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Development of herbal juice from *Centella asiatica*: antioxidant property, nutritional value and shelf life of product

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Abstract

Centella asiatica, Bua Bog, has been widely used in Thailand for more than a hundred years as an important medicinal herb. The objective of this study was to determine the effect of blanching time of *C. asiatica* on antioxidant activity. Developed-pasteurized *C. asiatica* juice was also evaluated for sensory evaluation, nutrition values, and shelf life. Fresh leaves were blanched in hot water for 0, 1, and 2 minutes, before taking to make a juice and the filtrates were determined total extractable phenolic content (TPC), total extractable flavonoid content (TFC), and antioxidant activities. The juice was divided to add with sugar at 1.2, 2.5 and 5% as low sugar (LS), medium sugar (MS), and high sugar (HS). All treatments were evaluated for sensory quality. Selected samples were pasteurized at 85 ± 1 °C for 15 seconds then microbial and sensory evaluation were conducted during storage at 8 °C and 25 °C. The results indicated that the juice obtaining from blanching time for 2 minutes showed the highest value of TPC and TFC, including antioxidant activity. The LS was selected to produce pasteurized juice product that could be stored at 8 °C for 10 days. Therefore, *C. asiatica* can be pasteurized as juice and used as functional drink.

Keywords: *Centella asiatica*; antioxidant property; herbal juice; functional drink.

Practical Application: *Centella asiatica*, an herbal plant in Thailand, is a source of antioxidants and bioactive compounds. Improving the extraction process could increase the levels of antioxidants from the leaves for producing a potent health drink.

1 Introduction

Nowadays, food consumption does not only consider calories and main nutrition but also other specific health effects, including antioxidants, antimicrobial, anti-inflammatory, immune-enhancing nutrients, blood glucose lowering, and so on (Barbosa et al., 2021; Eor et al., 2021; Iwansyah et al., 2021). Plants, including vegetables and herbs, have been used as an important source of bioactive compounds and ingredients for functional food (Mazahir et al., 2022; Wang et al., 2022). There has been a rapid growth of the functional food market in South East Asian countries, especially Thailand (Puranabhandu & Mullis, 2021). In Thailand, herbs have been used as an important source for producing various pharmaceuticals, health care purpose, and nutraceuticals, including functional products (Salawu et al., 2011).

Centella asiatica is a local Thai plant belonging to the Apiaceae family and widely spread in Southeast Asian countries such as Indonesia, Malaysia and Thailand (Orhan, 2012; Rattanakom & Yasurin, 2014). *C. asiatica* or "Bua Bog", as it is called Thai has been widely used as an important medicinal herb and functional food in Thailand and others around the world (Gohil et al., 2010; Seevaratnam et al., 2012; Yasurin et al., 2016). This herb contains many types of active compounds that exhibit antioxidant, antimicrobial, neuroprotective, and other properties. Moreover, *C. asiatica* was claimed to be one of the important medicinal plants in the international medicinal plant trade (Seevaratnam et al., 2012). However, utilization of *C. asiatica* extract as a commercial functional drink in Thailand is limited. Therefore, to increase market value, the effect of the blanching condition on the extraction of *C. asiatica* was studied including antioxidant activity and the influence of pasteurized *C. asiatica* juice on sensory evaluation, nutrition values, and shelf life.

2 Materials and methods

2.1 Chemicals

Most of the chemicals used for this experiment were purchased from Sigma-Aldrich, Seelze, Germany; Merck, Darmstadt, Germany; Ajax Finechem, Auckland, New Zealand; QRAC, Selangor, Malaysia; Fisher Scientific, Leicestershire, England; and LAB-SCAN, Dublin, Ireland.

2.2 Plant material

C. asiatica leaves in the right stage – that is, leaves which can still be folded without breaking easily - were purchased directly from the local market (Songkhla, Thailand) and transported to the laboratory within 24 hours of harvesting.

2.3 Sample preparation

The *C. asiatica* leaves were washed with tap water, drained and air-dried for 15 minutes. Then, the leaves were blanched in hot water at 85 ± 1 °C for 0, 1, and 2 minutes and cooled

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down to 4 °C. The blanched leaves were ground with water at a ratio 1:2 before being filtered through three layers of gauze. The filtrates were kept at -20 °C to determine TPC, TFC, and antioxidant activities.

