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STUDY ON PHYSIOLOGICAL AND BIOCHEMICAL PARAMETERS OF SACHA INCHI LEAF (*Plukenetia volubilis* L.) AND THE PROCESS OF MAKING TEA BAGS IN VIETNAM

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Abstract. The study consists of two experiments, the first one aims to determine the number of growth and physiological parameters of sacha inchi leaves (content of tannins, reducing sugars, minerals) to provide a basis for choosing leaf type (according to age) for the second experiment: a study on the effect of temperature and drying time on the perceived results of tea bags made from sacha inchi leaves as well as to find out the mixing ratio between sacha inchi leaf combined with licorice and pandan leaves to make a tea bag which has good sensory quality and quality in the tests. The main experimental methods used in this study were: Determination of the total chlorophyll content in sacha inchi leaves by using handheld chlorophyll meter; The dry weight of leaf samples was determined based on the dry weight after drying at 105ºC for 1 day 1 night by analytical balance; The content of tannins in the leaves by leventhal method also was measured; Minerals such as Mg, Ca and K were determined by wetting of leaf samples and measured with an AAS atomic absorption spectrometer. The factors affecting the quality and sensory of tea bags such as temperature tea drying time, and mixing ratio of sacha inchi: licorice: pandan leaves were also investigated. A study on the extraction time of tea solution for phenolic content and oxidation activity of finished tea product was also carried out. The results showed the highest concentration of tannins in old leaves (2.47 ± 0.02 % by dry weight) and lowest in young leaves (2.17 ± 0.02 % by dry weight), the highest concentration of reducing sugars in mature leaves (25.9 ± 5.67 mg/g). The concentration of mobile minerals such as Mg, and K was highest in young leaves (0.75 ± 0.04 and 33.62 ± 0.98 mg/kg, respectively) and lowest in old leaves (0.61 ± 0.01 and 29.41 ± 0.12 mg/kg, respectively) while non-mobile minerals as Ca were highest in old leaves (32.59 ± 0.16 mg/kg) and lowest in young leaves (28.27 ± 1.02 mg/kg). The phenolic content was highest and the antioxidant capacity SC_{50} was lowest in a 5-minute tea extract solution (126.2^a ± 8.9 mg gallic acid/1g and 4.34 ± 0.5 mg/ml. The tea treatment with the highest sensory survey results was the mature leaf sample, which was dried at 130℃ for 1 hour, with a mixing ratio of sacha inchi leaves: licorice leaves: pandan leaves of 1.6/0.4/0.2 for tea packages with a dry weight of 2.2 g/bag and extract the tea bag in hot water in 5 minutes is our recommendation.

Keywords*: licorice leaves, pandan leaves, growth, sacha inchi leaves, tea bag.*

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1. Introduction

The sacha inchi tree (*Plukenetia volubilis* L.) originates from the Amazon forest, in South America, and was brought to Vietnam for trial by the National Center for Testing of Seeds and Plant Products in November 2013 [1]. At present, it has been growing quite popularly and is very well suitable for the climate in our country. Sacha inchi leaves also have high nutritional content and are used as functional foods to help with anti-oxidants, anti-cancer, cardiovascular diseases, and blood pressure, and to support the treatment of insomnia.

Licorice (*Abrus precatorius* L.) is a traditional medicine with many widely used uses. It contains a sweetener called glycyrrhizin (saponin) which is 60 times sweeter than saccharose, which helps to increase the sweetness of tea, as well as medicinal flavors, making the medicine easier to use [2].

Pandaleaves (*Pandanus amaryllifolius* Roxb.) are used as a familiar flavoring material in food technology. Contains the main flavoring ingredient 2-acetyl-1-pyroline [2, 3].

Currently, the world and Vietnam are focusing on research on the nutritional composition of sacha inchi seeds [4-7]; the effects of lighting [8], irrigation [9], and fertilizer application on the growth of sacha inchi [10]; studied using sacha inchi oil to synthesize silver nanocatalysts [11]; comparison of physicochemical properties of different species of sacha inchi [12]. The sacha inchi tree has the advantage of having a broad spectrum of adaptation in both soil and climate (suitable temperature from 10-36 ^{o}C, rainfall from 800-1500 mm/year, altitude below 1700 m, does not require high farming technical skills and has a long economic cycle (20-30 years) [1, 6]. Another study result shows that the growth and root to stem mass ratio of sacha inchi had a lower sensitivity response to deficit irrigation, probably owing to their extremely low root mass fraction [8]. Especially, sacha inchi seeds contain approximately 45–50 % lipid, of which approximately 35.2–50.8 % is α-linolenic acid and approximately 33.4–41.0 % is linoleic acid [4, 6, 7, 11].

Besides, the study of Nascimento *et al*., 2013 showed that sacha inchi's leaf extracts could inhibit the HeLa (cervical cancer cells) and A549 (tumor cells from lung tissue) cancer cells. Terpenoids, saponins, and phenolic compounds (flavonoids) are the main bioactive compounds found in the leaf with antiproliferative activity against certain cancer cells [13]. This is one of the scientific concepts for researchers and for us to choose sacha inchi leaves to make tea.

