International Journal of Food Science and Nutrition
www.foodsciencejournal.com
ISSN: 2455-4898
Received: 01-08-2022, Accepted: 17-08-2022, Published: 03-09-2022
Volume 7, Issue 3, 2022, Page No. 146-152

Study on processing conditions for tea bags from *Codonopsis pilosula*

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Abstract
By the virtue of convenience and safety, tea bags are well-known among Vietnamese consumers. *Codonopsis pilosula*, also known as Dang Sam, has great potential in Vietnam; however, in order to introduce these products to global markets and to domestic consumers believing in its application, further researches need to be conducted. This study aimed to evaluate the processing conditions of each step of Dang Sam dried tea bags with the highest total saponin content (TSC) and soluble solid content (SSC). The methods used to investigate TSC, total phenolic content (TPC), and antioxidant activity (AA) were Vanillin-Sulfuric assay, Folin-Ciocalteau reagent, and DPPH assay, respectively. SSC was determined by using a refractometer. The drying conditions were identified using four drying temperatures (60, 70, 80, and 90ºC) and four drying durations (5, 6, 7, and 8 hours). The samples were then examined with different sieve sizes (500, 212, 125, and 63µm). As a result, the study revealed the optimal drying conditions of Dang Sam tea were dried at 90ºC for 7 hours and the optimal grinding size was all particles between 500 µm and 212 µm. In addition, the appropriate brewing time was from 7 to 8 mins, and the applicable ratio of tea: water was 1:100 w/v. In general, this study contributed greatly to the food industry with the updates of antioxidant activity, nutritional value and processing conditions of Dang Sam dried tea bags.

Keywords: dang sam, drying conditions, soluble solid content (SSC), tea, total saponin content (TSC)

Introduction

Codonopsis is a genus of dicotyledonous herbaceous plants belonging to the family *Campanulaceae*. *Codonopsis pilosula* (Franch.) Nannf., known as "Dang Sam" in Vietnamese, is reported as a substitute for Panax ginseng as a tonic showing similar but cheaper therapeutic effects. In Vietnam, it has been mostly found in Lai Chau, Lao Cai, Ha Giang, Quang Nam, Lam Dong, and Kon Tum. Different types of chemical constituents were already isolated. Among them, polyacetylenes, phenylpropanoids, alkaloids, triterpenoids, and polysaccharides are considered to be the major constituents and responsible for most biological activities of this genus [1]. Pharmacological research indicated that it functions in protecting against peptic ulceration and promoting its healing[2], improving learning and memory behavior [3], as well as enhancing growth factor-induced neurite outgrowth in PC12 cells [4].

Dang Sam's products are favored by those who cannot afford to spend millions of VND to buy Korean ginseng or Ngoc Linh ginseng. In Vietnam, Dang Sam is used in many different forms such as being used fresh, soaked with alcohol, mixed with medicinal herbs, and served as a healthy drink. Besides their use in herbal preparations, the roots are also used as a health food ingredient in the form of soup or broth with other herbs. It is claimed to be the treatment of body weakness, poor appetite, thirst, indigestion, chronic diarrhea, chronic anemia, and leukemia[5]. Although Dang Sam tea bags are not popular in the Vietnamese market compared to other herbal teas (artichoke tea, ginger tea, etc), several famous local brands such as L'angfarm, Thai Bao, and Vinh Tien company have sold this product in the market.

On the one hand, many studies related to the biological and chemical compounds of Dang Sam have been published worldwide. Saponins are constituents of many plants drug and folk medicines, therefore, extensive research about their pharmacological and biological properties has been done. It has been found to have analgesic, anti-nociceptive and antioxidant activities, causing hypoglycemia, acting as antifungal and antiviral agents, affecting reproduction in animals, and affecting the cell membrane permeability. Some are effective in treating gastric ulcers and dermatitis. Moreover, several studies revealed that inulin and sugar are also the main chemical component of the Dang Sam [6]. In the clinical aspect, saponins were said to help the body defend against cancer cells, and lower cholesterol levels and blood glucose response [7]. On the other hand, with the topic of the production of products from Dang Sam as well as preparation methods, the number of articles is negligible for both foreign and domestic. For a country with a rich source of herbal materials such as Vietnam, the development of Dang Sam must have taken place before. Moreover, in order for these tea products to develop into world markets as well as for domestic consumers to believe in the application of tea, researchers

need to invest and study more about nutritional value, antioxidant activities along with the variety of flavors and packaging of tea bag products.

