

Original Article

The Study of Citrus Peels (*Citrus amblycarpa*) Mass Ratio Substitution on Physicochemical of Rich-Antioxidant of Liang Tea

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ABSTRACT

The purpose of this study was to obtain the mass ratio substitution of citrus peel in the rich-antioxidant of liang tea formulation which produced the best physicochemical characteristics. The ingredients for making rich-antioxidant of liang tea are dried herbal plant ingredients consisting of muje leaves (*D. chinensis*), nanas kerang leaves (*Tradescantia spathacea* Sw.), origanum leaves (*Origanum vulgare* Wilder), pandan leaves (*Pandanus amaryllifolius*), stem bark from Secang wood (*Caesalpinia sappan*), midrib skin from Lidah Buaya (*Aloe vera chinensis*) and jeruk sambal peels (*Citrus amblycarpa*) with mass ratios according to treatment. The treatment was in the form of mass ratio of liang tea and citrus *amblycarpa* peel (100:0; 90:10; 80:20; 70:30; 60:40; 50:50 and 40:60). Parameters to observed are physical characters include color, pH and chemical characters include qualitative phytochemical testing, vitamin C content, total phenol content, total flavonoid content and antioxidant activity with DPPH inhibitory activity. The results showed that the formulation of the mass ratio of liang tea: citrus peel 70:30 had the highest total phenol content, total flavonoid and antioxidant activity ($63,82 \pm 3,70$ mg GAE/g ext, $47,73 \pm 4,34$ mg QE/g ext and $83,28 \pm 1,99$ % respectively). The formulation liang tea and citrus peel with mass ration 60:40 showed the highest pH value ($6,89 \pm 0,04$). The colour of tea formulation of liang tea and citur peel with mass ratio 70:30 showed colour characteristics are *L value ($32,07 \pm 0,06$), *a ($2,03 \pm 0,06$), *b ($0,6 \pm 0$), *C ($2,53 \pm 0,06$) and *h ($15,23 \pm 1,04$).

Keywords: Antioxidant, Liang Tea, Orange Peels, Physicochemical

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INTRODUCTION

Tea is a popular drink that originated from China and is consumed in worldwide due to its beneficial effect on health¹. Tea which is served by infusion is a beverage obtained from parts of the plant (dry leaves, flowers, and fruits) or the shoot of several herbs or aromatic plants². According to Kosinska and Andrauler (2014), tea is high valued because of its taste, aroma, health benefits, and cultural practice³.

A kind of tea is a herbal tea, and its formulation mainly consists of one herbal ingredient or a mixture of some herbal ingredients to provide a particular purpose, such as relief from a specific condition or rejuvenation⁴. One kind of herbal tea commeced at Pontianak City, Indonesia, namely liang tea, consists of a blend of herbs is

Dicliptera chinensis, *Pandanus amaryllifolius*, *Origanum vulgare* Wilder, *Aloe verachinensis*, *Tradescantia spathacea* Sw., and *Caesalpinia sappan*⁵. Each of the mentioned herbs was known to have a medicinal effect on the human body⁶⁻¹¹.

The interest in tea as a health-promoting beverage is growing, leading to the development of beverages (Vilaplana *et al.*, 2015). People usually consume tea for its attractive taste and aroma¹². One way to develop and improve tea's sensory characteristic is by adding citrus fruit peels. Citrus peels is a byproduct of orange fruit juice processing that generally treated as a waste and it has potential health promoting benefits. D-Limonene, which mostly present on citrus fruit peels is widely utilized as adjuvant and

flavoring agent in food and beverages industries as well as perfumes and other personal hygiene products.

This study's idea is to formulate tea sold in Pontianak City, with a different ratio of citrus peels. Citrus peels covering almost half of the fruit mass contains bioactive compounds such as polyphenols, carotenoids, and essential oils as well as pectin^{13,14}. Citrus peel powder has traditionally been used to add tea flavor and to prepare some traditional cake¹⁵. The research about phytochemical content and the bioactivity of tea is important to provide the scientific data to consumers about tea as a health-promoting beverage.

This research aims to determine the effect of adding *Citrus amblycarpa* peels in various ratios toward phytochemical content and antioxidant activity of herbal tea.

