

## The Development of Herbal Drink Mixed Collagen and Low-calorie Stevia

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### Abstract

The objective of this study was to develop an herbal drink mixed collagen and low-calorie stevia. Six formulas of the herbal drink contained kariyat juice, butterfly pea juice, ginger juice, and lime juice. It was found that the formulas of this herbal drink affected the scores of the colors, flavors, and tastes as well as the statically overall preferences ( $p < 0.05$ ). In terms of the 1 formula of the herbal drink, the preference scores of the color, flavor, taste, and overall preference were the most of all. According to the study of stevia content in this herbal drink, which could be equivalent to 6, 8, 10, and 12 °brix of sugar, it was found that the amount of stevia impacted on the scores of the taste and statically overall preferences ( $p < 0.05$ ). The herbal drink with stevia equivalent to 12 °brix of sugar gained the highest scores in taste and the overall preference. Regarding the study of the collagen content in the herbal drink at 4 levels 1,000, 2,000, 3,000, and 4,000 mg, the results showed that the collagen content did not affect the scores of the color, flavor, taste, and statically overall preference ( $p \geq 0.05$ ). However, the 4,000 mg collagen was selected to add into the herbal drink due to the fact that it was the highest content of all. The  $L^*$ ,  $a^*$ , and  $b^*$  values of the developed herbal drink were 7.55, 6.83, and -8.59, respectively. Moreover, in the developed formula, the total soluble solid (sugar) was 6 °brix; the total acidity was 1.74%; the pH value was 3.8; and the vitamin C content was 89.99 mg. Finally, the results of 50 general consumers' acceptance test revealed that most of the consumers (58%) moderately and highly liked the product.

**Keywords** Herbal drink, Collagen, Stevia

### Introduction

The pandemic of Coronavirus 2019 or COVID-19 began at the end of 2019 and spread throughout the world. It has extremely made people fear and concerned about the impacts on their health, society, and economy (Mishra et al., 2020). After the end of 2022, it will be the post COVID-19 pandemic, and this disease will become an endemic because fewer people get this virus, and its harms are reducing (Biancolella et al., 2022). However, this virus is still remaining in the common environment and can be spread again, especially in winter. There are various methods to prevent this COVID-19 such as social distancing, vaccinations, regular exercise, enough sleep, healthy minds, and useful diets. Particularly, some food containing herbs such as kariyat, gingers, limes, and butterfly peas can be consumed to promote good health, add more immune system, and prevent COVID-19 (Dahiya et al., 2022; Anh et al., 2020; Mohanapriya,

Ramaswamy & Rajendran, 2013; Vidana, Lim & Choo, 2021). Therefore, this innovative project about an herbal drink with collagen and low-calorie stevia was interestingly studied in order to modify Thai herbs to be a flavored beverage that is easily consumed, including the addition of collagen for the health of the skin of consumers, prevents diseases, strengthen the immune system, and benefit consumers in the post COVID-19.

## **Objective**

To develop an herbal drink with collagen and low-calorie stevia for consumers.

## **Materials and Methods**

### **1. Raw materials**

Kariyat was brought from no. 13, Weruwam Road, Sateng Subdistrict, Mueang District, Yala Province. Fresh butterfly peas, gingers, and fresh limes were from Market 4, Mueang District, Yala Province. Stevia (Green Sweet Brand) was from Green Food Asia Company Limited, no. 455-424, Charan Sanit Wong Road, Bang Khun Sri, Bangkok Noi, Bangkok. (stevia is used as a component of unrefined sugar 99.5% and Steviol glycosides 0.5% and reduces energy by 50%). A supplement (Ime Brand) of collagen peptide made from fish was a product by Medici International Company Limited, no.28, Soi Wachiratham Satit 51, Sukhumvit Road, Bang Chak, Phra Khanong, Bangkok.

### **2. Herbal drink preparation**

Kariyat juice was prepared by cleaning and chopping 10 grams of fresh kariyat leaves into pieces and mixing them with 500 grams of water. Then the mixture was blended in a blender for 1.5 minutes and filtered with a strainer.

Fresh butterfly pea petals were washed and weighed to make butterfly pea juice. The ratio of butterfly peas and water was 130:2,600 grams. After that, the mixture was boiled for five minutes before being filtered with a white cloth.

For ginger juice, gingers were washed, peeled, and sliced. The ratio of gingers and water was 1:15. Then the mixture was blended for 1.5 minutes and filtered with a strainer.

Finally, lime juice was prepared by cleaning fresh limes and cutting them in half. Their seeds were taken out before the limes were squeezed and filtered with a strainer.