Materials and Methods

1. Materials

Roots of two-year-old Dang Sam were collected from Kom Tum province in March 2019 with a length of approximately 20 centimeters and a width of nearly 3 centimeters. Dang Sam's exterior was light brown, sometimes branching, not moldy or damaged. The taste was described as a little sweet and slightly warm. After harvesting, the initial moisture content was up to 90%.

Chemicals such as Folin-Ciocalteu's reagent, gallic acid (98% purity), vanillin, absolute ethanol, and methanol were purchased from Hoa Nam Company; oleanolic acid was bought from Sigma Aldrich Chemical Co.; sodium carbonate was acquired from Bach Khoa Company; sulfuric acid and DPPH were supported by Applied Biochemistry Laboratory.

2. Experimental Design

a. Effect of Drying Conditions for TSC and SSC

500 grams of prepared sample were oven-dried at different temperatures of 60, 70, 80 and, 90ºC for different times such as 5, 6, 7 and, 8 hours. After drying, the sample was then subjected to a grinding and sieving process. Moisture content should be below 10% and be involved with the high TSC and SSC were was to conclude suitable drying conditions.

b. Effect of Grinding Size for SSC

First, 100 grams of sample was ground by a laboratory grinder and poured into a mass of sieves of different sizes (500, 212, 125, and 63 µm). Next, placed it on the sieve shaker, fixed the clamps, and accommodated times to 10 mins. After 10 mins, different sizes of the ground sample were collected. The sample with the highest value of SSC would be chosen to perform the next experiments.

c. Effect of the Brewing Process for SSC

Effect of Different Tea: Water Ratio for the SSC

Tea bags containing 2 grams of the ground sample with the appropriate size were soaked with different tea: water ratios (1:100, 1:125, 1:150, and 1:175 g/mL) for 6 mins at about 80ºC to choose the best tea: water ratio.

Effect of Different Brewing Times for the SSC

Tea bags with fitting tea: water ratio was immersed into boiled water at different times (5, 6, 7, and 8 mins). The best result was chosen based on measuring SSC.

3. Analytical Methods

a. Preparation of Dried Dang Sam Tea Extract

Samples prepared for SSC testing were formulated based on TCVN 5086:1990 (ISO 3103-1980). A sample of 3 grams of product was mixed with 140 mL of boiled water for 6 mins. Let the sample cool down to virtually ambient temperature and measure for the SSC with a refractometer.

The procedure of preparing sample extracts for testing TSC, TPC, and AA was determined using ethanol 80% as a solvent. First, 1 gram of Dang Sam powder was mixed with 30mL ethanol 80% in an Erlenmeyer flask. Next, the extraction was run in a shaking incubator with a shaking speed of 150 rpm at 70ºC for 2 hours. After that, the centrifuge was used to obtain only the supernatant at 8,000 rpm for 15 mins. The clear extract was then added to a beaker and put into 90ºC water bath in order to reject the ethanol. Finally, the sample extracts were mixed with distilled water to reach 25 mL in a volumetric flask and used for further analysis.

b. Determination of TSC

In this project, total saponin is determined as described by Hiai *et al*. (1976) with some modifications. First of all, the sample extract was diluted 8 times. Volume of 0.5 mL of diluted sample was pipette into a test tube, then 0.5 mL of 8% (w/v) vanillin in methanol was added. Test tubes were then put in ice bath and 5 mL of 72% (v/v) H_2SO_4 in water were added. Next, the mixture was heated at the temperature of 75ºC for 15 mins, then was cooled down to room temperature for 3 mins. Finally, the absorbance was measured at a wavelength of 544 nm by a spectrophotometer. Oleanolic acid was used to build the standard curve. Stock solution was prepared and the serial dilution was then done with different concentrations (0, 0.02, 0.04, 0.06, 0.08 and 0.1 mg/mL).