Tea is a traditional drink of Vietnamese people. At this time, tea bags have become popular because of their convenience, so sacha inchi tea bags combined with pandan leaves and licorice both meet the convenience and meet the health needs of people. consumers.

However, up to now, there is not much research on the process of making tea bags from sacha inchi leaves. In Vietnam, Truong An sacha inchi company in collaboration with the Institute of Chemistry of Natural Compounds - Vietnam Academy of Science and Technology launched a study on the tea production process from sacha inchi leaves [14]. Therefore, the study of sacha inchi leaves applied in the production of tea bags combined with other ingredients such as licorice and pandan leaves is new in Vietnam.

2. Content

2.1 Materials and research methods

2.1.1. Materials

Sacha inchi leaves were selected from 5-year-old sacha inchi trees grown at the Experimental Garden of Biology Faculty, Hanoi National University of Education. Licorice leaves and pandan leaves were collected from plants which were grown also at the Experimental Garden of Biology Faculty. The selected leaves from these plants are pest free, remove impurities, wash up carefully, gently dry (wither) at 45 ^{o}C for 1 hour, and wither for 5 to 6 hours.

2.1.2. Research Methods

**** Experiment 1: Study on physiological and biochemical parameters of sacha inchi leaves***

Determination of dry matter content of leaves (the ratio of leaf dry mass to fresh mass) by conventional measurement method (repeated 3 times); chlorophyll content in leaves was determined by handheld chlorophyll meter SPAD-502 (repeated 3 times) [15]; the leventhal method was used to determine the tannin content in leaves (repeated 6 times) [16]; Atomic absorption spectroscopy (AAS) method was used to measure mineral content in sacha inchi leaves (replicated 3 times) [17-TCVN 10916:2005] and reducing sugar content in sacha inchi leaves was determined by DNS method (8 repeated) [18-Nguyễn Văn Mùi].

**** Experiment 2: Study on the process of making tea bags from sacha inchi leaves, licorice, and pandan leaves***

- *Experiment 2.1: Studying the effect of drying time and temperature on the quality of tea bags made from sacha inchi leaves (repeated 3 times):* to the right of drying temperature and time of drying for the best quality of tea.

The experiment was arranged with sacha inchi leaves at 3 different ages (young leaves, mature leaves, and old leaves), conducted at 4 drying temperature levels (100 ^{o}C, 110 ^{o}C, 120 ^{o}C, 130 ^{o}C) with 4-time levels (1 hour, 2 hours, 3 hours, 4 hours) for each of the above temperature levels.

Process: Sacha inchi leaves after being harvested will be washed under running tap water 2-3 times and rinsed with distilled water; then leaves are dried at 45 ^{o}C for 1 hour, left at room temperature for 5-6 hours (withered). Next, bring the tea at 80 ^{o}C (just stir in the cast iron pan and gently roll the sacha inchi leaves, every 5 minutes, turn off the stove, rolling the leaves evenly), repeat these steps 3 times. After obtaining the semi-finished products, put them in small paper packages (2 g) and then put them in trays to dry at 4 heat levels and 4-time levels as above. Conduct a survey of the obtained semi-finished products (survey on 10 students with a scorecard built based on industry standard 10TCN 459:2001) [19]. From there, the type of leaves and the drying temperature and time were selected for the next experiment.

- *Experiment 2.2: Study on the effect of mixing ratio between sacha inchi leaves and licorice on the quality of tea bags (repeated 3 times):* to select the tea blend ratio for the highest sensory value, to meet the needs and tastes of consumers.

Process:

After selecting the formulas with the highest sensory value in experiment 2.1, continue to mix with sacha inchi and licorice leaves at different ratios (1.4/0.6; 1.6/0.4; 1.8/0.2) (the ratio between sacha inchi and licorice leaves based on the ratio of dry weight and different ratios in this experiment was accorded on the research of Nguyen Tien Dung *et al*., 2018 [20].

Sacha inchi and licorice leaves were dried according to time and temperature in the recipe with the highest sensory score selected in experiment 2.1.

Mix selected sacha inchi recipes with licorice in 3 ratios: 1.4/0.6; 1.6/0.4; 1.8/0.2. Then, break in a ceramic pot, collect opinions from 10 Biology teachers and 4 students (with evaluation sheet based on standards, and record results) to find out the best mixing ratio of sacha inchi licorice leaves.

- *Experiment 2.3: Studying the ratio of pandan leaves added to sacha inchi and licorice leaves on the quality of sacha inchi tea bags (repeated 3 times)*: to choose the ratio of mixing these ingredients for the highest sensory value, meeting the needs and tastes of consumers.

Process: After selecting the formulas with the highest sensory value in experiment 2.2, continue to mix with glutinous leaves at different ratios. Sacha inchi, licorice, and pandan rice leaves were copied and dried according to time and temperature in the recipe with the highest sensory scores selected in experiment 2.1. Mixing recipes of sacha inchi: licorice selected in experiment 2.2 with pandan leaves at 6 levels: 0.05; 0.075; 0.1; 0.15; 0.2 and 0.25 g (based on dry weight). Then, brewing tea, collecting opinions from 30 students of the Biology Faculty and 50 students in the area of Hanoi National University of Education (with a standard-based assessment sheet, recording the results) to find out the ratio of mixing sacha inchi-licorice leaves with pandan leaves which gave the highest sensory scores.