### **3. Methods**

#### **3.1 The study of herbal drink formulas**

The proportion of six formulas was studied to have the herbal drink formulas as shown in Table 1. Next, sugar with 8 °brix of sweetness was added into each formula before the mixture was pasteurized at 85 °C for five minutes. The final product of each formula was poured into glass bottles (sterilized by boiling in hot water for 10 minutes) while it was still hot. After each bottle was capped, they were immediately brought to soak in water at room temperature. Each herbal drink formula was tested for sensory testing of food via 9-point Hedonic Test in terms of colors, flavors, tastes, and overall preferences. This test was conducted with 30 samples who were not trained. In addition, Randomized Complete Block Design (RCBD)

was applied to plan the experiment, Analysis of Variance (ANOVA) was employed to analyze the data, and Duncan's New Multiple Range Test (DMRT) was used to compare the mean at 95% of the confidence level. Then the formula that was liked the most by the samples was used in the next step.

**Table 1** Six formulas of herbal drink

Formula	proportion			
	Butterfly pea juice	Ginger juice	Kariyat juice	Lime juice
1	5	2.875	0.125	2
2	5	3.875	0.125	1
3	5	3.75	0.25	1
4	5	2.75	0.25	2
5	5	2.5	0.5	2
6	5	3.5	0.5	1

### 3.2 The study of the stevia content in herbal drink

The suitable stevia content mixed with the selected herbal drink formula was then studied. The selected stevia needed to be the ingredient between 99.5% unrefined sugar and 0.5% steviol glycosides. Therefore, this stevia contained 50% of normal calories. In fact, two teaspoons of sugar give 32 kilocalories, which is equivalent to one teaspoon of stevia power which is only 16 kilocalories. In the experiment, the stevia contents which were similar to 6, 8, 10, and 12 °brix (3, 4, 5, and 6 % (w/v) respectively) of sugar were added to the herbal drink. The mixtures were pasteurized at 85 °C for five minutes before being filled into hot glass bottles (sterilized by boiling in hot water for 10 minutes). The herbal drink bottles were immediately brought to immerse in water at room temperature after being capped. The 9-point Hedonic Test in terms of colors, flavors, tastes, and overall preferences was conducted for each herbal drink bottle with 30 samples who were not trained. Besides, Randomized Complete Block Design (RCBD) was applied to plan the experiment, Analysis of Variance (ANOVA) was used to analyze the data, and Duncan's New Multiple Range Test (DMRT) was conducted to compare the mean at 95% of the confidence level. Then the most favorite herbal drink mixed with the suitable stevia was brought to conduct in the next step.

### 3.3 The study of collagen contents added to herbal drink

In this step of the study, there were four levels of collagen contents added to the herbal drink: 1,000, 2,000, 3,000, and 4,000 milligrams for 250 ml of the herbal drink. The mixed liquids were pasteurized at 85 °C for 5 minutes before they were filled into hot glass bottles (sterilized by boiling in hot water for 10 minutes). The herbal drink bottles were capped and then immediately brought to immerse in water at room temperature. Later, the 9-point Hedonic Test was used to test the colors, flavors, tastes, and overall preferences of each herbal drink bottle with 30 samples who were not trained. Besides, Randomized Complete Block Design (RCBD) was applied to plan the experiment, Analysis of Variance (ANOVA) was

conducted to analyze the data, and Duncan's New Multiple Range Test (DMRT) was used to compare the mean with 95% of confidence level. Then the most favorite herbal drink mixed with the suitable stevia and collagen was brought to conduct in the next step.

### 3.4 The study of the properties of the developed herbal drink mixed collagen and stevia.

The herbal drink mixed collagen and stevia was analyzed for its Color value ( $L^*$ ,  $a^*$  and  $b^*$ ), total soluble solid (TSS), acidity (as citric acid), pH, and Vitamin C content which were determined according to AOAC. (2002).

### 3.5 The acceptance test by common consumers

The acceptance test was conducted ask 50 common consumers through the 9-point Hedonic scale (1: the most disliked to 9: the most liked). After that, the results were evaluated in percentage.

## Results

### 1. The study of herbal drink formulas

Six formulas of the herbal drink were prepared as shown in Table 1. The findings revealed that the formulas could significantly affect the scores of colors, flavors, tastes, and statistically overall preferences ( $p < 0.05$ ). As displayed in Table 2.