c. Determination of TPC

Folin-Ciocalteu method was widely used for measuring TPC, which is easy to perform, rapid, and applicable in routine laboratory use and at low cost [8]. At first, 0.5 mL of 1:5 diluted sample was added with 2.5 mL of 10% Folin reagent and 2 mL of 7.5% sodium carbonate, respectively. The solution was then mixed well and kept in dark condition at room temperature for 30 mins to form a blue complex. The absorbance was measured at 760 nm by spectrophotometer. Gallic acid was used as the reference standard and the results were expressed as mg of Gallic acid equivalents per g of the dry weight of the sample.

d. Measurement of antioxidant activity

DPPH (2,2-diphenyl-1-picrylhydrazyl-hydrate) assay provides an easy and rapid way to evaluate antioxidants by spectrophotometry. First, DPPH ethanol solution (0.15 mM) was prepared. In a test tube, 2 mL of DPPH ethanol solution and 2 mL of 20 mg/mL sample extracts with different dilutions of 0, 2, 4, and 8 were merged. It then vortexed well and was kept in a dark holder at room temperature for 30 mins. Finally, the absorbance was measured at 517 nm by a spectrophotometer. The volume of 2mL of ethanol 70^{o} served as blank.

e. Measurement of SSC

Degree of Brix is the sugar content of an aqueous solution. The soluble solid content (SSC (%), ^{o}Brix) was ascertained by the index of refraction and is measured by a refractometer.

f. Measurement of Ash Content

First of all, the crucible was heated to constant mass, cooled down in a desiccator, and weighed it. Secondly, weigh exactly 2 grams of product, put in the crucible, and spread evenly. The product was then burned completely. Placed the crucible in the muffle furnace at 550 – 600^{o}C until the matter was no longer black. Lastly, allowed it to cool down in a desiccator for about 30 mins and weighed [9].

g. Sensory evaluation

After the Dang Sam tea bags making process is accomplished, the sensory test is assessed. About 30 untrained panelists are invited to check the quality of the product. Each member inspected each individual indicator (appearance, taste, smell, and color) according to the standard transcript and scored from 1 (dislike very much) to 5 (like very much).

4. Statistical Analysis

A one-way ANOVA test was performed using SPSS version 20 with 95% confidence interval. All measurements were done in triplicate and the parameters were expressed as mean ± standard deviation. Data with the same letter did not differ significantly at the 0.05 level through Duncan's multiple-range test.