- *Experiment 4: Study on polyphenol content and antioxidant activity in tea extract solution at different time points (repeated 3 times)*

+ Polyphenol content: determined by the Folin-Ciocalteu reagent method [21].

+ Antioxidant activity*:* investigated by the DPPH free radical scavenging method [22].

2.1.3. Sensory evaluation method

This is the simplest and most convenient method of evaluating the quality of tea products, based on sensory analysis of the receptors (smell, sight) combined with the analyst's experience to give conclusions about product quality. The survey form to collect the opinions of evaluation participants was built based on the TCVN 10TCN459:2001 standard [19].

2.1.4. Data processing method

Using SPSS 16.0 and Microsoft Excel 2016 software to process data. One-way analysis of variance and Turkey's - b test to evaluate the difference was statistically significant at the $\alpha = 0.05$ level.

2.2. Results and discussion

2.2.1. Study on physiological and biochemical parameters of sacha inchi leaves

**** Chlorophyll content***

In the same leaf layer, there is a difference in chlorophyll content according to leaf age. Specifically: total chlorophyll content was highest in mature leaves (43.4 ^{a} ± 0.36 mg/dm^2), gradually decreased in old leaves (35.8 b ± 0.38 mg/dm2), and lowest in young leaves (28.7 c ± 0.67 mg/dm^2). The reason is that when the leaves are young, the photosynthetic apparatus is not complete, and the chloroplasts do not contain much chlorophyll. Mature leaves have the highest chlorophyll content because when the photosynthetic apparatus is complete, the amount of chlorophyll accumulates a lot. The chlorophyll content of old leaves decreases because chlorophyll is degraded or destroyed when the light intensity is too strong.

Table 1. Results of determining physiological and biochemical indicators sacha inchi leaves

Indicators		Leaf age		
		Young leaves	**Mature leaves**	**Old leaves**
Total chlorophyll content (*mg/dm^2*)		28.7 ^{c} ± 0.67	43.4 ^{a} ± 0.36	35.8 ^{b} ± 0.38
Dry matter content (*g*)		2.20 ^{c} ± 0.02	2.6 ^{b} ± 0.04	2.81 ^{a} ± 0.08
Tannin content *(% by dry weight)*		2.17 ^{a} ± 0.02	2.31 ^{b} ± 0.01	2.47 ^{c} ± 0.02
Mineral content (*mg/kg*)	Mg	0.75 ^{a} ± 0.04	0.66 ^{b} ± 0.01	0.61 ^{c} ± 0.01
	Ca	28.27 ^{c} ± 1.02	30.57 ^{b} ± 0.74	32.59 ^{a} ± 0.16
	K	33.62 ^{a} ± 0.98	30.19 ^{b} ± 0.22	29.41 ^{c} ± 0.12
Reducing sugar content (*mg/g*)		17.4 ^{b} ± 4.47	25.9 ^{a} ± 5.67	24.83 ^{a} ± 6.88

Note: Comparing the experimental formulas in the same row, different letters (a, b, c) represent a statistically significant difference, the same letters indicate no significant statistical difference.

**** Dry matter content***

The dry matter content in leaves gradually increased from young leaf samples (2.2 g) to mature leaf samples (2.6 g, accounting for 118.2 % compared with young leaf samples) and was highest in old leaves (2.81 g, accounting for 127.7 % compared to the young leaf sample) (Table 1). That is because the volume of dry matter gradually accumulates over time through photosynthesis, related to the accumulation of substances such as proteins, lipids, saccharides, etc. in the leaves.

**** Tannin content***

The tannin content has a direct effect on the taste of the finished sacha inchi tea. From the results of Table 1 above, it is found that the tannin content in old leaves was highest (113.82% compared to young leaves), followed by mature leaves (106.4 % compared to young leaves). These results show that the tannin content in leaves increases with growing time.

**** Mineral content***

The concentration of mobile minerals such as Mg, and K was highest in young leaves ($0.75^{a} \pm 0.04$ and $33.62^{a} \pm 0.98$ mg/kg, respectively) and lowest in old leaves ($0.61^{c} \pm 0.01$ and $29.41^{c} \pm 0.12$ mg/kg, respectively). This difference between the magnesium and potassium mineral content values in the formulas in this study is statistically significant. Meanwhile, non-mobile minerals such as Ca were highest in old leaves ($32.59^{a} \pm 0.16$ mg/kg) and lowest in young leaves ($28.27^{c} \pm 1.02$ mg/kg*).*

**** Reducing sugar content***

The results in Table 1 showed the reduced sugar content was highest in mature leaves and lowest in young leaves. This result may be because the reduced sugar content is directly proportional to the intensity and efficiency of photosynthesis.