METHOD

The leaves of *Aloe vera*, *Pandanus amaryllifolius*, *Orthosiphon aristatus*, *Rheo discolor*, *Dicliptera chinensis*, wood of *Caesalpinia sappan* and fruit of *Citrus amblycarpa* were brought from local marketplace. *Citrus amblycarpa* peels were removed from the fruit, and the peels and other materials were washed, cleaned carefully, and dried using a cabinet dryer at 50°C for 12 hours. The dried materials were finely powdered with the grinder and sieved to 80-mesh size. The powder was stored in the plastic jar at room temperature.

The chemicals and reagents used were analytical grades. Gallic acid, sodium carbonate (Na₂CO₃), folin-ciocalteu reagent, ethanol, Potassium iodide, amylum, iodine,

aluminum (III) chloride (AlCl₃), NaNO₂, quercetin, sodium hydroxide (NaOH), were obtained from Merck (Darmstadt, Germany). 1,1-Diphenyl-2-picrylhydrazyl (DPPH) was obtained from Sigma (Sigma-Aldrich, Germany). Aquades were obtained from a local chemical store.

There are eight formulations of *Liang tea* that were used in this present study. The mass ratio of the herbs of *Dicliptera chinensis*, *Pandanus amaryllifolius*, *Caesalpinia sappan*, *Aloe vera*, *Orthosiphon aristatus*, *Rheo discolor* were 10:1:4,5:1:1:2,5 respectively, and the ratio of *Citrus amblycarpa* peels were 0, 10, 20, 30, 40, 50 and 60% of mass weight of mixed herbal tea.

Tea compositions are shown in Table 1. The herbs powder was mixed according to the treatment, and the tea was prepared by performed by Dewi *et al.* (2021) with some modifications. One sachet of liang tea, according to the treatment, was transferred to beaker glass then heated with 200ml aquadest at 80-90°C and stirred with magnetic rod for 6 minutes. All of the teas were added to xylitol as a sweetener⁵.

The tea as stored in a refrigerator with 4°C for 24 hours and filtrated through Whatman paper no.1. The filtrats of liang tea were then stored at freezer until further analysis.

RESULTS

Physicochemical characteristic of Rich Antioxidant Liang Tea with Various Substitutions of Citrus Peels

Physicochemical characteristic for each component of rich-antioxidant liang tea has been analyzed and the data shown in Table 2.

Table 1 Herb Composition for Each Formulation in Tea Bag

Ingredients	Liang tea: Citrus peels Mass Ratio						
	100:0	90:10	80:20	70:30	60:40	50:50	40:60
<i>Dicliptera chinensis</i>	1	0.90	0.80	0.7	0.6	0.5	0.4
<i>Pandanus amaryllifolius</i>	0.20	0.18	0.16	0.14	0.12	0.10	0.08
<i>Caesalpinia sappan</i>	0.45	0.405	0.36	0.315	0.27	0.225	0.18
<i>Aloe vera chinensis</i>	0.10	0.09	0.08	0.07	0.06	0.05	0.04
<i>Origanum vulgare</i> Wilder	0.10	0.09	0.08	0.07	0.06	0.05	0.04
<i>Tradescantia spathacea</i> Sw.	0.25	0.225	0.20	0.175	0.15	0.125	0.10
<i>Citrus amblycarpa</i>	0	0.20	0.40	0.60	0.80	1.0	1.20

Table 2. Physicochemical Characteristics of Fresh Ingredients of Rich-Antioxidant Liang Tea

Ingredients	Moisture content (%)	Ash content (%)	Vitamin C
<i>Dicliptera chinensis</i> *	81,26±0,83	0,15±0,03	2,42±0,38
<i>Pandanus amaryllifolius</i> *	66,19±5,79	0,31±0,05	2,2±0,44
Stem bark of <i>Caesalpinia sappan</i> **	9,43±0,94	0,58±0,22	2,68±0,32
<i>Aloe vera</i> ***	91,37±0,04	0,08±0,00	0,95±0,13
<i>Origanum vulgare</i> Wilder*	81,65±3,11	0,14±0,02	2,13±0,34
<i>Tradescantia spathaceae</i> Sw.*	91,70±1,54	0,09±0,01	0,88±0,44

Description * leaf, ** dried stem bark (commercial), *** midrib

pH

pH value of tea with various formulation were shown in figure 1. The pH value ranged to

6,71 ± 0,01 to 6,89 ± 0,04 which tea with 60% citrus peel weight ratio has the highest value.

