THE CHARACTERISTICS AND ANTIOXIDANT ACTIVITIES OF CHABA MAPLE (HIBISCUS ACETOSELLA) HOMEMADE JAM

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Received: 12 January 2019, Revised and Accepted: 19 July 2019

ABSTRACT

Objective: This study aimed to characterize physicochemical and chemical characteristics of Chaba maple (Hibiscus acetosella) homemade jam (CHJ) and determine its antioxidation ability.

Methods: The physicochemical and chemical characteristics of CHJ were investigated. The color, viscosity, and pH were observed as physicochemical data while chemical properties were obtained from sugar content and total polyphenol content (TPC), determined using high-performance liquid chromatography refractometer and Folin–Ciocalteu assay, respectively. The antioxidant activities of CHJ were identified using 1,1-diphenyl-2-picrylhydrazyl (DPPH) radical scavenging, ferric reducing antioxidant power (FRAP), and nitric oxide (NO) radical scavenging ability methods.

Results: The color and viscosity of CHJ were purple-red and 34,483.33±152.75 cp, respectively. The pH was at 3.78. The total sugar was not detected in CHJ. The TPC of CHJ showed the highest (47.18±1.80 mg gallic acid equivalent [GAE]/g of jam) followed by Streamline (SL) (23.66±0.32 mg GAE/g of jam), Doikham (DK) (21.99±0.50 mg GAE/g of jam), and Best food (BF) (9.75±0.38 mg GAE/g of jam), respectively. Antioxidant activities of CHJ with %1,1-diphenyl-2-picrylhydrazyl (DPPH) radical scavenging of 100.00±1.39% and FRAP value of 1690.70±8.26 μM. Both of activities exhibited the highest activity and significantly different when compared with other jams. The %NO scavenging activity of CHJ and SL was 72.3±1.93% and 73.8±1.66%, respectively, which higher than DK and BF.

Conclusion: This study shows good in both physicochemical and chemical characteristics of CHJ. The CHJ presents the highest TPC as well as antioxidant activities. Thus, a homemade jam of Chaba maple may be considered as a good source of antioxidants and functional foods.

Keywords: Chaba maple (Hibiscus acetosella), Homemade jam, Antioxidation, Total polyphenol content.

INTRODUCTION

Jam is one of the most popular products in the market made from several fruits such as apricot, pineapple, grape, strawberry, blueberry, cranberries, and black currant for preserving the food. There are many parts of plants which have been used to prepare a jam and the different part of plant can provide a varied bioactive compounds, phenolics, flavonoids, and anthocyanins [1]. Typically, jam is composed of at least 40% of fruit mixed with sugar and geling agent, using by thermal processing [2]. Low sugar and low-calorie fruit jams have increased in the market for protecting obesity and diabetes [3]. Therefore, sweeteners as sorbitol, xylitol, steviol glycosides, and erythritol have been replaced.

Chaba maple (Hibiscus acetosella) is the plant found in Pathum Thani, Sanburi, Nonthaburi, and Nakhon Nayok, Thailand. Chaba maple is in Malvaceae family and well known in African mallow, false roselle, maroon mallow, cranberry hibiscus, or red-leaved hibiscus [4]. This plant is a perennial subshrub and ornamental plant as well as fresh food. All parts of this plant are purple-red color. In previous studied, flowers and leaves of Hibiscus species and Chaba maple showed biological activities including antioxidant, antityrosinase, and antibacterial activities [5-9]. All of these activities were obtained from phenolic compounds such as anthocyanins (i.e., cyanidin, delphinidin, and malvidin), flavonols (quercetin, kaemperol, and myricetin) in flowers, and caffeoyl-hydroxycitric acid and neochlorogenic acid in leaves. These compounds show a wide range of antioxidant activities which prevent to degenerate of neuronal disorders, cardiovascular disease, cancer, and diabetes.

In this study, the attractive purple-red color of Chaba maple parts was made to jam. To ensure the quality as antioxidation of Chaba maple jam, the total polyphenol content (TPC) and antioxidant activities of Chaba maple homemade jam (CHJ) were evaluated. Moreover, these results were compared with three commercial products.

METHODS

Plant material

The fresh flowers and leaves of Chaba maple were collected from Pathum Thani, Thailand, on September 2018. The petals and leaves were used for CHJ preparation.