2.4 Determination of total extractable phenolic and flavonoid contents and antioxidant activities

Total extractable phenolic content (TPC)

The TPC was measured using the Folin–Ciocalteu method (Tan & Kassim, 2011). Briefly, 500 µL of the extract was added to the tube, then 2.5 mL of Folin-Ciocalteu reagent (10% v/v) and 2 mL of sodium carbonate (Na_2CO_3) solution (7.5% w/v) were added and mixed thoroughly. After incubation for 30 minutes in the dark at ambient temperature, the absorbance was measured at 765 nm using a spectrophotometric microplate reader. The measured values were compared with a standard curve prepared with gallic acid and expressed as mg of gallic acid equivalents/g dry extract (GAE/g dry extract).

Total extractable flavonoid content (TFC)

The TFC content was measured by the colorimetric method (Imam et al., 2011) with some modifications. Briefly, 800 µL of distilled water were added to 200 µL of the extract, followed by 60 µL of 5% (w/v) sodium nitrite ($NaNO_2$) solution and 60 µL of 10% (w/v) aluminum chloride ($AlCl_3$) solution. The mixture was allowed to stand at ambient temperature for 5 minutes then 400 µL of 1 M sodium hydroxide (NaOH) solution was added. Thereafter, the volume of the reaction mixture was made up to 2 mL with distilled water and mixed thoroughly. The absorbance of solutions was measured with a spectrophotometric microplate reader at 510 nm. The TFC content was calculated from the standard curve of catechin and expressed as mg of catechin equivalent (CE)/g dry extract.

2,2-diphenyl-1-picrylhydrazyl (DPPH) radical scavenging activity

DPPH radical scavenging activity was determined by the DPPH assay using the modified method from Raj et al. (2016). The extract (150 µL) was added to 150 µL of 0.2 mM DPPH in 95% ethanol. The mixture was shaken lightly and stood at ambient temperature for 30 minutes in the dark. The absorbance was determined at 517 nm, using a spectrophotometric microplate reader. The activity was calculated from the calibration curve of gallic acid and expressed as mg of gallic acid equivalent (GAE)/g dry extract.

2,2'-azino-bis (3-ethylbenzothiazoline-6-sulphonic acid (ABTS) radical scavenging

The procedure used for the ABTS assay was as the modified method of Pereira et al. (2014). The stock solutions included 7.4 mM ABTS solution and 2.6 mM potassium persulfate ($K_2S_2O_8$) solution. The working solution was prepared by mixing the two stock solutions in equal quantities and allowing them to react for 12-14 hours at room temperature in the dark. The solution was then diluted by mixing 1 mL of ABTS solution with 48 mL of distilled water to obtain an absorbance of 1.1 ± 0.02 units at 734 nm. Fresh ABTS solution was prepared and used within 2 hours. The prepared extract (15 µL) was mixed with 285 µL of ABTS solution and the mixture was kept at room temperature in the dark for 2 hours. The absorbance was then measured at 734 nm using a spectrophotometric microplate reader. A standard curve of gallic acid was prepared. The activity was expressed as mg equivalent of GAE/g dry extract.

Ferric ion reducing antioxidant power (FRAP) activity

The FRAP assay was conducted according to the method of Tan & Chan (2014) with some modifications. The stock solutions included 300 mM acetate buffer [3.1 g sodium acetate trihydrate ($C_2H_3NaO_2.3H_2O$) and 16 mL acetic acid ($C_2H_4O_2$)], pH 3.6, 10 mM of 2, 4, 6- tripyridyl-s-triazine (TPTZ) solution in 40 mM HCl, and 20 mM Iron (III) chloride hexahydrate ($FeCl_3.6H_2O$) solution. The fresh working solution was prepared by mixing 25 mL acetate buffer, 2.5 mL TPTZ solution, and 2.5 mL $FeCl_3.6H_2O$ solution and then warmed up at 37°C before use. The extract (15 µL) was allowed to react with 285 µL of the FRAP solution for 30 minutes in the dark. Readings of the colored product were then taken at 593 nm using a spectrophotometric microplate reader. The standard curves were prepared using gallic acid and reported as mg equivalent of GAE/g dry extract.