Results and discussion

1. Effect of Drying Conditions for TSC and SSC

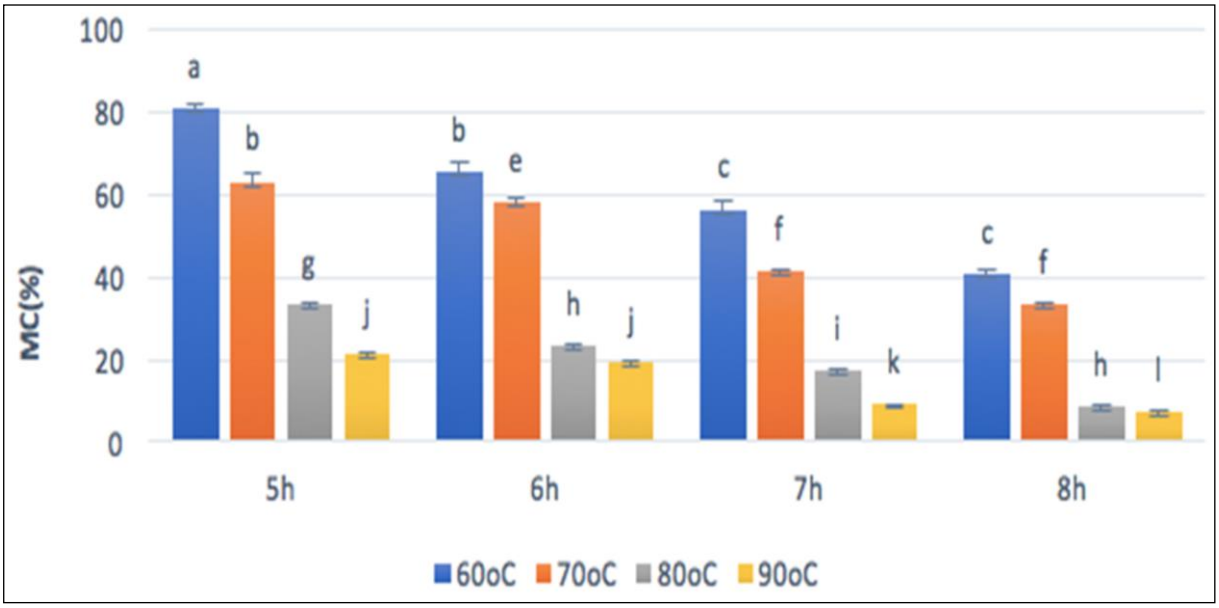

Fig 1: The moisture changes at different drying conditions

As an overall trend, it was clear that the moisture content was significantly declined after the drying process. In the same duration, when the temperature increased gradually from 60 to 90^{o}C, the moisture remaining in the sample reduced. Simultaneously, in the same temperature, the longer the duration, the lower the moisture content. While conducting high drying temperature for a long duration, it created good conditions for moisture evaporation and the sample has a low moisture content, dry and brittle. Due to the fact that the moisture content must be below 10% according to TCVN 7975:2008, only samples 7 and 8h at 80 and 90^{o}C were eligible to perform the next tests. The remaining samples had humidity above the standard level so that it caused the phenomenon of viscosity and microbial growth.