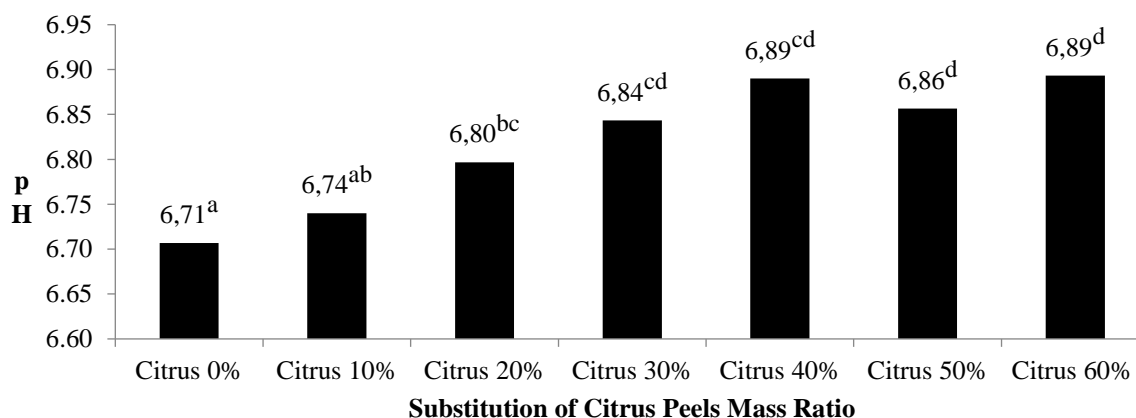


Figure 1. pH of Liang Tea with Various Substitution of Citrus Peels Mass Ratio

Vitamin C

Vitamin C content of rich-antioxidant liang tea with various formulation of citrus peel weight ratio has been shown in figure 2.

Vitamin C value ranged from 1,76 ± 0,0 to 6,01 ± 0,25 which the highest result obtained from liang tea with 0% citrus peel weight ratio.

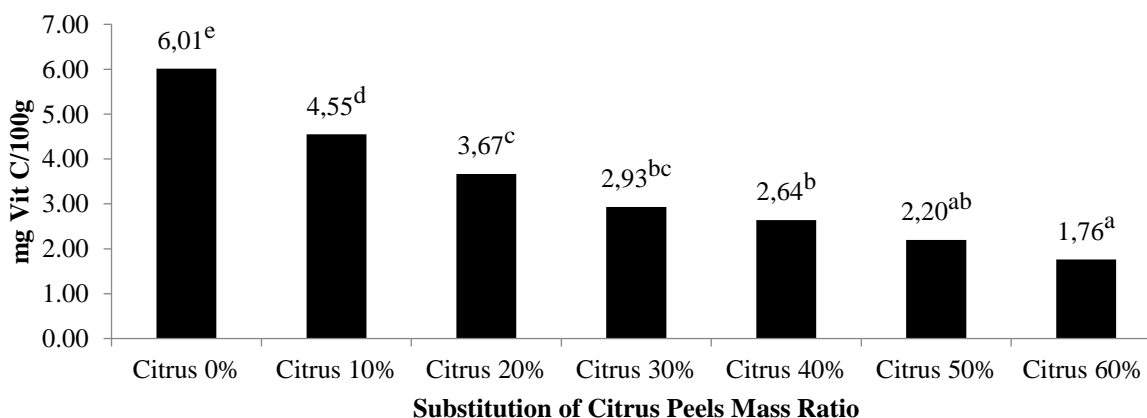


Figure 2. Vitamin C Content of Liang Tea with Various Substitution of Citrus Peels Mass Ratio

Total Phenolic Content

The amount of total phenolic content of rich-antioxidant liang tea with various formulation has been shown in figure 3. Total

phenolic content of the liang tea varied from 20,07 ± 0,94 to 63,82 ± 3,70 mg GAE/g extract which the highest value obtained from tea with 30% citrus peel weight ratio.