Chemicals

Folin–Ciocalteu’s phenol reagent, gallic acid, L-ascorbic acid, aluminum chloride, DPPH, and sodium nitroprusside (SNP) were purchased from Sigma Chemical Co. (St. Louis, MO, USA). Griess Reagent Kit was obtained from Promega Corporation (Promega, State, USA). All of chemicals in the study were analytical grade. The available jams in the market were purchased from Tesco Lotus supermarket (Pathum Thani, Thailand) including, Best food (BF), Doikham (DK), and Streamline (SL) jam.

CHJ preparation

The jam formula was 44.44% of petals, 35.56% of leaf juice (fresh leaves/water, 1:4), 17.78% of erythritol, 1.78% of lemon juice, and 0.44% of salt. First, the leaves were boiled for 15 min and filtered. The filtrated solution was then used and heated to 70–80°C after that the petals were added and allowed to boil for 10 min. The lemon juice,
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Int J App Pharm, Vol 11, Special Issue 5, 2019

Characterization of CHJ

Color
The color of CHJ was evaluated by organoleptic and instrument. For the instrument, the measurement based on CIEL*a*b*, system of the color parameter using spectrophotometer (Colorquest XE, HunterLab, USA). The l* (lightness), a* (greenness [-] to redness [+]), and b* (blueness [-] to yellowness [+]) were measured.

Viscosity
The viscosity of CHJ was measured using a Brookfield Viscometer (DV2T, Brookfield Engineering Laboratories, USA) at 25°C. Viscometer was adjusted to zero and the spindle LV6 was set in the instrument.

pH measurement
The sample of CHJ was blended with deionized water (1:9 jam:water, w/v) for 1 min using vortex mixer (WiseMix®, Korea) and then was filtrated before measuring. The pH of filtered solution was determined using a pH meter (SP-2100, Suntex, Taiwan).

Total sugar
The total sugars were analyzed using high-performance liquid chromatography (HPLC) with refractive index detector. One gram of CHJ was dissolved in 25 ml acetonitrile:water (50:50 v:v). This solution was then centrifuged for 10 minutes at 8000 rpm. The supernatant was filtered using 0.45 nylon filter. Separation were carried out on an amino-bonded column with a mobile phase of acetonitrile:water:triethylamine (75:25:0.2) and sugar content were determined by Refractive Index Detector against the standard solution (0.1, 0.2, 0.5, 0.8 and 1.0 ppm fructose, glucose, sucrose, maltose, and lactose). The column and the refractive index detector were maintained at 30°C. The injection volume was 10 μl and flow rate was 1.5 ml/min.

Sample preparation for phytochemical analysis
The CHJ and commercial jams, i.e., BF, DK, and SL were extracted with water solvent and assisted by ultrasonic method. The 250 mg of jams were mixed with 5 ml distilled water and immersed in a temperature controlled ultrasonic bath (Elmasonic Easy 60 H, Germany) at 30°C for 5 min. Then, the extracts were centrifuged (Universal 320 R, Germany) at 9000 rpm for 10 min. The supernatants were collected to obtain the extract samples and kept at −20°C before analysis.

Determination of TPC
The amount of TPC of CHJ, BF, DK, and SL was determined by Folin–Ciocalteu assay which modified from Kamtekar et al. [10]. Briefly, 10 μl of 10 mg/ml extract samples were filled into 96-well microplate and then 100 μl of 1:10 diluted Folin–Ciocalteu reagent was added. After incubation at room temperature for 7 min, 80 μl of 7% w/v of sodium carbonate was added. After storing in dark room temperature for 2 h, the mixture was recorded under microplate reader (GloMax-Multi Detection System, USA) at 750 nm. TPC was estimated from a calibration curve of standard gallic acid solution. All extracts were measured in 3 times and the results were expressed as milligram gallic acid equivalent (GAE)/g of jam.

Determination of antioxidant activities

DDPH radical scavenging assay
Free radical scavenging activity of jam was measured by 1,1-diphenyl-2-picrylhydrazyl (DPPH) according to a modified method of Lee et al. In brief, 75 μl of jam solutions was mixed with 150 μl of 0.2 mM DPPH the jam solutions. The reaction of mixtures was carried out at room temperature for 30 min. After incubation, the mixtures were measured at 517 nm using microplate reader (GloMax-Multi Detection System, USA). L-ascorbic acid was used as a positive control and water was used as a blank. The experiment was done in triplicate. The antioxidant capacities of jams were expressed as % DPPH radical scavenging.

\[
\% \text{ DPPH radical scavenging} = \left[ \frac{A_{517 \text{ blank}} - A_{517 \text{ sample}}}{A_{517 \text{ blank}}} \right] \times 100
\]

Where, \(A_{517 \text{ blank}}\) is absorbance of negative control (water) and \(A_{517 \text{ sample}}\) is absorbance of jam solution.