Ferrous ion chelating (FIC) activity

The FIC activity on ferrous (Fe^{2+}) was determined using the method of Mau et al. (2003) with some modifications. 1 mL of extract solution was mixed with 3.7 mL of distilled water. The mixture was then reacted with 0.1 mL of 2 mM iron (II) chloride ($FeCl_2$) and 0.2 mL of 5 mM ferrozine for 20 minutes at ambient temperature. The absorbance was read at 562 nm, using a spectrophotometric microplate reader. The control was prepared in the same manner except that distilled water was used instead of the sample. A standard curve was prepared using ethylene diamine tetra-acetic acid (EDTA). The activity was expressed as mg EDTA equivalent (EDTAE)/g dry extract.

2.5 Preparation of the C. asiatica juice

Four formulas of the juice of *C. asiatica* leaves were set up. Two main ingredients, salt and sugar, were used for juice production. 0.00% salt and 0.00% sugar (control), 0.20% salt, and 0.00% sugar (salt control, SC), 0.20% salt and 1.25% sugar (low sugar, LS), 0.20% salt and 2.50% sugar (medium sugar, MS), and 0.20% and 5.00% sugar (high sugar, HS). All samples were pasteurized at 85 ± 1 °C for 15 seconds before taken to hot fill in the resisted container and cooled down to 4 °C.

2.6 Sensory evaluation

The panelists were recruited through a sensory evaluation class at Prince of Songkla University. Before the experiment, all panelists signed and returned the consent form to the research team. All methods used in this part of the experiment were carried out following the ethical sensory evaluation guidelines and regulations (Meilgaard et al., 2007). The sample juices (10 mL)

were coded randomly with three-digit codes and one of each sample was served randomly to the panelists. In between sample testing, the panelists were served some water to drink. The sensory quality of product samples was determined by 50 panelists with a 9-point Hedonic scale as 1 (dislike extremely); 2 (dislike very much); 3 (dislike moderately); 4 (dislike slightly); 5 (neither like nor dislike); 6 (Like slightly); 7 (like moderately); 8 (like very much) and 9 (like extremely). The preference rating score of the juice products was evaluated in terms of appearance, color, odor, flavor, and overall acceptability (Meilgaard et al., 2007).

2.7 Antioxidant and microbiological properties of the C. asiatica pasteurized juice

The selected sample juice of *C. asiatica* was analyzed for TPC (Tan & Kassim, 2011), TFC (Imam et al., 2011), and ABTS activities (Pereira et al., 2014). For microbiological properties, the juice was evaluated in terms of total viable count (TVC) (U.S. Food and Drug Administration, 2001a), yeast and mold (U.S. Food and Drug Administration, 2001b), and *Clostridium perfringens* (U.S. Food and Drug Administration, 2001c). *Staphylococcus aureus* (U.S. Food and Drug Administration, 2016), *Bacillus cereus* (U.S. Food and Drug Administration, 2020a), *Escherichia coli* and coliform bacteria (U.S. Food and Drug Administration, 2020b), and *Salmonella* Sp. (U.S. Food and Drug Administration, 2020c).

2.8 Nutrition contents of the C. asiatica pasteurized juice

The selected sample product was analyzed for proximate compositions and nutrition contents, including protein, fat, fiber, carbohydrate, ash, sugar, vitamins (vitamin A, B1, and B2) and mineral contents (sodium, calcium, and iron) according to the method in Association of Official Analytical Chemists (2019).

2.9 Microbial and sensory evaluation of pasteurized C. asiatica juice during storage

The selected juice sample of *C. asiatica* was pasteurized and kept at 25 °C and 8 °C for 0, 5, 10, and 15 days. Thereafter, each product was analyzed for microbial content as TVC (U.S. Food and Drug Administration, 2001a) and sensory evaluation by 50 panelists was conducted for appearance, color, odor, flavor, and overall acceptability with a 9-point Hedonic scale (Meilgaard et al., 2007).

2.10 Statistical analysis

A completely randomized design (CRD) was chosen for this experiment. The data were subjected to analysis of variance (ANOVA). A comparison of means was carried out by Duncan's multiple range tests. The significance was declared at $p<0.05$ using the statistical software.