2.2.2. Research on the production process of tea bags from sacha inchi leaves combined with pandan leaves and licorice

**** The effects of temperature and drying time on the quality of sacha inchi tea***

We conducted parallel studies on the effect of 4 temperature levels (100 ^{o}C; 110 ^{o}C; 120 ^{o}C; 130 ^{o}C) and 4 different drying time levels on the quality of sacha inchi tea (1, 2, 3, and 4 hours). A total of 48 treatments were investigated, with scoring sheets included (based on industry standard 10TCN 459: 2001). The results showed that: sensory scores ranged from 1.1 to 4.91; where color ranges from 1.1 to 3.9; smell ranges from 1.27 to 4.78; and taste ranges from 1.1 to 4.91 (Table 2). Among them, there were 5 formulas with the highest sensory scores (mature leaves were dried at 130 ^{o}C for 1 hour and 2 hours; mature leaves were dried at 120 ^{o}C for 3 hours and 4 hours, and old leaves were dried at 130 ^{o}C for 1 hour). Thus, our research shows that the highest drying temperature at which we dry tea is 130 ^{o}C, much lower than the 160 ^{o}C used by Truong An Sacha Inchi Joint Stock Company [14]. We also tested some trial batches to select the drying temperature range and at the temperature was 140 ^{o}C, the sacha inchi leaves had a burnt smell.

Table 2. Sensory scores (color, smell, taste) of sachi leaves after drying at different temperatures and times

Formulars	Sensory scores		
	Color	***Smell***	***Taste***
Young leave 100 ^{o}C 1hr	$3,16^{e} \pm 0,14$	$2,7^{h} \pm 0,07$	$1,85^{i} \pm 0,05$
Young leave 100 ^{o}C 2 hrs	$1,5^{h} \pm 0,02$	$1,45^{i} \pm 0,07$	$1,67^{i} \pm 0,02$
Young leave 100 ^{o}C 3 hrs	$2,3^{f} \pm 0,54$	$2,43^{k} \pm 0,07$	$1,56^{i} \pm 0,28$
Young leave 100 ^{o}C 4 hrs	$3,3^{e} \pm 0,14$	$2,23^{h} \pm 0,53$	$2,1^{h} \pm 0,01$
Young leave 110 ^{o}C 1 hr	$2,4^{f} \pm 0,12$	$1,99^{m} \pm 0,06$	$1,9^{i} \pm 0,02$
Young leave 110 ^{o}C 2 hrs	$2,5^{f} \pm 0,03$	$1,95^{m} \pm 0,07$	$1,9^{i} \pm 0,12$
Young leave 110 ^{o}C 3 hrs	$1,99^{f} \pm 0,12$	$2,44^{k} \pm 0,01$	$2,23^{h} \pm 0,03$
Young leave 110 ^{o}C 4 hrs	$2,5^{f} \pm 0,03$	$2,45^{k} \pm 0,01$	$2,5^{h} \pm 0,024$
Young leave 120 ^{o}C 1 hr	$1,5^{h} \pm 0,12$	$1,56^{o} \pm 0,07$	$2,45^{h} \pm 0,12$
Young leave 120 ^{o}C 2 hrs	$3,7^{c} \pm 0,03$	$3,7^{d} \pm 0,01$	$2,6^{h} \pm 0,024$
Young leave 120 ^{o}C 3 hrs	$3,53^{d} \pm 0,07$	$2,74^{h} \pm 0,02$	$2,1^{h} \pm 0,12$
Young leave 120 ^{o}C 4 hrs	$2,81^{f} \pm 0,024$	$2,13^{k} \pm 0,32$	$2,4^{h} \pm 0,35$