*Ferric reducing antioxidant power (FRAP) assay*
FRAP assay was measured according to Abolhasami et al. [11] method with some modifications, based on the reduction of Fe⁴⁺-TPTZ to a blue-colored Fe²⁺-TPTZ. First, the FRAP reagent was prepared by mixing of 10 ml of 0.3 M sodium acetate buffer solution, 1.0 ml of 10 mM TPTZ, and 1.0 ml of 20 mM FeCl₃ and then incubated at 37°C for 4 min. The 150 μl of FRAP reagent was mixed with 20 μl of each jam solution (50 mg/ml) and incubated at 37°C for 30 min. The absorbance of the reaction mixture was measured at 700 nm using a microplate reader (GloMax-Multi Detection System, USA). The calibration curve was prepared using standard 1 mM FeSO₄ solution. The FRAP value of the sample was expressed as μM.

*Nitric oxide (NO) radical scavenging assay*
NO donor was generated from SNP interacts with oxygen. This assay is modified from Sasikumar and Kalaisezhiyen [12]. Exactly, 10 mM of SNP in a phosphate-buffered solution (pH 7.4) was incubated with 1 ml of jam solution at 25°C for 3 h. A 100 μl of the testing solution was withdrawn to react with a Griess Reagent Kit, whereby the solution was reacted with 20 μl sulfanilamide for 10 min and then 20 μl N-(1-naphthyl) ethylenediamine dihydrochloride for another 10 min. The reaction mixture absorbance was measured at 560 nm and the NO concentrations were determined as the nitrite (NO⁻²) concentrations from the standard curve of a standard nitrite solution. Distill water and L-ascorbic acid were used as the negative and positive controls, respectively. Percentage inhibition of the nitrite ions generated is observed as NO scavenging capacity of jam solution following below equation.

\[
\% \text{ NO radical scavenging} = \left[ \frac{A_{560 \text{ blank}} - A_{560 \text{ sample}}}{A_{560 \text{ blank}}} \right] \times 100
\]

Where, \(A_{560 \text{ blank}}\) is absorbance of negative control (water) and \(A_{560 \text{ sample}}\) is absorbance of jam solution.

Statistical analysis
Data are reported as the mean ± standard deviation of triplicated experiments. TPC and antioxidant activities were analyzed by one-way analysis of variance followed by Tukey’s (post hoc) using the SPSS 22.0 software. Significance was accepted at p<0.05.

RESULTS AND DISCUSSION

Characteristics of CHJ
The characteristic of CHJ is shown in Fig. 1. For physical characteristics, the color of jam is purple-red, similar to their petal and leave’s color. For the color analysis, CHJ showed dark purple-red by visual observation and showed low L* value (1.90±0.01) that represented dark color while a* showed high value (13.36±0.08) with redness (dark red color) and b* showed quite blue color (Table 1). The pH of CHJ was at 3.78. The CHJ

Fig. 1: The color of Chaba maple homemade jam by organoleptic observation (a), the petals (b), and leaves (c) of Chaba maple

salt, and erythritol were added, respectively, and mixed together. The mixture was mixing at 60°C for 1 h and allowed to cool down at room temperature. This jam was stored under 2–4°C until analysis.
TPC (mg GAE/g of jam)

2. The TPC of CHJ showed the highest -9.75±0.38 100.00±1.39 c 3.27±0.02 -540.58±5.05 -1706.36±0.002 13.36±0.08 th Not detected FRAP values (μM) 1690.70±8.26 87.72±0.79 Viscosity (cP) 63.89±1.27 73.82±1.66 pH 72.43±1.93 a 90.06±0.09 % NO radical scavenging 34,483.33±152.75

Total sugar (g/100 g)

Antioxidant activities

Table 1: Determination of physicochemical and chemical characteristics of Chaba maple homemade jam

| L* | a* | b* | pH | Viscosity (cP) | Total sugar (g/100 g) |
|----|----|----|----|---------------|----------------------|
| 1.90±0.01 | 13.36±0.08 | 3.27±0.02 | 3.78 | 34.883±3±152.75 | Not detected |

*Values are expressed as means±SD (n=3)