3 Results and discussion

3.1 TPC and TFC contents and antioxidant activities of the C. asiatica pasteurized juice

The TPC and TFC, including antioxidant activity, determined by DPPH, ABTS, and FRAP assays in *C. asiatica* juice are shown in Table 1. The results of the TPC and TFC, including the antioxidant activity, showed the highest value of blanching time for 2 minutes followed by 1 and 0 minutes respectively. It indicated that the blanching process could increase the extractability and affect the release of bioactive compounds from plant cells that may be due to thermal disruption or breakdown of the polyphenol–protein complexes (Xiao et al., 2017). Gliszczynska-Swiglo et al. (2006) reported that using steam blanching of broccoli for 10 minutes could extract and increase the total polyphenol content by 52% compared with untreated samples; while Hiranvarachat et al. (2013) found that the β-carotene and total carotenoid contents, as well as antioxidant activities, of blanched carrots were significantly higher than the un-blanched samples. Moreover, the antioxidant activities in the juice exhibited the highest value in the ABTS assay, followed by the FRAP and DPPH assay, respectively. As expected, using water for the extraction in this plant provided more high polarity antioxidant compounds, which are easily determined by ABTS and/or FRAP assays; while the DPPH assay has a high affinity for lipophilic antioxidants (Martysiak-Żurowska & Wenta, 2012; Berker et al., 2013). Generally, phenolics are believed to be responsible for primary antioxidant activity, while flavonoids can provide both primary and secondary antioxidant activity (Lim et al., 2007). *C. asiatica* has been reported to contain several phenolic acids, such as *p*-hydroxybenzoic acid, vanillic acid, *p*-coumaric acid, *o*-coumaric acid, and *trans*-cinnamic acid, and flavonoid derivatives, such as quercetin, kaempferol, patuletin, rutin, apigenin, castilliferol, castillicetin, and myricetin (Kuroda et al., 2001; Matsuda et al., 2001; Subban et al., 2008; Orhan, 2012). Therefore, these results suggest that *C. asiatica* juice is suitable for functional drink products and a source of other antioxidants.

3.2 Sensory qualities of the C. asiatica pasteurized juice

The sensory acceptability scores of the *C. asiatica* juice after pasteurization evaluated in terms of appearance, color, odor, flavor, and overall liking are presented in Figure 1. The results showed that each attribute from all samples ranged between 4.6 ± 1.7 to 7.2 ± 0.9. Moreover, the samples with sugar added ranging from low (LS), medium (MS) to high (HS) concentrations were acceptable and yielded sensory scores between 5.5 ± 1.2 to 7.2 ± 0.9 with no significant difference ($p\geq0.05$). In addition, the result of sensory

Table 1. TPC, TFC, and antioxidant activities of *C. asiatica* juice.

Activities	Blanching time (min)		
	0	1	2
TPC (mg GAE/g dry extract)	42.68 ± 2.0^{c}	79.26 ± 4.1^{b}	137.49 ± 3.3^{a}
TFC (mg CE/g dry extract)	4.98 ± 0.19^{c}	62.04 ± 0.80^{b}	69.79 ± 0.42^{a}
DPPH (mg GAE/g dry extract)	0.88 ± 0.08^{c}	4.64 ± 0.73^{b}	7.87 ± 0.20^{a}
ABTS (mg GAE/g dry extract)	6.03 ± 0.41^{c}	25.16 ± 0.85^{b}	29.61 ± 1.01^{a}
FRAP (mg GAE/g dry extract)	2.93 ± 0.19^{c}	25.85 ± 0.62^{b}	27.87 ± 1.00^{a}
FIC (mg EDTAE/g dry extract)	1.62 ± 0.49^{c}	5.06 ± 0.16^{b}	6.04 ± 0.92^{a}

TPC means total extractable phenolic content; TFC means total extractable flavonoid content; GAE means gallic acid equivalent; CE means catechin equivalent and EDTAE means EDTA equivalent. $^{a-c}$Mean within a row with different letters are significantly different ($P<0.05$). Values are represented as mean ± standard deviation (n=3).

