other hand, the yellowness of the mixed teas decreased sequentially from 15.8 to 9.9 with a decrease in the MGTI ratio due to yellow being the predominant color parameter of the MGTI. The L* values of RBTI, MGTI, and the mixed teas were in the range of 46.3 and 63.3, indicating that the tea color was rather dark.

Table 2 Physical and chemical properties of RBTI, MGTI and the mixed teas

RBTI: MGTI	Color parameter			TAC	TPC^{ns}	DPPH
	L*	a*	b*	(mg/L)	(mg GAE/L)	(%)
100:0	47.1 ± 0.3^{de}	8.1 ± 0.4^{b}	9.5 ± 0.6^{c}	15.5 ± 0.6^{a}	16.2±0.5	49.6 ± 3.5^{a}
0:100	56.9 ± 1.8^{b}	-1.3 ± 0.2^{e}	12.9 ± 0.2^{b}	*nd*	15.7±1.2	40.9 ± 2.6^{b}
50:50	63.3 ± 1.1^{a}	5.1 ± 0.3^{d}	15.8 ± 0.9^{a}	9.3 ± 0.7^{e}	14.3±1.4	51.2 ± 2.8^{a}
60:40	49.2 ± 1.8^{cd}	7.4 ± 0.5^{c}	12.5 ± 0.6^{b}	12.1 ± 0.5^{d}	14.8±0.8	52.9 ± 1.9^{a}
70:30	46.3 ± 1.3^{e}	8.4 ± 0.2^{b}	10.7 ± 0.4^{c}	13.2 ± 0.4^{c}	15.6±0.7	50.0 ± 1.2^{a}
80:20	51.2 ± 1.7^{c}	9.6 ± 0.5^{a}	9.9 ± 0.9^{c}	14.4 ± 0.7^{b}	15.1±1.2	49.2 ± 2.4^{a}

ns = insignificantly different
nd = not detected, Means±sd in the same column with different superscript letters are significantly different ($p \leq 0.05$)

Anthocyanin content in the stock RBTI was 15.5 mg/L (Table 2) while it was not detected in the MGTI. As a result, the TAC in the mixed teas increased consecutively from 9.3 to 14.4 mg/L with the increase of the RBTI proportion from 50 to 80. In the case of the phenolic content, the TPC values of RBTI, MGTI, and all four mixed teas were insignificantly different ($p > 0.05$) and ranged from 14.3 to 16.2 mg GAE/L. The DPPH antioxidant activity of the RBTI (49.6 %) was approximately 1.2 times higher ($p \leq 0.05$) than that of the MGTI (40.9 %). However, the DPPH antioxidant values of all mixed teas (49.2 - 52.9 %) were not significantly different ($p > 0.05$).

5. Sensory evaluation

The liking scores of the sensory characteristics of the RTD mixed teas with different proportions of RBTI: MGTI (50:50, 60:40, 70:30, and 80:20) are presented in Table 3. The color (6.3 - 7.5 scores) and clarity (7.3 - 7.6 scores) attributes of all four mixed teas were insignificantly different ($p > 0.05$). The bitterness of the 50:50 received the lowest liking score

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(4.0 scores), however, it was insignificantly different from the score (5.1 scores) of the 60:40 formula. With higher proportions of RBTI, the 80:20 formula received the highest liking score (6.5 scores). Similarly, the 80:20 formula in which the RBTI ratio was higher than the MGTI received the highest ($p \leq 0.05$) liking score for the overall flavor (6.9 scores) and aftertaste (6.1 scores) attributes. The 80:20 formula was selected as the most preferable product. Although its color, TPC, DPPH activity and sensory properties were insignificantly different from the 70:30 formula, the 80:20 formula showed the highest TAC value indicating better health promoting value.

Table 3 Liking scores for sensory attributes of the mixed teas with different ratio of RBTI and MGTI

Sensory attribute (score)	RBTI:MGTI			
	50:50	60:40	70:30	80:20
Color^{ns}	7.4±0.9	7.5±0.9	6.9±0.9	6.3±1.0
Clarity^{ns}	7.6±0.8	7.5±0.6	7.3±0.9	7.4±0.9
Bitterness	4.0±0.9^{c}	5.1±0.7^{bc}	6.2±0.4^{ab}	6.5±0.4^{a}
Overall flavor	4.2±0.7^{c}	5.2±0.9^{bc}	6.4±0.8^{ab}	6.9±0.5^{a}
Aftertaste	4.1±0.8^{b}	4.0±0.9^{b}	6.0±0.8^{a}	6.1±0.8^{a}
Overall liking	4.0±0.9^{b}	4.2±0.5^{b}	6.6±1.0^{a}	6.9±0.4^{a}

ns = insignificantly different, Means±sd in the same row with different superscript letters are significantly different ($p \leq 0.05$)