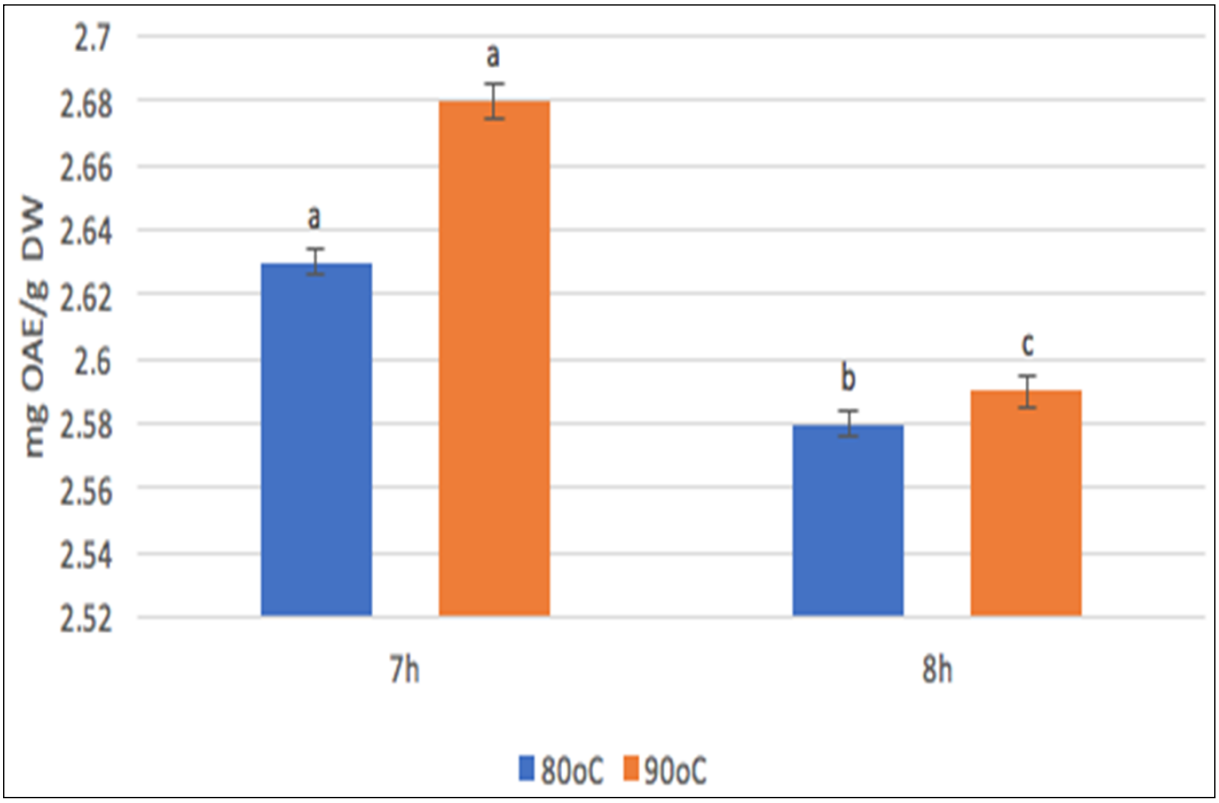

Fig 2: TSC changes at different drying conditions

Figure 3.2 showed the change of SSC from 7 to 8h at 80 and 90ºC. The highest value

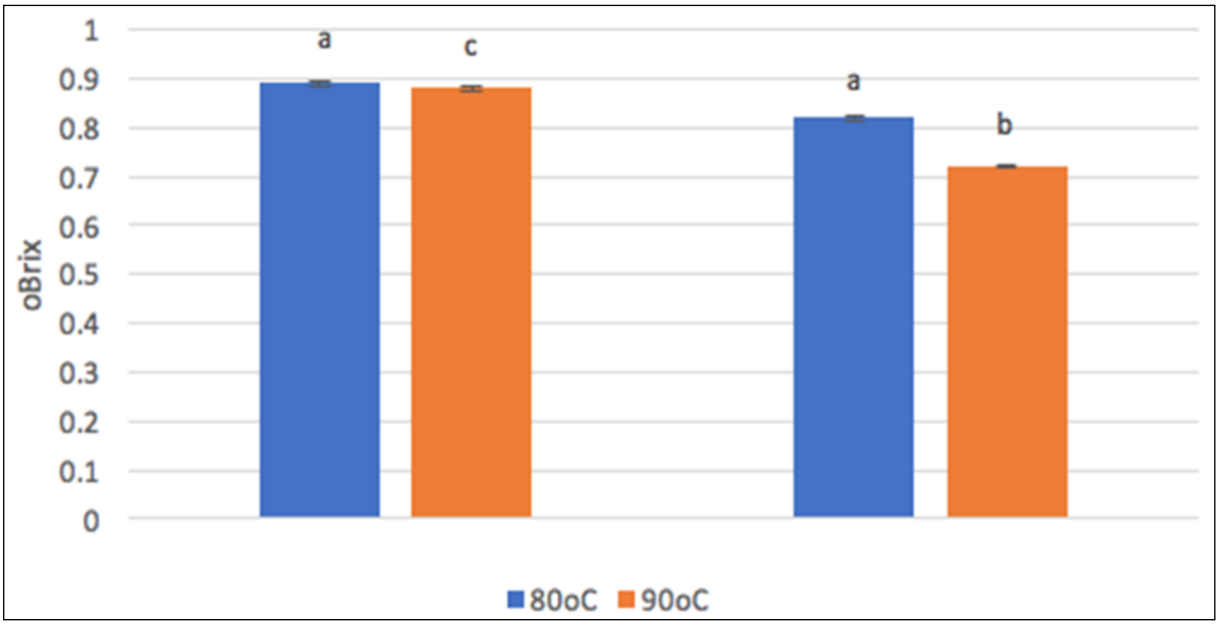

Fig 3: SSC changes at different drying conditions

was recorded in samples dried at 80ºC for 7h (0.89 ± 0.01^{a}°Brix) and the minimum value was recorded at 90ºC for 8h (0.72 ± 0.00^{b}°Brix). During the same duration, the SSC was slumped with the increase in temperature, it may be a result of the degradation of sugars. This is also consistent with the findings of Yusufe, who reported that an increase in drying temperature could lead to the reduction in SSC [10].