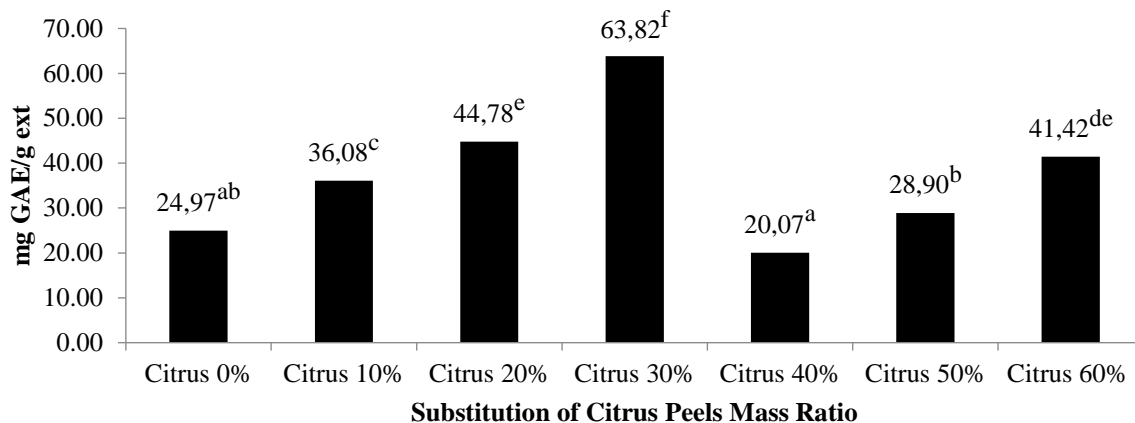


Figure 3. Total Phenolic Content of Liang Tea with Various Substitution of Citrus Peels Mass Ratio

Total Flavonoid Content

The amount of total flavonoid content of rich-antioxidant liang tea with various formulation has been shown in figure 4. Total

flavonoid content of the liang tea varied from $17,31 \pm 0,96$ to $47,73 \pm 4,34$ mg QE/g extract which the highest value obtained from tea with 30% citrus peel weight ratio.

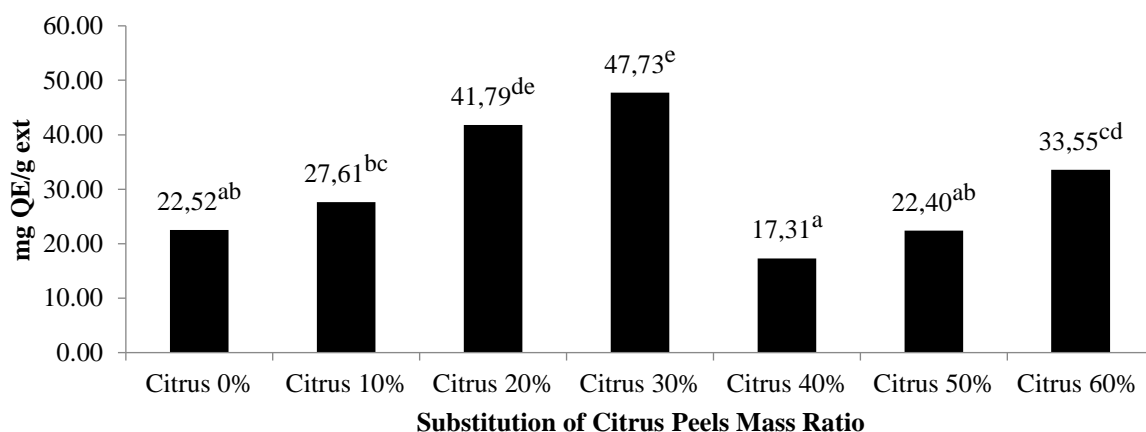


Figure 4. Total Flavonoid Content of Liang Tea with Various Substitution of Citrus Peels Mass Ratio

Antioxidant by DPPH Scavenging Activity

Antioxidant activity by DPPH radical scavenging method of rich-antioxidant liang tea are shown in figure 5. Antioxidant activity of

the liang tea varied from $67,34 \pm 1,77$ to $83,28 \pm 1,99$ % which the highest value was obtained from citrus peel 30% formulation.