6. Properties of the RTD mixed tea during storage

The color of the RTD mixed tea containing RBT: MGTI at an 80:20 ratio decreased in intensity (Table 4). The tea showed an increase of lightness indicated by subsequently increasing ($p \leq 0.05$) of L* value at 10, 15, and 20 days of storage in which the L* values were 50.7, 57.2, and 60.2, respectively. The a* and b* values of the teas were stable after 5 days of storage. These color values showed a decreasing trend upon further storage. The tea stored at 15 days showed a lower a* value ($p \leq 0.05$) compared to the five-day-stored tea, however, it was insignificantly different ($p > 0.05$) from the ten-day-stored one. After the tea was stored for 20 days, it showed the lowest ($p \leq 0.05$) a* value at 7.3. The change in b* value showed almost the same decreasing trend as a* value (insignificant change ($p > 0.05$) after 10 days of storage). After that, b* values decreased to 8.6 and 7.0 on days 15 and 20, respectively. The total soluble solid content of the tea showed a decreasing trend during storage. The teas stored for five days and more showed significantly lower ($p \leq 0.05$) TSS values than that of the zero-day-stored one. The TSS values of the teas showed a decreasing trend upon further storage. However, the TSS values of the teas stored for 5 to 20 days were insignificantly different ($p > 0.05$).

Table 4 Physical and chemical properties of the mixed teas containing RBTI:MGTI at 80:20 ratio during storage

Day	Color			TSS	TAC	TPC	DPPH
	L*	a*	b*	(°Brix)	(mg/L)	(mg GAE/L)	(%)
0	45.2±1.2^{d}	10.1±0.6^{a}	11.0±0.5^{a}	0.30±0.06^{a}	14.3±0.1^{a}	15.2±0.1^{a}	53.4±1.3^{a}
5	46.9±1.4^{d}	10.5±0.9^{a}	10.2±1.0^{a}	0.27±0.06^{bc}	14.7±1.1^{a}	15.8±0.1^{a}	50.5±1.4^{ab}
10	50.7±1.1^{c}	9.3±0.7^{ab}	9.9±0.8^{ab}	0.23±0.01^{bc}	13.6±1.1^{a}	14.9±0.9^{a}	49.7±1.8^{b}
15	57.2±1.0^{b}	8.1±0.5^{bc}	8.6±0.9^{b}	0.20±0.06^{c}	10.7±1.2^{b}	12.8±0.7^{b}	35.3±1.5^{c}
20	60.2±0.7^{a}	7.3±0.6^{c}	7.0±0.8^{c}	0.18±0.01^{c}	9.6±0.5^{b}	10.0±0.3^{c}	34.4±1.9^{c}

Means±sd in the same column with different superscript letters are significantly different ($p \leq 0.05$)

The total anthocyanin content of the teas changed insignificantly ($p > 0.05$) during ten days of storage in whereby the values ranged between 13.6 to 14.7 mg/L. On days 15 and 20 of storage, TAC decreased to the lowest values ($p \leq 0.05$) of 10.7 and 9.6 mg/L, respectively. The change in TPC value was almost on the same trend as that of the TAC value. During 10 days of storage, TPC values of the teas were stable ($p > 0.05$) with values of 14.9 to 15.8 mg GAE/L. On days 15 and 20 of storage, TPC values subsequently decreased ($p \leq 0.05$) to 12.8 and 10.0 mg GAE/L, respectively. The decrease in DPPH activity occured earlier than those of TAC and TPC. The DPPH activity of the five-day-stored tea (50.5 %) tended to decrease as compared to the zero-day-stored tea (53.4 %), however, the change was statistically insignificantly different ($p > 0.05$). After further storage for 10, 15, and 20 days, the DPPH activity of the tea decreased ($p \leq 0.05$) to 49.7, 35.3, and 34.4 %, respectively.

Discussion

1. Physical and chemical properties of RBT and MGT

The properties of the teas (Table 1), RBT and MGT changed significantly as affected by the heat processing. The moisture content of both teas (7.1 - 7.5 %) agreed with the standard values of dried mulberry for infusion (10 %) [18] or tea (8 %) [1] products. The RBT was darker compared to the raw material Riceberry rice as indicated by significantly ($p \leq 0.05$) lower L*, a*, and b* values. This decrease in color parameters was a result of roasting at a high temperature which developed color via the Maillard reaction [16]. The color of the mulberry leaves and MGT was green, indicated by the negative a* and b* values. The kneading of the mulberry leaves increased the negative value of the a* parameter of the MGT by stimulating the enzymatic browning process [19].

Anthocyanin is the predominant pigment in unpolished Riceberry rice although it was not detected in the mulberry leaves. As a result, TAC was presented only for the Riceberry rice and RBT. Heat treatment significantly reduced the TAC values of the Riceberry rice because anthocyanins are suspectable to heat. Arora, S., Virdi, I. K., Sharanagat, V. S., Kheto, A., Dhua, S., Suhag, R., Kumar, R., Kumar, Y., and Patel, A. [16] reported that heat

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treatment could reduce TAC value by approximately 10 times compared to the original value.