Young leave 130 °C 1 hr	$1,3^{h} \pm 0,14$	$1,54^{o} \pm 0,07$	$2,1^{h} \pm 0,28$
Young leave 130 °C 2 hrs	$1,45^{h} \pm 0,02$	$2,1^{m} \pm 0,02$	$1,87^{i} \pm 0,02$
Young leave 130 °C 3 hrs	$2,5^{f} \pm 0,07$	$2,78^{h} \pm 0,14$	$3,11^{h} \pm 0,03$
Young leave 130 °C 4 hrs	$2,34^{f} \pm 0,015$	$2,19^{m} \pm 0,01$	$2,87^{f} \pm 0,12$
Mature leave 100 °C 1 hr	$1,22^{i} \pm 0,02$	$1,34^{p} \pm 0,024$	$1,97^{i} \pm 0,05$
Mature leave 100 °C 2 hrs	$1,76^{f} \pm 0,07$	$1,89^{n} \pm 0,05$	$2,11^{h} \pm 0,07$
Mature leave 100 °C 3 hrs	$2,56^{f} \pm 0,07$	$2,55^{h} \pm 0,14$	$2,7^{h} \pm 0,01$
Mature leave 100 °C 4 hrs	$2,33^{f} \pm 0,05$	$2,43^{k} \pm 0,024$	$2,78^{f} \pm 0,28$
Mature leave 110 °C 1 hr	$2,4^{f} \pm 0,03$	$2,31^{l} \pm 0,024$	$2,56^{h} \pm 0,05$
Mature leave 110 °C 2 hrs	$1,96^{g} \pm 0,01$	$1,9^{n} \pm 0,07$	$2,12^{h} \pm 0,12$
Mature leave 110 °C 3 hrs	$1,13^{i} \pm 0,12$	$1,27^{p} \pm 0,12$	$1,98^{i} \pm 0,024$
Mature leave 110 °C 4 hrs	$1,32^{i} \pm 0,02$	$1,65^{o} \pm 0,07$	$1,46^{n} \pm 0,05$
Mature leave 120 °C 1 hr	$2,1^{f} \pm 0,01$	$2,13^{m} \pm 0,12$	$1,99^{h} \pm 0,12$
Mature leave 120 °C 2 hrs	$2,3^{f} \pm 0,015$	$2,56^{i} \pm 0,015$	$2,3^{h} \pm 0,05$
Mature leave 120 °C 3 hrs	$2,67^{f} \pm 0,14$	$3,11^{g} \pm 0,12$	$2,87^{g} \pm 0,07$
Mature leave 120 °C 4 hrs	$3,90^{b} \pm 0,12$	$3,75^{c} \pm 0,02$	$4,15^{d} \pm 0,07$
Mature leave 130 °C 1 hr	$4,76^{a} \pm 0,14$	$4,78^{a} \pm 0,07$	$4,91^{a} \pm 0,015$
Mature leave 130 °C 2 hrs	$4,67^{a} \pm 0,015$	$3,99^{b} \pm 0,015$	$4,87^{b} \pm 0,024$
Mature leave 130 °C 3 hrs	$3,46^{d} \pm 0,03$	$3,25^{e} \pm 0,01$	$2,99^{f} \pm 0,015$
Mature leave 130 °C 4 hrs	$3,1^{e} \pm 0,07$	$2.67^{h} \pm 0,12$	$2,54^{h} \pm 0,05$
Old leave 100 °C 1 hr	$3,11^{e} \pm 0,07$	$2,1^{m} \pm 0,02$	$1,8^{i} \pm 0,024$
Old leave 100 °C 2 hrs	$1,1^{i} \pm 0,12$	$1,33^{p} \pm 0,07$	$1,89^{i} \pm 0,024$
Old leave 100 °C 3 hrs	$1,67^{g} \pm 0,015$	$1,6^{o} \pm 0,03$	$1,89^{i} \pm 0,12$
Old leave 100 °C 4 hrs	$1,3^{h} \pm 0,12$	$1,6^{o} \pm 0,12$	$1,1^{p} \pm 0,05$
Old leave 110 °C 1 hr	$1,45^{h} \pm 0,015$	$1,63^{o} \pm 0,07$	$1,74^{k} \pm 0,015$
Old leave 110 °C 2 hrs	$1,56^{h} \pm 0,02$	$1,6^{o} \pm 0,015$	$2,8^{g} \pm 0,02$
Old leave 110 °C 3 hrs	$1,3^{h} \pm 0,14$	$1,63^{o} \pm 0,024$	$1,4^{n} \pm 0,024$
Old leave 110 °C 4 hrs	$1,5^{h} \pm 0,03$	$1,56^{o} \pm 0,015$	$1,77^{i} \pm 0,12$
Old leave 120 °C 1 hr	$2,1^{f} \pm 0,015$	$2,44^{k} \pm 0,024$	$1,6^{m} \pm 0,03$
Old leave 120°C 2 hrs	$3,1^{e} \pm 0,12$	$3,1^{g} \pm 0,12$	$2,5^{h} \pm 0,015$
Old leave 120 °C 3 hrs	$1,5^{h} \pm 0,12$	$1,8^{n} \pm 0,12$	$2,05^{h} \pm 0,12$
Old leave 120 °C 4 hrs	$1,45^{h} \pm 0,015$	$1,66^{o} \pm 0,015$	$2,31^{h} \pm 0,05$
Old leave 130 °C 1 hr	$1,5^{h} \pm 0,02$	$1,45^{o} \pm 0,07$	$1,67^{l} \pm 0,015$
Old leave 130 °C 2 hrs	$3,65^{b} \pm 0,14$	$3,78^{c} \pm 0,12$	$4,27^{c} \pm 0,024$
Old leave 130 °C 3 hrs	$3,34^{b} \pm 0,56$	$2,8^{d} \pm 0,42$	$2,7^{g} \pm 0,01$
Old leave 130 °C 4 hrs	$1,7^{f} \pm 0,12$	$2,01^{m} \pm 0,12$	$1,89^{i} \pm 0,015$

Note: Compare the experimental formulas in the same column, the letters (a, b, c, d, e, f, g, h, i, k, l, m, n, o, p, q) are different which means the value is a statistically significant difference, if the same letters which mean the value is not a statistically significant difference.