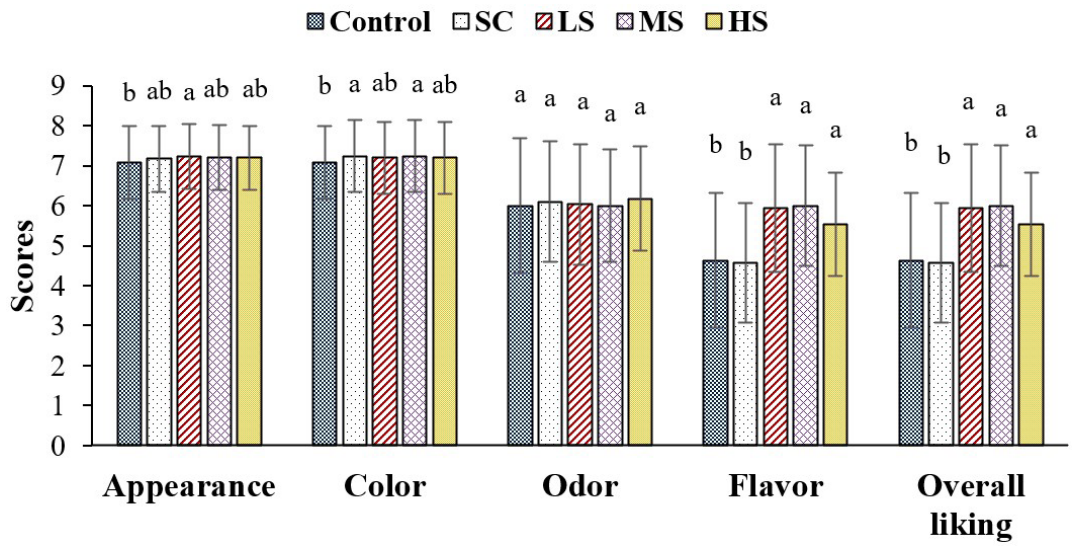

Figure 1. Sensory acceptability of the *C. asiatica* pasteurized juice. Control means control sample; SC means salt control sample; LS means low sugar sample; MS means medium sugar sample; HS mean high sugar sample. [a-b]Mean within parameter with different letters are significantly different (p<0.05). Values are represented as mean ± standard deviation (n=50).

scores pointed out that the panelists more accepted the LS sample than the HS sample of the C. *asiatica* juice. This phenomenon may reflect the awareness of consumers who better realize and understand the meaning of functional food, which should not normally contain high sugar. Therefore, the LS sample, with 0.20% of salt and 1.25% of sugar add, was selected for assessment of the antioxidant activity, microbiological qualities, and nutritional values during storage.

3.3 TPC, TFC, antioxidant activity, and microbiological qualities of the C. asiatica pasteurized juice

TPC, TFC, and antioxidant activity as determined through the ABTS assay of the pasteurized juice are shown in Table 2. The results showed that all aforementioned values of the sample juice slightly decreased after pasteurization. Aydogan-coskun et al. (2020) reported the effect of pasteurization at 90 °C for 15 seconds in astragalus honey showed no significant difference in phenolic and flavonoid contents such as 2,5-dihydroxybenzoic, chlorogenic, *p*-coumaric, ferulic acids, apigenin, rutin, kaempferol-3-glucoside, isorhamnetin-3-glucoside, quercetin, luteolin, and kaempferol compared with the non-pasteurized sample. However, using a high temperature for a short time (85 ± 1 °C, 15 seconds) for pasteurization in this experiment may destroy some bioactive compounds, especially heat-sensitive compounds leading to lower TPC, TFC, and antioxidant activity compared with non-pasteurized juice. Moreover, the antioxidant activity depends on the type of phenolic compounds (Zapata et al., 2022). Sanchez et al. (2020) reported the bioactive compounds of *Passiflora setacea* fruit with pasteurizing at 82 °C for 20 seconds provided the highest levels of antioxidant activity, flavonoids, and vitamin C, compared with using temperature at 82 °C for 40 seconds and 63 °C for 30 minutes but these were still lower than the non-pasteurized sample. Even though pasteurization caused a reduction of some phytochemicals and antioxidant activity containing in the juice, the values of ABTS, TPC and TFC still remained at least 68%,73%, and 92% respectively (Table 2).