The phenolic content was also affected by the heat treatment. Arora, S., Virdi, I. K., Sharanagat, V. S., Kheto, A., Dhua, S., Suhag, R., Kumar, R., Kumar, Y., and Patel, A. [16] reported a reduction of the TPC value in the Riceberry rice by a factor of 2.9 as a result of heat treatment. The TPC value of MGT was approximately 1.9 times lower than that of the fresh mulberry leaves. Wanyo, P., Siriamornpun, S., and Meeso, N. [17] also reported a similar reduction factor (1.9 times) of the TPC in commercial mulberry leaf teas as compared to the fresh leaf.

2. Physical and chemical properties of RBTI, MGTI, and the mixed teas

The physical and chemical properties of the tea infusions, RBTI and MGTI, and the mixed teas with different RBTI:MGTI ratios: 50:50, 60:50, 70:30, and 80:20 v/v, were analyzed. The color of RBTI was in the area of red as indicated by positive a* and b* values. The b* of the MGTI was predominant with a positive value indicating the green color area. The color of RBTI and MGTI represented their corresponding raw materials. The color of the mixed teas changed significantly when the ratio of the RBTI increased. Contrary, the b* values of the mixed teas decreased significantly as the RBTI increased, however, the ratio of 70:30 and 80:20 showed insignificantly different b* values.

The anthocyanin content of the mixed teas increased proportionally by the factor of 1.1 - 1.3 depending on the ratio of RBTI. This was because anthocyanin content was present only in RBTI. However, the TPC value of the mixed teas changed insignificantly because both RBTI and MGTI contained insignificantly different TPC values.

Although the DPPH antioxidant activity of RBTI was higher than that of MGTI, the activity of all mixed teas was insignificantly different. This could be because anthocyanins are weak antioxidants as tested by the DPPH assay. Jiamyangyuen, S., Nuengchamnong, N., and Ngamdee, P. [9] reported that the extract of black rice of the first stage of grain development contained lower TPC and detected no TAC, showing equal DPPH antioxidant activity to the fully developed grain, which contained higher TPC and TAC.

3. Sensory evaluation RTD mixed teas

The color and clarity attributes of the RTD mixed teas were insignificantly different. The liking scores on these attributes were in the range of 6.3 - 7.6 scores which were in the level of like slightly to like very much. The 50:50 and 60:40 mixed teas received the lowest liking scores, which were translated to the level of dislike slightly to neither like nor a dislike, on bitterness, overall flavor, aftertaste, and overall liking attributes. The 70:30 and 80:20 mixed teas received insignificant liking scores of 6.0 - 6.9 scores which indicated the liking level of like slightly to like moderately. Angki, T. A., Saikim, F. H., Dawood, M. M., Ahmad, A. H., Godoong, E., Mahyudin, A., Bagul, A. H. B. P., Matanjun, P. C., Noor, N. Q. I. M., Janaun, J. A., Neoh, Y. Y., Sirinus, E., and Gibin, G. [4] reported that the liking level of Tudan's organic mulberry leaf tea was in the level of slightly like. The sensory evaluation result indicated that consumers tended to dislike mixed teas with a higher ratio of MGTI. This could be due to individual flavor and test characteristics of the mulberry leaf tea.

4. Storage stability of the RTD mixed tea

Storage stability of the RTD mixed tea with 80:20 of the RBTI:MGTI ratio stored at 4 ^{o}C for 20 days was evaluated. The a* and b* values of the RTD mixed tea changed significantly on days 15 and 20 of storage. This decrease was associated with the reduction of TSS, TAC, and TPC values. The precipitate pigment could be the cause of the reduction of these values. Dorris, M. R. and Bolling, B. W. [20] reported that soluble anthocyanin and polyphenolic molecules can react and form polymeric precipitate. This causes the reaction of soluble molecules which subsequently reduces the TSS value. Some anthocyanins and other polyphenols can combine to form a polymeric precipitate which causes the reduction of TAC and TPC values. This phenomenon subsequently caused the reduction of the DPPH activity of the tea due to the loss of some antioxidant molecules. The study of Baek, N., Kim, Y., Duncan, S., Leitch, K., and O’Keefe, S. [15] also reported the color change behavior of RTD green tea which agreed with the results from this study. After the tea was stored for 20 days, they found an increase in L* values while the values of a* and b* had decreased.

Conclusion

The tea infusion from Riceberry rice was red and contained anthocyanin as a predominant active compound which was not found in the mulberry leaf tea infusion. The RTD mixed teas containing a different ratio of the Riceberry rice tea and mulberry leaf tea infusions were prepared. The color of the RTD mixed tea changed toward red as the ratio of the Riceberry rice tea infusion as well as the anthocyanin content increased. However, the variation of the tea ratio showed no effect on the total phenolic content and DPPH antioxidant activity. The RTD mixed tea with a ratio of the Riceberry rice tea and the mulberry leaf tea infusion at 80:20 was selected due to consumer preference along with its high health-promoting property. During the storage, color intensity, TSS, TAC, TPC, and DPPH antioxidant activity of the RTD mixed tea showed a significant decrease on day 15. These results indicated that the RTD mixed tea from Riceberry rice and mulberry leaf tea could be an alternative RTD product with health benefits.

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