Figure 3.3 showed the TSC value at 80 and 90ºC during 7 and 8 hours. The yield of TSC was not significantly affected by the interactions of both drying temperature and duration. The highest value of TSC (2.68 ± 0.01^{a} mg OAE/g DW) was observed at 90ºC for 7 hours and the lowest value was at 80ºC for 8 hours (2.58 ± 0.02^{b} mg OAE/g DW). In general, this amount of saponins was not too high due to the fact that the outcome of oven-dried saponins does not show a great amount compared to the other methods of drying, such as microwave drying or freeze-drying [11]. From the above moisture content, SSC, and TSC results, the optimal drying conditions were chosen at 90ºC for 7 hours.

2. Effect of Grinding Size for SSC

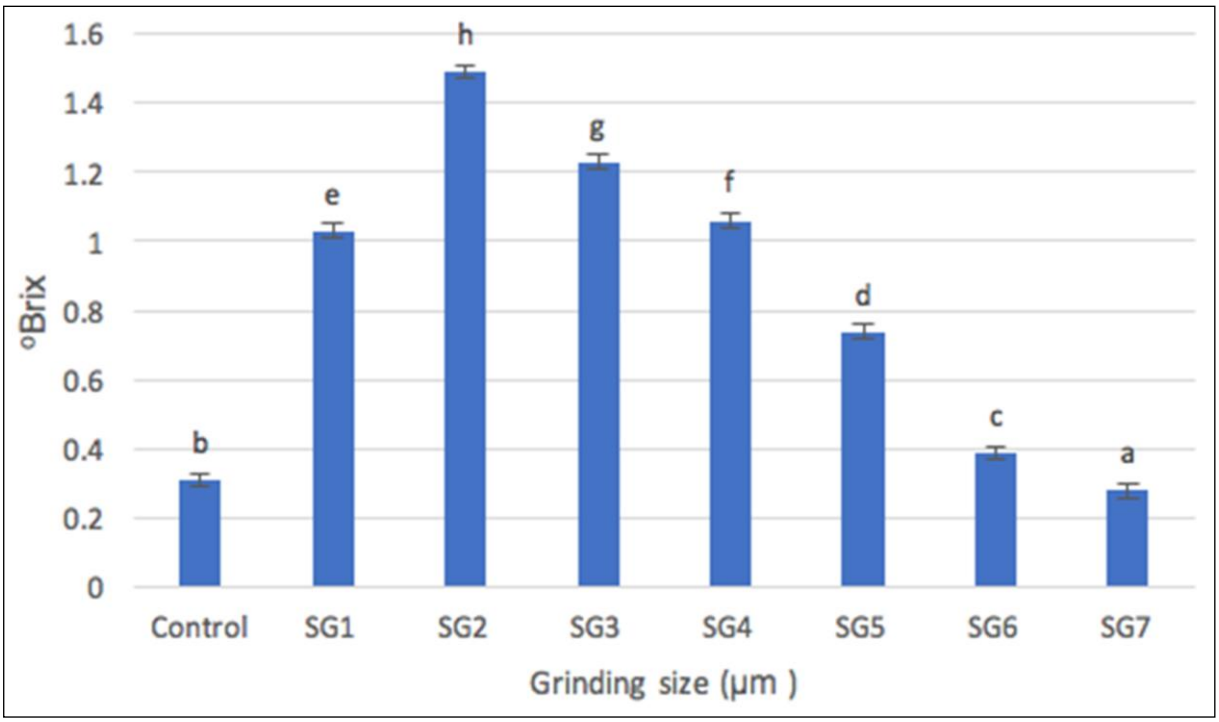

Fig 4: Effect of grinding sizes on SSC

According to Figure 3.4, the SSC reached the peak at the particles size between 500 µm and 212 µm (1.49 ± 0.01^{h}) and the bottom at particle sizes which was lower than 63 µm (0.28 ± 0.01^{a}). Also, significant differences ($p < 0.05$) were observed between all samples. The size reduction resulted in greater mass transfer because it increased the surface area [12]. However, finer particle size did not promise a higher extraction rate due to the agglomeration of water. Therefore, the most suitable grinding size was the size that took out the most medicinal ingredients of the plant materials, yet prevent the agglomeration. On the other hand, as the particle sizes decreased, the sensory evaluation of the product also declined. The sample S_{G2} gave high sensory value because the product was clear, nice odor, and has no tea dust. Overall, the combination of all samples between 500 and 212 µm was chosen as the appropriate size.

Assume:

$S_{G1} = x \geq 500$ µm

$S_{G2} = 500$ µm $> x \geq 212$ µm

$S_{G3} = x \geq 212$ µm

$S_{G4} = x \geq 125$ µm

$S_{G5} = 212$ µm $> x \geq 125$ µm

$S_{G6} = 125$ µm $> x \geq 63$ µm

$S_{G7} = x < 63$ µm

3. Effect of the brewing process for the SSC

a. Effect of different tea: water ratio for the SSC

Table 1: Effect of extraction ratio for the SSC

Extraction ratio	SSC (° Brix)
1:100	1.34 ± 0.02^{d}
1:125	0.98 ± 0.02^{c}
1:150	0.85 ± 0.02^{b}
1:175	0.71 ± 0.01^{a}