Table 2. TPC, TFC, and antioxidant activity of the *C. asiatica* pasteurized juice.

Activities	Pasteurized juice	Non-pasteurized juice
TPC (mg GAE/g dry extract)	101.97 ± 0.40[b]	137.49 ± 3.30[a]
TFC (mg CE/g dry extract)	64.03 ± 0.66[b]	69.79 ± 0.42[a]
ABTS (mg GAE/g dry extract)	20.47 ± 0.70[b]	29.61 ± 1.01[a]

TPC means total extractable phenolic content; TFC means total extractable flavonoid content; GAE means gallic acid equivalent; CE means catechin equivalent. [a-b]Mean within a row with different letters are significantly different (p<0.05). Values are represented as mean ± standard deviation (n=3).

Table 3. Microbiological qualities of the *C. asiatica* pasteurized juice.

Parameters	Pasteurized juice	Thai Industrial Standards (TIS)
TVC (CFU/mL)	<25	$<1\times10^4$
Yeast & Mold count (CFU/mL)	Not found	Must not be found
Staphylococcus aureus (CFU/mL)	Not found	Must not be found
Bacillus cereus (CFU/mL)	Not found	<100
Clostridium perfringens (CFU/mL)	Not found	<100
Coliform bacteria (MPN/100 mL)	Not found	<2.2
Escherichia coli (MPN/mL)	Not found	<100
Salmonella Sp. (25 mL)	Not found	Must not be found

Various microorganisms are known to cause food spoilage and food-borne diseases in human beings (Seevaratnam et al., 2012). However, following the Thai Industrial Standards Institute (TIS) (Thai Industrial Standards Institute, 2009), the pasteurized juice passes all microbiological requirements as shown in Table 3. TVC in pasteurized juice was less than 25 CFU/mL, while microbial pathogens, including *Staphylococcus aureus*, *Bacillus cereus, Clostridium perfringens*, *Salmonella* spp., coliform bacteria, and *Escherichia coli* were not detected. This suggests the heating

process used for pasteurized juice is an effective and suitable technique to destroy microbial hazards. Moreover, the decrease in the development of microorganisms is related to some bioactive compounds, which are high in antioxidant activity (Prestes et al., 2022). As mentioned above that *C. asiatica* juice contains multiple bioactive compounds such as phenol, flavonoid, and tannin substances which affect the antioxidant and antimicrobial activities (Kumar et al., 2014; Maisetta et al., 2019). This finding was in agreement with Thatoi et al. (2008) who reported that water extracts of *C. asiatica* leaves showed antibacterial activity against *Staphylococcus aureus*, *Pseudomonas aeruginosa*, *Escherichia coli*, *Bacillus subtilis*, *Shigella flexneri*, and *Candida kruesi*.

3.4 Nutritional values of the C. asiatica pasteurized juice

The nutritional values of the pasteurized juice of *C. asiatica* are shown in Table 4. The results showed that carbohydrates, proteins, and ash in the 100 mL of pasteurized juice were 2.33, 0.43, and 0.48 g while vitamin A, iron, and calcium as 0.20, 0.15, and 53.18 mg, respectively. Previous studies reported that *C. asiatica* is rich in niacin and carotene (Jamil et al., 2007). The findings of this study revealed that the pasteurized juice was high in ash content and can be a good source of various micronutrients. This experiment was similar to other work that indicates that *C. asiatica* was good source for chloride, sulphate, phosphate, iron, calcium, magnesium, sodium, and potassium (Bhavana & Jyoti, 2011). However, proximate composition of *C. asiatica* can be affected by the location and environmental conditions of plant growth (Seevaratnam et al., 2012). With low energy, non-fat, and cholesterol therefore this pasteurized juice product may be great and suitable for consumers on a diet and a functional drink, as well as a healthful ingredient for other products.

Table 4. Nutrition values of the *C. asiatica* pasteurized juice.