Table 2: Determination of TPC and antioxidant activities in various jams

| Test samplea | TPC (mg GAE/g of jam)b | Antioxidant activitiesb | % radical scavenging | FRAP values (μM) | % NO radical scavenging |
|--------------|------------------------|------------------------|---------------------|------------------|------------------------|
| BF | 9.75±0.38c | 70.40±2.79c | 540.58±5.05c | 17.58±1.72c |
| DK | 21.99±0.50c | 97.01±0.39c | 1169.3±20.05c | 63.89±1.27c |
| SL | 23.66±5.32c | 95.64±1.82c | 1179.03±6.52 | 73.82±1.66 |
| CHJ | 47.18±1.80 | 100.00±1.39 | 1690.70±8.26 | 72.43±1.93 |
| 1 mM L-ascorbic acid | - | - | 1706.3±6.00 | - |
| 0.1 mg/ml L-ascorbic acid | - | 90.06±0.09 | - | - |
| 0.0625 mg/ml L-ascorbic acid | - | - | - | 87.72±0.79 |

*aTest sample: BF: Best food, DK: Doikham, SL: Streamline, CHJ: Chaba maple homemade jam. Values are expressed as means±SD (n=3).

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**REFERENCES**

1. Lee DJ, Lee H, Lee SH, Lee CY, Kim DO. Effects of jam processing on anthocyanins and antioxidant capacities of Rubus coreanus Miquel berry. Food Sci Biotechnol 2013;22:1607-12.

2. Basu S, Shivhare US. Rhological, textural, micro-structural and sensory properties of mango jam. J Food Eng 2010;100:357-65.

3. Bana's A, Korus A, Korus JB. Texture, color, and sensory features of low-sugar gooseberry jams enriched with plant ingredients with probiotic properties. J Food Qual 2018;2018:1-12.

4. Mahr S, editor. In: Hibiscus acetosella. Wisconsin Master Gardener. Wisconsin: University of Wisconsin; 2008.
5. Tsumbu CN, Deby-Dupont G, Tits M, Angenot L, Frederich M, Kohnen S, et al. Polyphenol content and modulatory activities of some tropical dietary plant extracts on the oxidant activities of neutrophils and myeloperoxidase. Int J Mol Sci 2012;13:628-50.

6. Puckhaber LS, Stipanovic RD, Bost GA. Analyses for flavonoid aglycones in fresh and preserved Hibiscus flowers. In: Janick J, Whipkey A, editors. Trends in New Crops and New Uses. Alexandria: ASHS Press; 2002. p. 556-63.

7. Kepepula PM, Kabamba Ngombe N, Tshisekedi Tshibangu P, Tsumbu C, Franck T, Mouithys-Mickalad A, et al. Comparison of metabolic profiles and bioactivities of the leaves of three edible congolese hibiscus species. Nat Prod Res 2017;31:2885-92.

8. Brain A, John M. Phenolic content and antioxidant activity of selected Ugandan traditional medicinal foods. Afr J Food Sci 2014;8:427-34.

9. Wong SK, Lim YY, Chan EC. Evaluation of antioxidant, anti-tyrosinase and antibacterial activities of selected Hibiscus species. Ethnobot Leafl 2010;14:781-96.

10. Kamtekar S, Keer V, Patil V. Estimation of phenolic content, flavonoid content, antioxidant and alpha amylase inhibitory activity of marketed polyherbal formulation. J Appl Pharm Sci 2014;4:61-5.

11. Abolhasani A, Barzegar M, Sahari MA. Effect of gamma irradiation on the extraction yield, antioxidant, and antityrosinase activities of pistachio green hull extract. Radiat Phys Chem 2018;144:373-8.

12. Sasikumar V, Kalaisezhiyen P. Evaluation of free radical scavenging activity of various leaf extracts from Kedrostis foetidissima (Jacq.) Cogn. Biochem Anal Biochem 2014;3:1-7.

13. Esmaeili MA, Sonboli A. Antioxidant, free radical scavenging activities of Salvia brachyantha and its protective effect against oxidative cardiac cell injury. Food Chem Toxicol 2010;48:846-53.

14. Vilela TC, Leffa DD, Damiani AP, Damazio DDC, Manenti AV, Carvalho TJG, et al. Hibiscus acetosella extract protects against alkylating agent-induced DNA damage in mice. An Acad Bras Cienc 2018;90:3165-74.

15. Skrovankova S, Sumczynski D, Mlcek J, Jurikova T, Sochor J. Bioactive compounds and antioxidant activity in different types of berries. Int J Mol Sci 2015;16:24673-706.

16. Shahidi F, Ambigaipalan P. Phenolics and polyphenolics in foods, beverages and spices: Antioxidant activity and health effects a review. J Funct Foods 2015;18:820-97.