Through table 3.1, there was a significant difference between different tea: water ratios. 1:175 extraction rate resulted in the lowest SSC value (0.71 ± 0.01^{a}°Brix), while the remaining rates were reasonably high, especially at 1:100 (1.34 ± 0.02^{d}°Brix). The dissolution of solute into water stopped when the concentration of the solution was saturated. The low amount of water used to extract led to a higher Brix value. Theoretically, the more substances dissolved into the solution, the higher the sensory value. However, in this experiment, the sensory values in these 4 ratios were comparable, which meant that the extract was all yellowish-brown and had subtle

aromatic of Dang Sam. From the above results, the optimal parameter for the ratio of tea and water to make the best tea was 1:100 (w/v).

b. Effect of different brewing times for the SSC

Table 2: Effect of brewing time for the SSC

Brewing time (min)	SSC (° Brix)
5	1.29 ± 0.01^{a}
6	1.37 ± 0.00^{b}
7	1.39 ± 0.01^{c}
8	1.39 ± 0.01^{c}

According to table 3.2, there were no significant differences between samples extracted for 7 and 8 mins, which both got the SSC values of 1.39 ± 0.01^{c} °Brix. Values of samples at 6, 7 and 8 mins were not altered, which were 1.37 ± 0.00^{b} °Brix, 1.39 ± 0.01^{c} °Brix, and 1.39 ± 0.01^{c} °Brix, respectively. Prior research substantiated the belief that the longer extraction time produced the higher yield. However, the solubility of the substance into the solution will be stopped when equilibrium was accomplished. Since extraction in the same volume of water was required, the longer extraction time may not affect the SSC values when equilibrium was reached. Moreover, immersing tea bags in water for a long time leads to more tea dust outspread into the final product. Consequently, the sensory values were reduced. To clarify, the best time to brew tea was from 7 to 8 mins.

4. Determination of the Antioxidant Activity of the Final Product

DPPH radical scavenging method is widely used in measuring antioxidant activity with more than 17.000 related articles. When comparing the antioxidant activity of the final product and ascorbic acid, the results in Figure 3.5 indicated that the antioxidant activity of the tea product was lower than the ascorbic acid. This was understandable because ascorbic acid is one of the highest natural antioxidants while the tea sample had undergone several processing stages; therefore, the antioxidant activity has been affected through each stage. However, from the above results, the Dang Sam tea final product can be concluded as having a fairly high antioxidant capacity.