Nutrition information	Quantities (per 100 mL of pasteurized juice)
Total energy (kcal)	11.04
Total energy from fats (kcal)	0.00
Carbohydrates (g)	2.33
Fibers (g)	0.00
Proteins (g)	0.43
Total fats (g)	0.00
Saturated fats (g)	0.00
Cholesterol (mg)	0.00
Sugar (g)	2.03
Vitamin A (mg)	0.20
Vitamin B1 (mg)	0.00
Vitamin B2 (mg)	0.00
Sodium (mg)	123.88
Calcium (mg)	53.18
Iron (mg)	0.15
Ash (g)	0.48

3.5 TVC content and sensory qualities of the C. asiatica pasteurized juice during storage

The results related to microbial quality in terms of TVC and the sensory qualities of pasteurized juice product during storage are presented in Table 5. It was found the pasteurized juice had a short shelf-life with less than 5 days when stored at 25 °C; while storage at 8 °C helps it keep longer - approximately 3 times or 15 days, based on microbial quality and sensory scores. The sensory acceptability (appearance, color, odor, flavor, and overall acceptability) of the pasteurized juice kept at 8 °C for 10 days determined as was above 6 while TVC was followed the standard with less than 25 CFU/mL. Thus, this pasteurized juice still needs chilled storage to prevent bacterial growth. However, more than 40% of panelists started to note and remark to reject the juice when stored at 8 °C for 15 days due to precipitation, color fading, flavor and change in taste, compared with fresh one, even though the microbial quality still complied in TIS standard ($<1\times10^4$). The findings revealed that the major quality deterioration of the juice kept at low temperature involves chemical and biochemical reaction. For example, blanching can degrade chlorophyll by damaging tissue (Koca et al., 2007) and, during storage, the pH of the product juice changes, resulting in the loss of its green color. Moreover, the presence of precipitate in juice is due to tannins-protein binding. Tannins can bind to proteins, forming a tannin coating of the protein and this can lead to precipitation of the tannin-protein complex (Adamczyk et al., 2012).

Table 5. TVC content and sensory qualities of the *C. asiatica* pasteurized juice during storage at 25 °C and 8 °C.

Storage times (Day)	TVC (CFU/mL)	Sensory scores				
		Appearance	Color	Odor	Flavor	Overall liking
Day 0TH						
25 °C	<25	6.5 ± 1.5^{ab}	6.9 ± 1.1^{a}	6.2 ± 1.5^{a}	5.7 ± 1.6^{ab}	6.0 ± 1.4^{a}
8 °C	<25	6.5 ± 1.4^{ab}	6.8 ± 1.2^{a}	6.1 ± 1.5^{a}	5.7 ± 1.6^{ab}	5.9 ± 1.4^{ab}
Day 5TH						
25 °C	3.9×10^9	Not tested	Not tested	Not tested	Not tested	Not tested
8 °C	<25	6.8 ± 1.4^{a}	6.6 ± 1.6^{a}	5.9 ± 1.6^{a}	5.9 ± 1.6^{ab}	6.1 ± 1.4^{a}
Day 10TH						
25 °C	Not tested	Not tested	Not tested	Not tested	Not tested	Not tested
8 °C	<25	6.6 ± 1.3^{ab}	6.5 ± 1.4^{a}	6.3 ± 1.5^{a}	6.4 ± 1.2^{a}	6.3 ± 1.2^{a}
Day 15TH						
25 °C	Not tested	Not tested	Not tested	Not tested	Not tested	Not tested
8 °C	<25	5.8 ± 1.6^{b}	6.2 ± 1.9^{a}	5.6 ± 1.7^{a}	5.3 ± 1.85^{b}	5.3 ± 1.8^{b}

$^{a-b}$Mean within a column with different letters are significantly different ($p<0.05$). Values are represented as mean ± standard deviation (n=50).

4 Conclusion

The findings from this research indicate that *C. asiatica* juice provided the highest value of TPC and TFC including the antioxidant activity with blanching time for 2 min followed by 1 and 0 min respectively. The LS juice, with 0.20% of salt and 1.25% of sugar added, yielded the highest sensory qualities and was selected to produce the prototype product. The final product passed the microbial quality requirements of the Thai Industrial Standards Institute. Taking the pasteurized juice containing proteins, ash, vitamin A, iron, and calcium, as well as phytochemicals and antioxidants, can provide more health benefits. For 10 days of storage, the pasteurized juice needed to be store at 8 °C. Therefore, shelf life needs to be prolonged for further study into manufacturing a functional drink.

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