**Table 2** Sensory data for six herbal drink formulas by the hedonic test

Formula	Scores of Hedonic Test*			
	Colors	Flavors	Tastes	Overall Preferences
1	8.06±0.90 <sup>a</sup>	7.90±0.80 <sup>a</sup>	8.10±0.84 <sup>a</sup>	8.33±0.71 <sup>a</sup>
2	7.33±1.10 <sup>b</sup>	7.13±1.00 <sup>b</sup>	7.50±1.14 <sup>b</sup>	8.03±1.07 <sup>a</sup>
3	7.13±0.90 <sup>b</sup>	6.23±0.80 <sup>c</sup>	6.17±0.79 <sup>c</sup>	6.30±0.92 <sup>c</sup>
4	8.03±0.90 <sup>a</sup>	6.67±1.20 <sup>bc</sup>	6.50±1.11 <sup>c</sup>	6.87±1.07 <sup>b</sup>
5	8.16±0.80 <sup>a</sup>	4.87±1.60 <sup>d</sup>	3.50±1.33 <sup>d</sup>	3.13±1.14 <sup>d</sup>
6	7.33±1.00 <sup>b</sup>	4.43±1.90 <sup>d</sup>	2.23±0.94 <sup>e</sup>	2.43±0.97 <sup>e</sup>

Mean ± standard deviation, \* 30 samples

<sup>a-d</sup> The numbers with these letters in the same column were significantly different ( $p < 0.05$ ).

### 2. The study of the stevia content in herbal drink

After the study of the stevia contents at four levels which were equivalent to 6, 8, 10, and 12 °brix of sugar, it was found that the stevia contents significantly affected the scores of tastes and statistically overall preferences ( $p < 0.05$ ). In Table 3.

**Table 3** Sensory data for the herbal drinks mixed with four levels of stevia by the hedonic test.

Stevia sweetness is equivalent to sugar (°brix)	Scores of Hedonic Test*			
	Colors <sup>ns</sup>	Flavors <sup>ns</sup>	Tastes	Overall Preferences
6	7.67±1.03	7.57±1.07	6.37±1.07 <sup>c</sup>	6.53±1.25 <sup>c</sup>
8	7.57±1.04	7.53±1.07	6.80±1.19 <sup>bc</sup>	6.93±1.14 <sup>bc</sup>
10	7.40±0.89	7.50±0.94	7.23±1.04 <sup>b</sup>	7.43±1.10 <sup>b</sup>
12	7.53±1.25	7.57±1.19	8.43±0.77 <sup>a</sup>	8.60±0.56 <sup>a</sup>

Mean ± standard deviation, \* 30 samples

<sup>a-c</sup> The numbers with these letters in the same column were significantly different ( $p < 0.05$ )

<sup>ns</sup> The numbers with these letters in the same column were not significantly different ( $p > 0.05$ ).

### 3. The study of collagen contents added to herbal drink

According to four levels of the collagen content 1,000, 2,000, 3,000, and 4,000 milligrams for 250 milliliters of the herbal drink, it was found that all the collagen content levels did not affect the scores of the colors, flavors, tastes, and statistically overall preferences ( $p < 0.05$ ) (Table 4).

**Table 4** Sensory data for the herbal drinks mixed with four levels of collagen content by the hedonic test.

Collagen content (milligrams)	Scores of Hedonic Test*			
	Colors <sup>ns</sup>	Flavors <sup>ns</sup>	Tastes <sup>ns</sup>	Overall Preferences <sup>ns</sup>
1,000	7.40±1.19	6.87±1.14	7.07±1.17	7.00±0.80
2,000	7.43±1.10	6.80±1.13	7.00±1.17	6.93±0.66
3,000	7.40±0.86	6.73±0.78	7.00±0.95	7.00±0.56
4,000	7.40±1.30	6.80±1.40	7.13±1.36	7.00±0.65

mean ± standard deviation,\* 30 samples

<sup>ns</sup> The numbers with these letters in the same column were not significantly different ( $p > 0.05$ ).