**** The effects of mixing ratio between sacha inchi and licorice on the quality of sacha inchi tea bags***

From the 5 recipes selected in the results of experiment 1, we mixed sacha inchi and licorice at 3 ratios of sacha inchi: licorice of 1.4/0.6; 1.6/0.4, and 1.8/0.2. After examining the sensory scores in the formulas, the following results were obtained:

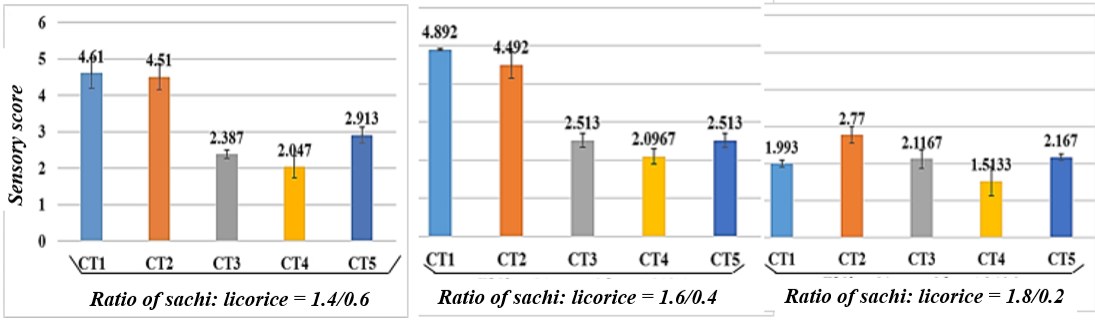

Figure 1. Sensory score comparison chart in 5 recipes when mixing sacha inchi: licorice with 3 different ratios

The results in Figure 1 showed that the sensory scores in all 5 recipes increased when the percentage of licorice added to sacha inchi tea increased from 0.2; to 0.4 and decreased with the addition of licorice at the rate of 0.6. The results show that the sensory scores ranged from 1,993 to 4,892. In which, sensory scores were highest in formula 1 and formula 2 when adding licorice with the ratio: 1.4 / 0.6 and 1.6 / 0.4. After 3 times of surveys, we selected 2 formulas: CT1: mature leaves 130 ^{o}C 1 hour and CT2: mature leaves 130 ^{o}C 2 hours with the ratio of sacha inchi: licorice is 1.4: 0.6 and 1.6: 0.4.

**** The effects of the percentage of pandan leaves added to sacha inchi and licorice on the quality of sacha inchi tea bags***

From the 2 formulas and ratios selected through experiment 2, we continued to investigate the additional percentage of pandan leaves with a series of 6 different ratios (0,05; 0,075; 0,1; 0,15; 0,2; 0,25) and obtained the following results:

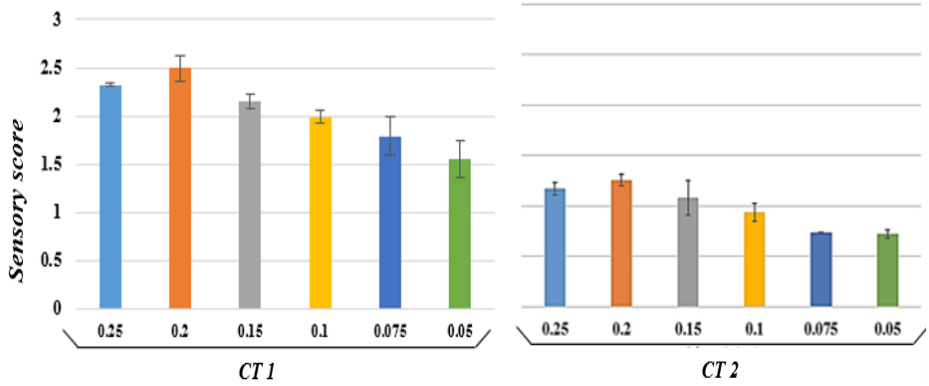

Figure 2. The sensory scores between formula 1 (mature leaves - 130 ^{o}C - 1 hour) and formula 2 (mature leaves - 130 ^{o}C - 2 hours) with a ratio of sacha inchi: licorice of 1.4 /0.6 when adding pandan leaves at 6 different ratios

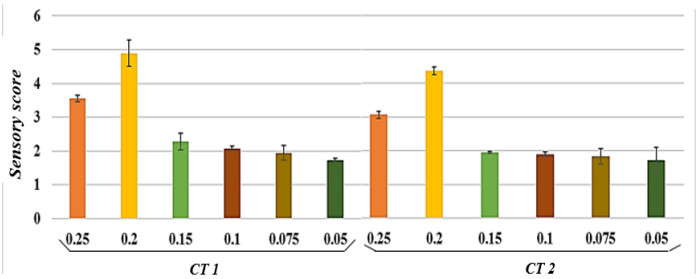

Figure 3. The sensory scores between formula 1 (mature leaves - 130 °C - 1 hour) and formula 2 (mature leaves - 130 °C - 2 hours) with a ratio of sacha inchi: licorice of 1.6 /0.4 when adding pandan leaves at 6 different ratios

Thus, through the results of surveying the effect of the percentage of additional pandan leaves and sacha inchi and licorice on the quality of sacha inchi tea, we have selected 2 recipes as follows: Mature leaves - 130 °C - 1 hour with mixing ratio of sacha inchi: licorice: pandan leaves is 1.6/0.4/0.2 and mature leaves - 130 °C - 2 hours with the mixing ratio of sacha inchi: licorice: pandan leaves is 1.6 /0.4/0.2. To make sure the results, we continued to survey 50 students of Hanoi National University of Education. The results are shown in Figure 4 below:

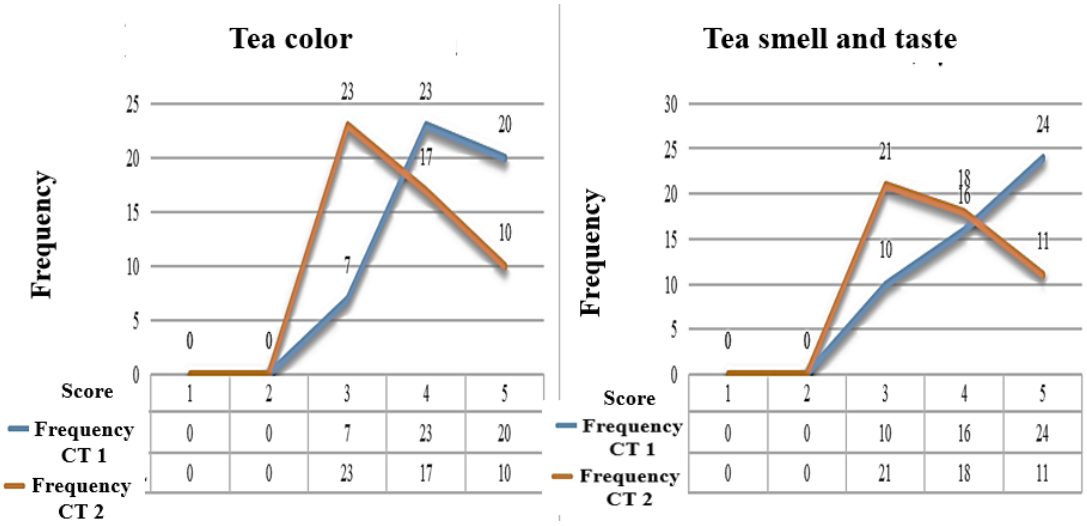

Figure 4. The results of a sensory survey on mature leaves of sacha inchi : licorice: pandan hours with the mixing ratio 1.6/0.4/0.2 drying at 130 °C for 1 hr (CT1) and 2 hrs (CT2)

The results show that formula 1 (CT1): mature leaves 130 °C 1 hour had a greater score than that in formula 2 (CT2): mature leaves at 130 °C 2 hours) on color and taste of tea when mixed with the ratio of sacha inchi: licorice: pandan leaves is 1.6: 0.4: 0.2.

Therefore, we proposed the sacha inchi tea production process for the best quality as follows:

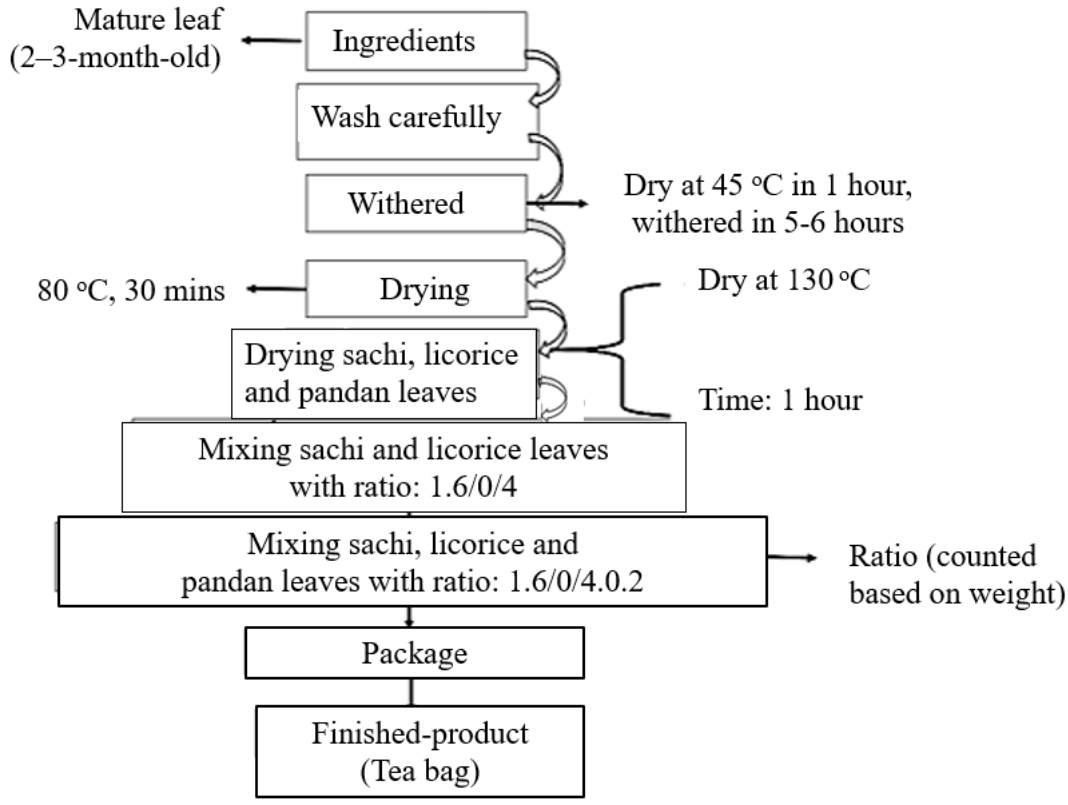

Figure 5. Schematic summary of the process of making tea bags from sacha inchi leaves, licorice, and pandan leaves

**** Determination of polyphenol content and antioxidant activity in tea extract at different time points***

To have more information related to tea water quality from mixing sacha inchi leaves with licorice and pandan leaves as mentioned above, an experiment on polyphenol and antioxidant activity was carried out.