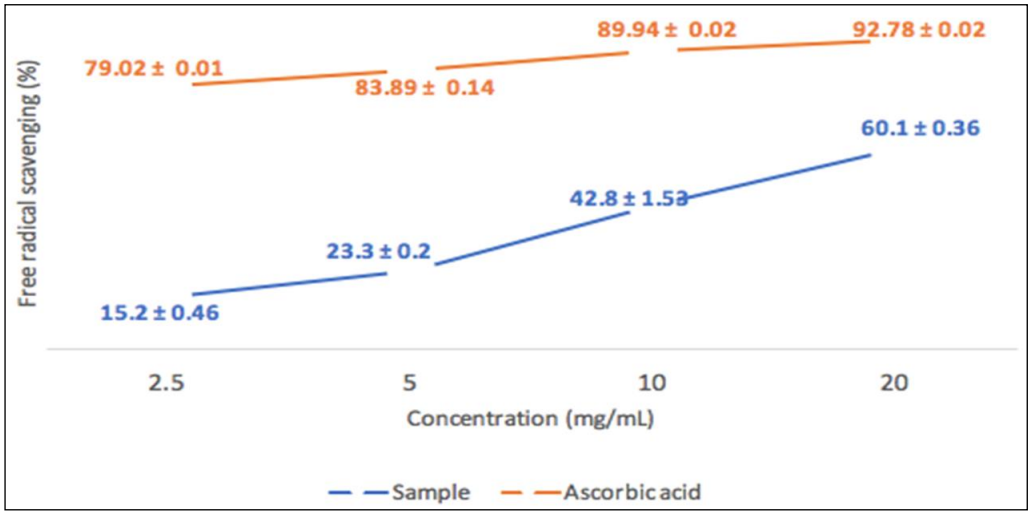

Fig 5: The comparison of antioxidant activity between final product and ascorbic acid

5. Final Quality Evaluation

a. Physical and chemical parameters

Table 3: Physical and chemical parameters of final dried product

No.	Parameters	Result	TCVN standards
1	MC (%)	9.27	< 10% (TCVN 7975:2008)
2	Ash (%)	5.01	< 8% (TCVN 7975:2008)
3	Organic matter (%)	85.72	-
4	SSC (° Brix)	0.88	-
5	TSC (mg OAE/ g DW)	2.68	-
6	TPC (mg GAE/g DW)	2.93	-

b. Sensory Evaluation

The Dang Sam dried tea bag had a sensory score of 15.5. Therefore, this product could be classified as a fairly good product based on TCVN 3218-2012. It had the highest sensory value of flavor. With the total score of 4.5, it was said to have a natural, earthy sweetness and no strange taste. However, it was still limited in color which means that the solution was still opaque, and the residues were at the bottom of the glass. If this problem could be solved, this Dang Sam dried tea bags would be preferred by consumers.

Conclusion

This research was conducted to determine the process of producing Dang Sam tea bags with the highest TSC and SSC. The optimum drying conditions were at 90^{o}C for 7 hours with the highest TSC value was 2.68 ± 0.01 mg OAE/g DW and the SSC value was 0.88 ± 0.01 ^{o}Brix. The moisture content was 9.27% in order to preserve the product from harmful microorganisms as well as extend its shelf life. Particle size not only affects the extractability but also affects the sensory quality of the product; hence, figuring out the optimal particle size which was all particles between 500 and 212 µm contributes greatly to the progress. Moreover, finding out of appropriate brewing time (7-8 mins) and the ratio of tea: water (1:100 w/v) should be labeled on the product packaging as a guide for customers to follow. Several parameters of final tea products such as TPC (2.93 mg GAE/g DW) and ash content (5.01%) were also identified in this research. Last but not least, the sensory value, which could be described as grading the product through the senses, was fairly high (15.5). In general, it can be concluded that this Dang Sam dried tea product meets the requirements of a good product and can be developed in the market.

Acknowledgment

We would like to express our faithful thanks to the School of Biotechnology and Food Technology Department of International University which provided valuable support in both facilities and spiritual perspectives during the accomplishment of this project. We also appreciate everyone who is involved directly and indirectly in this study.

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