### 4. The study of the properties of the developed herbal drink mixed collagen and stevia.

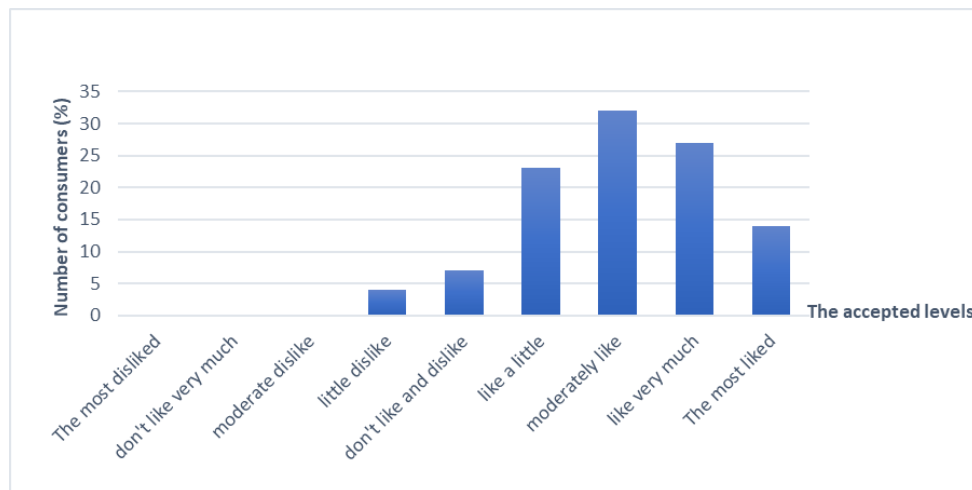
The results showed that L\*, a\*, and b\* values of this develop herbal drink were 7.55, 6.83, and -8.59, respectively. Moreover, its total soluble solid (sugar) value was equal to 6 °brix of sugar; its pH value was 3.8; its vitamin C content was 89.99 milligrams/100 g. The results showed in table 5.

**Table 5** Physical and chemical properties of herbal juice for supplementing collagen with stevia, developed formula

Property of product	Herbal Drink Mixed Collagen and Low-calorie Stevia
Color value	
L*	7.55±0.90
a*	6.83±0.15
b*	-8.59±0.23
Total soluble solid (°brix)	6.00±0.10
Total acidity (%)	1.74±0.57
pH	3.80±0.10
Vitamin C (milligrams/ 100 g)	89.99±0.02

### 5. The acceptance test by common consumers

According to the survey of the acceptance test, 50 consumers participated in testing the herbal drink mixed with collagen and low-calorie stevia. As shown in Figure 1.



**Figure 1** The accepted levels of the herbal drink mixed with collagen and low-calorie stevia by 50 Consumers

## Discussion

### 1. The study of herbal drink formulas

Six formulas of the herbal drink were prepared as shown in Table 1 and tested by Duncan's New Multiple Range Test (DMRT) at 95% of confidence level. The findings revealed that the formulas could significantly affect the scores of colors, flavors, tastes, and statistically overall preferences ( $p < 0.05$ ). As displayed in Table 2, the first herbal drink formula received the highest scores in the color, flavor, taste, and overall preference. The ratio of this formula contained butterfly pea juice: ginger juice: kariyat juice: and lime juice at 5: 2.875: 0.125: 2. In fact, butterfly peas contain a pigment called anthocyanin which is

one of the antioxidants with high activity and immune system to a human's body (Vidana, Lim & Choo, 2021). In terms of ginger, their taste is hot and spicy, which can increase the immune system to prevent influenza viruses, so some local people consume them to kill influenza. Plus, gingers contain zingerol and gingerol which can attack the main protease (Mpro) to restrain the cell division of Coronavirus 2019 (Anh *et al.*, 2020). Kariyat includes Andrographolide, and its properties can help to prevent coughing, pain, inflammation, oxidants, and some viruses, as well as increase the immune system (Dahiya *et al.*, 2022). Regarding limes, they contain high content of vitamin C which plays an important role in antioxidants, inflammation reduction, and cell recovery from infections (Mohanapriya, Ramaswamy & Rajendran, 2013). It could clearly be seen that these four herbal plants have a lot of benefits for people's health nowadays.

## **2. The study of the stevia content in herbal drink**

After the study of the stevia contents at four levels which were equivalent to 6, 8, 10, and 12 °brix of sugar, it was found that the stevia contents significantly affected the scores of tastes and statistically overall preferences ( $p < 0.05$ ). In Table 3, the herbal drink mixed with the stevia which was equivalent to 12 °brix of sugar gained the highest scores. Its main taste was sweet and sour, and it was also slightly bitter from kariyat. It was indicated that stevia is a natural sweetener plant with fewer calories and impacts people's health. According to US Food and Drug Administration (FDA), stevia was considered and announced to be a safe acceptable sugar substitute. Its leaves contain nutritious values, and it has medically been accepted to have no toxins. A human's body can itself expel this herb without storage; therefore, it is suitable for consumers who are health-conscious and diabetic; or control weight or blood sugar. A sativoside is a natural sweetener used instead of sugar or sucrose, and it is as 300 times sweet as sugar (Gupta, Yadav & Rastogi, 2012; Gupta, Purwar, Sundaram & Rai, 2013). Therefore, it was selected and added to the herbal drink as a low-calorie sweetener for consumers to easily drink. In this study, the stevia content which was equivalent to 12 °brix of sugar was selected.