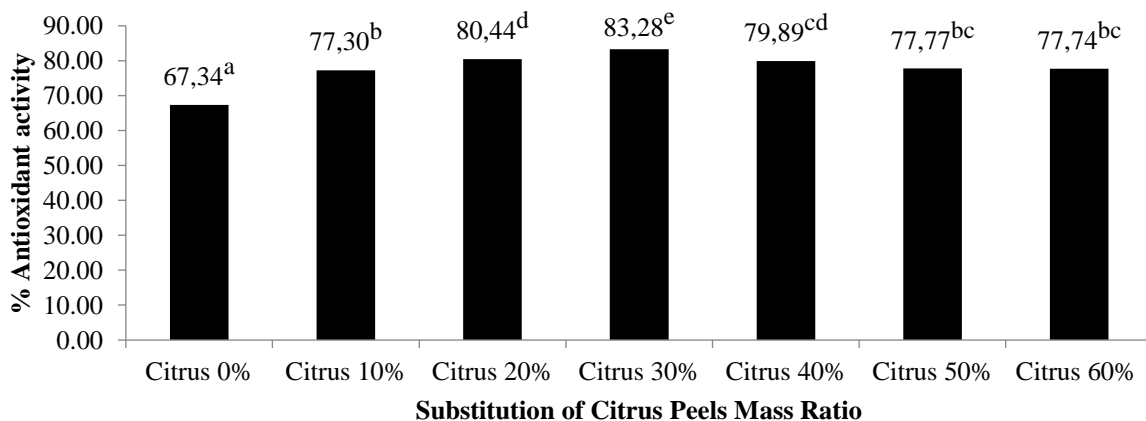


Figure 5. Antioxidant Activity of Liang Tea with Various Substitution of Citrus Peels Mass Ratio

Table 3. Phytoscreening of Riach-Antioxidant Liang Tea with Various Substitution of Citrus Peels

% Substitution of Citrus Peels	Alkaloids	Flavonoids	Phenols	Tannins	Terpenoids
0	+	+	+	+	-
10	+	+	+	+	-
20	+	+	+	+	-
30	+	+	+	+	-
40	+	+	+	+	-
50	+	+	+	+	-
60	+	+	+	+	-

Phytochemical Screening

Phytoscreening of riach-antioxidant Liang Tea with various substitution of citrus peels are shown in Table 3.

Colorimetric of Rich-Antioxidant Liang Tea

Color was determined based on International Commission on Illumination (CIE) L*a*b* system, where L* for lightness, a* for redness or greenness, and b* for

yellowness or blueness. The colorimetric of liang tea with various citrus peel weight ratio formulation are shown in Table 4. The result showed that the lightness of tea were increased as the much as the citrus peel ratio in tea formulation, fluctuate heading toward to green colour as higher the ratio of citrus peel in tea formulation, and fluctuate heading toward to yellow colour as the ratio of citrus peel in tea formulation increased.

Table 4. Colorimetric of Rich-Antioxidant Liang Tea with Various Substitution of Citrus Peels

% Substitution of Citrus Peels	L	a	b	c	h
0	27,47 ± 0,17 ^a	6,97 ± 0,38 ^e	2,7 ± 0 ^e	7,5 ± 0,36 ^g	23,87 ± 0,81 ^d
10	30,5 ± 0,17 ^b	3,8 ± 0,17 ^c	0,8 ± 0,17 ^{ab}	4,27 ± 0,4 ^e	12,87 ± 2,75 ^a
20	32,07 ± 0,06 ^d	2,03 ± 0,06 ^b	0,6 ± 0,06 ^a	2,53 ± 0,06 ^c	15,23 ± 1,04 ^{ab}
30	30,73 ± 0,31 ^{bc}	5,53 ± 0,25 ^d	1,9 ± 0,2 ^d	5,73 ± 0,25 ^f	18,68 ± 1 ^{bc}
40	31,07 ± 0,12 ^c	3,73 ± 0,15 ^c	1,33 ± 0,25 ^c	3,37 ± 0,15 ^d	21,27 ± 0,12 ^{cd}
50	32,5 ± 0,17 ^d	1 ± 0,1 ^a	0,6 ± 0,2 ^a	1,17 ± 0,06 ^a	40,4 ± 1,73 ^e
60	32,27 ± 0,06 ^d	1,27 ± 0,06 ^a	1,17 ± 0,06 ^{bc}	1,73 ± 0,21 ^b	45,27 ± 0,99 ^f

Value of Effectivity Index

Value of the effectivity index of rich-antioxidant liang tea with various substitutions

of citrus peels was shown in Table 5.

Table 5. Effectivity Index of Rich-Antioxidant Liang Tea with Various Substitutions of Citrus Peels

% Substitution of Citrus Peels	Effectivity Value
0	0,45
10	0,37
20	0,46
30	0,71
40	0,4
50	0,37
60	0,5

DISCUSSION

pH value increased simultaneously as much as the higher weight ratio of citrus peel in the tea formulation. According to Irkin *et al.* (2015) citrus peel extract pH value was around 6,62 ± 2,2¹⁶. The increment of pH value of tea might be due to the citrus peel's pH, which affects the pH of the citrus peel's pH and the tea's overall pH. Vitamin C value decreased simultaneously as much as the higher weight

ratio of citrus peel in the formulation of the tea. Reduction of the tea's vitamin C content as the citrus peel mass ratio increases due to the drying process of citrus peels in the preparation step. According to the USDA National Nutrient database, the peel of citrus fruit possess more vitamin C than its juice¹⁷. Ascorbic acid is sensitive vitamin against heat treatments. The reported percentage of vitamin C loss of C. valenciana peels due to heat treatment by

microwave, solar and air oven methods was 46,64, 52,95 and 68,83%, respectively¹⁷. Several factors also affect the loss of vitamin C due to oxidation process as well as also affect the loss of vitamin C due to the oxidation process and heat treatment that is as the exposure of light, the presence of oxygen, metal ions, and pH¹⁸.