Table 2. Polyphenol content in tea extract solution at different time points

Sample tea solution	**Polyphenol content (mg gallic acid/1g)**	**Antioxidant activity SC_{50} (mg/ml)**
After 3 minutes of extraction	$123.7^{a} \pm 6.7$	$5.97^{b} \pm 0.57$
After 5 minutes of extraction	$126.2^{a} \pm 8.9$	$4.34^{c} \pm 0.5$
After 7 minutes of extraction	$105.8^{b} \pm 11.2$	$7.10^{a} \pm 0.37$

Note: Compare the experimental formulas in the same column, different letters (a, b, c) show statistically significant differences, same letters show no significant statistical difference.

The results in Table 2 showed that the polyphenol content was different between the tea samples. After 3 minutes, the tea had a polyphenol content in of extraction $123.7^{a} \pm 6.7$ mg/g;

while after 5 minutes, tea had the highest polyphenol content in extraction, reached 126.2 ^{a} ± 8.9 mg/g, but the difference between tea samples after 3 minutes of extraction and 5 minutes of extraction was not statistically significant. Besides that tea had the lowest polyphenol content in extraction after 7 minutes, just 105.8 ^{b} ± 11.2 mg/g and this result was significantly different from 2 other formulas.

The reason may be that the shorter the time, the shorter the contact between the tea material and the solvent, so only small organic compounds may dissolve into the solvent. When increasing the extraction time, the contact time between the raw materials and the solvent also increases. At this time, the compounds with large molecular weight under the influence of the solvent's polarity will be extracted from the raw materials. On the other hand, raw materials soaked in solvents for a long time will swell, blocking the pores of the cell membranes, and hindering the solvent's ability to penetrate the raw materials, so the total phenolic extraction efficiency decreases (Ho *et al.*, 2012) [23]. Nascimento *et al*., 2013 also reported that total phenol in different solvents used to get leaf sacha inchi extract ranged from 5.34 to 10.35 % [13], these results are not much different from our results shown in Table 2 (polyphenol content was 105.8 ^{b} ± 11.2 to 126.2 ^{a} ± 8.9 mg gallic acid/1g, corresponding to from 10,58 to 12,62 %).

Concerning antioxidant activity, all 3 samples of tea extract solution at 3 time points: 3, 5, and 7 minutes showed antioxidant activity. Among them, the sample of tea extraction at 5 minutes had the smallest SC_{50} value (4.34minus 5 mg/ml), which was the sample for the best antioxidant capacity in the three time points of tea brewing. When comparing the antioxidant activity with the polyphenol content of the tea extracts, it was found that the polyphenol content and SC_{50} were negatively correlated, that is, the higher the polyphenol content, the lower the SC_{50} and higher oxidation resistance.

3. Conclusions

Dry weight and tannin content increased gradually from young leaves to mature leaves and were highest in older leaves of sacha inchi. Total chlorophyll content increased gradually from young leaves to old leaves and was highest in mature leaves. Besides, mobile mineral elements (Mg, K) have the highest concentration in young leaves > mature leaves > old leaves, while non-motile mineral elements (Ca) have the highest concentration in old leaves > leaves mature > young leaves. The reduced sugar content increased gradually from young leaves to mature leaves and old leaves. Then, mature leaves of sacha inchi are suitable to be applied in making tea bags.

The sacha inchi tea bag production process was set up by the following steps: select mature sacha inchi leaves, remove impurities, wash; dry at 45 ^{o}C for 1 hour, then leave at room temperature for 5-6 hours (withered); Dry tea at a temperature of 80 ^{0}C for 30 minutes, stir well in a cast iron pan while gently rubbing tea leaves, every 5 minutes, turn off the stove and rub the tea leaves. Mix sacha inchi leaves: licorice leaves: and pandan leaves with a ratio of 1.6/0.4/0.2 for tea packages with a total dry weight of 2.2 g/bag.

For the quality of tea water, the 5-minute tea extraction solution had the highest polyphenol content and antioxidant activity. Thus, consumers should drink sacha inchi tea bags combined with licorice leaves and pandan leaves after steeping the tea with boiling water for 5 minutes.

Further study on the tea fermentation time (after the withering step) on the quality of sacha inchi tea bags combined with licorice leaves and sticky leaves is needed. Polyphenol content and antioxidant activity in sacha inchi leaf also need to be evaluated.

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