## **3. The study of collagen contents added to herbal drink**

According to four levels of collagen content 1,000, 2,000, 3,000, and 4,000 milligrams for 250 milliliters of the herbal drink, it was found that all the collagen content levels did not affect the scores of the colors, flavors, tastes, and statistically overall preferences ( $p < 0.05$ ) because the fish collagen used in the study has no color, smell, and taste. A similar result was reported by Krasnova *et al.* (2020) showed that the sensorial properties of fruit beverages containing fish collagen hydrolysates proved to be comparable to control samples produced without fish collagen hydrolysates. The addition of fish collagen hydrolysate did not affect the color of the product. Also, what is very important, the beverages had a rich fruit taste without a specific fish taste and smell. The panelists noted that the addition of fish collagen hydrolysate had virtually no effect on the consistency of the beverages. In Table 4, the 4000 milligrams of collagen were selected to add to the product since this content was the highest and probably the most useful for consumers. The collagen peptides from fish were the most commonly found protein in a human's body, which is a short-form amino acid containing collagen and 8 out of 9 essential amino acids. These big collagen molecules were conducted by hydrolysis which is a water reaction to dissolve big molecules until they are broken into small molecules, so it is easy for a human's body to absorb them. In addition, a

human's body needs collagens to strengthen tissues, skin, bones, cartilage, joints, and blood vessels. Collagen peptides are rich in 90% of protein and three amino acids glycine, hydroxyproline, and proline. Importantly, collagens from fish have been considered the best resource because of the higher amount of collagen type 1. This collagen type is commonly (90%) found in the skin, hair, nails, bone organs, and tendons; therefore, it is good for the skin, and it is consumed for beauty. It is also easy to be absorbed due to its smallest and lightest molecules when compared to collagens from other animals. It could be clear that the collagen content from fish is higher than that of other resources, and a human's body can absorb and digest it easily so that it can be faster to flow into the blood, especially the hydrolyzed collagen. Besides, the collagen from fish has various benefits such as strengthening and making organs flexible, particularly bones, blood vessels, joints, cartilages, tendons, and skin. It can also relieve inflammation and pain around cartilage cells, make skin flexible, reduce wrinkles, minimize scarring, and add more moisture to the skin (Nasri, 2019; Sato, Jimi & Kusubata, 2019; Sato, Jimi & Kusubata, 2019). According to the Thailand Food and Drug Administration (FDA), the appropriate collagen content in one day is 5 - 7 grams (5,000 - 7,000 milligrams), and it should not be taken more than 10 grams (10,000 milligrams) a day in order to prevent some dangers to consumers (DISTHAI, 2017). The collagen used in this study was certified by Halal, so Muslim people can consume this herbal drink.

#### **4. The study of the properties of the developed herbal drink mixed collagen and stevia.**

The results showed that  $L^*$ ,  $a^*$ , and  $b^*$  values of this developed herbal drink was 7.55, 6.83, and -8.59, respectively. Moreover, its total soluble solid (sugar) value was equal to 6 °brix of sugar; its pH value was 3.8; its vitamin C content was 89.99 milligrams. A similar result was reported by Puranik et al. (2013) showed that vitamin C was reduced from 134 milligrams /100 grams to 62.6 milligrams /100 grams in the herbal beverage. It was indicated that vitamin C, pH, and TSS all gradually decreased, while acidity increased; all these three factors have a strong correlation. Since vitamin C is soluble in water and oxidation sensitive, it gradually decreased; this is the main reason for lowering the value of acidity and TSS.

#### **5. The acceptance test by common consumers**

According to the survey of the acceptance test, 50 consumers participated in testing the herbal drink mixed with collagen and low-calorie stevia. It was found that most of them (58%) accepted this product with rating scores from moderate to the high level, with the average preference at 7.06. as shown in Figure 1. It can be considered a useful herbal drink and has been well accepted by consumers.

### **Conclusion**

The developed herbal drink mixed with collagen and low-calorie stevia contained 47 grams of butterfly pea juice, 27.025 grams of ginger juice, 1.175 grams of karyat juice, 18.8 grams of lime juice, 6 grams of stevia, 4,000 milligrams of collagen from fish, and 89.99 grams of vitamin C. The results of the acceptance test by 50 consumers revealed that 58% of them moderately and highly liked the product, with the average preference at 7.06.

### **Suggestion**

Herbal Drink Mixed with Collagen and Low- calorie Stevia products should have a shelf-life study.



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