Total phenolic content tend to be increased as the citrus peel weight ratio increased and reached its peak at 30% weight ratio. According to Park *et al.* (2014) total phenolic content of citrus peels ranged from 1,39 mg GAE/100g to 1,85 mg GAE/g, higher than the fruits¹⁹. Arora and Kaur (2013) reported the highest total phenolic content was extracted from citrus peel with aqueous solution (210 mg GAE/g peels)²⁰. Major phenolic compounds present in the citrus include hydroxycinnamic acid (HCA) and flavonoids, among which flavanones are the most prevalent²¹.

Total flavonoid content also increased as the citrus peel weight ratio increased and reached its peak at 30% weight ratio. Flavanones, flavones and flavonols are three types of flavonoids which occur in citrus fruit. The main flavonoids found in citrus species are hesperidine, narirutin, naringin and eriocitrin¹³. Citrus flavonoids, especially hesperidin, have a wide range of therapeutic properties including anti-inflammatory, antihypertensive, diuretic, analgesic and hypolipidemic activities¹⁹.

Antioxidant defined as substance that capable to prevent or delaying the oxidation process of molecules such as lipids, proteins and nucleic acids. Antioxidant constituents act as radical scavengers and converting radicals into less reactive species and can be found in natural source such as fruits, vegetables and meats. Several common natural antioxidants which are found in daily foods are vitamin C (ascorbic acid), vitamin E (tocopherols), vitamin A (carotenoids), polyphenols including flavonoids and anthocyanins. Antioxidant activity of tea increased and reached its peak at 30% citrus peel weight ratio. The highest radical scavenging activity treatment showed a high phenolic and flavonoid, similar result reported from previous study¹³. According to the same report, there was no correlation between both total phenolic and flavonoid content against radical scavenging activity in linear regression analysis. On the contrary, several reports showed a strong correlation on

phenolic and flavonoid contents of citrus fruit peel extracts against DPPH assay^{22,23}. In general, the fruit's peel contains a higher concentration of antioxidant substances than the fruit's flesh²⁴. Synergistic effects and other substances such as vitamin C, carotenoids, and pigments, especially from tea ingredients may have contributed to the DPPH Radical Scavenging Activity²⁵.

A qualitative phytochemical screening test showed significant indication of certain metabolites' presence²⁶. Many scientific articles stated that phytochemical content and composition varies due to different factors and one of the factors indicated is environmental difference and variety of the plant²². The presence of phytochemical such as alkaloid, flavonoid, phenol, tannin and terpenoid in rich-antioxidant liang tea.

Chroma (C*), considered the quantitative indicator of colorfulness, is used to determine the degree of difference in a hue compared to a grey color with the same lightness. The higher the chroma values, the higher color intensity of samples is perceived by humans, which means the higher amount of citrus peel added in tea, the lower intensity of the color of tea. Hue angle (h*), considered the qualitative indicator of color, is an attribute according to which colors have been traditionally defined as reddish, greenish, and is used to define the difference of a certain color with the reference to grey color of the same lightness. The higher amount of citrus peel in tea formulation, the higher the h value. A higher hue angle represents a lesser yellow character in the assays and a greater redness intensity²⁷. Based on the effectivity index of rich-antioxidant liang tea with various substitutions of citrus peels, the mass ratio of 30% citrus peel substitution in formulation showed that the best formulation to produce rich-antioxidant liang tea.

CONCLUSION

Tea mainly consumed as the substitution of citrus peel in rich-antioxidant liang tea formulation affect both phytochemical and physicochemical characteristic of tea itself. Mass Ratio 30% citrus peel substitution in rich-antioxidant liang tea was the best result as it had the highest total phenolic, flavonoid and antioxidant activity (63,82 ± 3,70 mg GAE/g extract, 47,73 ± 4,34 mg QE/g extract and 83,28 ± 1,99 % respectively).

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