


Research Article

The Effects of Fixation Methods on the Composition and Activity of Sea Buckthorn (*Hippophae rhamnoides* L.) Leaf Tea

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Fixation is the key step to improve the quality of sea buckthorn leaf tea. Composition and activity are important indexes to evaluate the quality of sea buckthorn leaf tea. Comparing the effects of fixation methods on components and activities provides a theoretical basis for the contemporary, controllable, and continuous production of sea buckthorn leaf tea. The effects of six different fixed methods, pan-firing fixed (PF), steaming fixed (SF), boiling water fixed (BF), hot air fixed (HF), microwave fixed (MWF), and infrared fixed (IRF) for sea buckthorn leaf tea in terms of α -glucosidase inhibitory activity, lipase inhibitory ability, and the antioxidant capacity were studied. The total flavonoids (TF) content, total soluble phenolics (TP) content, water-soluble carbohydrate (WSC) content, the inhibitory activity of α -glucosidase, lipase inhibitory ability, and the antioxidant capacity of fixed sea buckthorn leaf tea were significantly higher ($p \leq 0.05$) compared with sea buckthorn leaf. IRF and MWF samples had higher ($p \leq 0.05$) contents of TF (92.48 mg RE/g and 79.20 mg RE/g), TP (115.37 mg GA/g and 135.18 mg GA/g) and WSC (4.24% and 4.39%). The DPPH radical scavenging activity of the SF sample was the strongest one, followed by the MWF sample and IRF sample ($p \leq 0.05$). The hydroxyl radical scavenging ability and reducing power of IRF were the strongest one, followed by the MWF sample ($p \leq 0.05$). The IRF sample had the strongest α -glucosidase inhibitory activity ($p \leq 0.05$), and the MWF sample had the strongest lipase inhibitory ability while samples contained the same amount of total polyphenols ($p \leq 0.05$). Principal component analysis results showed that the IRF sample, MWF sample, and SF sample had higher comprehensive principal component values. MWF takes less time than IRF, which operated at 2,450 MHz (full power of 700 W) for 2 min. Therefore, MWF was the most suitable fixation method for sea buckthorn leaf tea. *Practical Applications.* Leaf tea is the main product of sea buckthorn leaf. However, at present, the quality of sea buckthorn leaf tea in the market is uneven, the processing methods are diverse, and there is no certain quality standard. This paper provides some data support and theoretical support for the production, processing, and purchase of sea buckthorn leaf tea.

1. Introduction

Sea buckthorn (*Hippophae rhamnoides* L.) belongs to the *Elaeagnus* family, which is widely distributed over Asia and Europe [1], and China is one of the main producing areas. Sea buckthorn fruits, seeds, leaves, stem skins, and roots are rich sources of phenolic components, and those parts have antibacterial and antioxidant activities [2, 3]. The sea buckthorn fruits have long been used for tonifying the

spleen, helping digestion, relieving cough, eliminating phlegm, promoting blood circulation, and removing blood stasis as a dual-purpose food for medicine and food in China [4]. Compared with the berries, sea buckthorn leaves also have been used as edibles and herbal medicines for a long time in Tibetan and Mongolian of China. In 2013, the National Health and Family Planning Commission of People's Republic of China regulated sea buckthorn leaves as common food [5]. The study by Saggu et al. [6] showed that

the water extract of sea buckthorn leaves has no toxic effect. The sea buckthorn leaves are abundant in phenolics [7–13], essential oil [9, 14], carotenoid [8, 15], tocopherol [16], sugar [9, 16], and ascorbic acid [16]. Masoodi et al. [17] reported that water extract from sea buckthorn leaves can inhibit cellular proliferation, wound healing, and reduce the expression of prostate-specific antigens in prostate cancer cells in *vitro*. It has been reported that extracts of the sea buckthorn leaves have antioxidant [11, 18, 19], cytoprotective [19], antibacterial [14, 18, 19], anti-inflammatory [20], antifatigue [21], antivisceral obesity [1], inhibiting glioma cell growth [22], promoting apoptosis [22], and stronger antiplatelet properties in *vitro* [23]. In addition, extracts of the sea buckthorn leaves are used in the manufacture of extracts, tea, animal feed, drugs, and cosmetics in some countries [1, 24].

In recent years, there has been an increased interest in sea buckthorn leaf teas because of their beneficial health properties. According to tea manufacturing process, teas are classified as green, yellow, white, oolong, black, and dark tea [25, 26]. Green tea is prepared by fixation, rolling, shaping, and drying. Fixation is the key step to ensure the quality of green tea, which can result in inactivating phenol oxidases and other enzymes, emitting moisture, volatilizing grass flavor, forming aroma, and softening leaves. The most commonly used traditional fixation techniques for green tea are pan-firing (PF), steaming fixation (SF), and boiling water fixation (BF) [27–29]. The modern fixation techniques for green tea are hot air fixation (HF), microwave fixation (MWF), and infrared fixation (IRF) [29, 30]. In the process of PF [31], the dry pan is exposed to a high-temperature source (100–200°C). The disadvantage of the PF is that the temperature is not well controlled, resulting in yellow and brown leaves and a smoke smell. In the process of SF, heat is used through water steam, and the temperature is around 100°C [32]. It not only completely avoids the smell of smoke caused by the PF but also eliminates the bitterness of summer and autumn tea to a great extent, of which high content of tea polyphenol caused bitterness. The disadvantage of the SF is that the moisture content of the fixed leaves is higher than that of the PF. In the process of BF [33], the activity of polyphenol oxidase in the older fresh leaves can decrease sharply in a short time, while there is more loss of the water-soluble extracts. In the process of the HF [31], the fixed fresh leaves have a high-temperature (100–200°C), so they should be cooled quickly; otherwise, the color of the fixed leaves will turn yellow and dark and the quality of the tea will decrease. In the process of the MWF [34–36], the microwave with high frequency (300 MHz–300 GHz) can rapidly inactivate the enzymes in fresh leaves. The moisture content of the leaves by MWF is lower than those by SF, which is favorable to the following rolling process. In the process of the IRF [37], tea leaves absorb a large amount of infrared rays, which cause the inner molecules to vibrate and change into heat energy, so that the tea strips can be heated evenly.

Although more and more interest was found in the tea of sea buckthorn leaves [11], the effects of different fixation methods on the composition and activities of sea buckthorn

leaves tea have not been widely studied. This study aimed to find the most suitable fixation method for sea buckthorn leaves based on the quantification of bioactive compounds, antioxidant capacity, and the inhibition of α -glucosidase.

2. Materials and Methods

2.1. The Preparation of Sea Buckthorn Leaf Tea. Fresh leaves of sea buckthorn (*Hippophae rhamnoides* L.) were picked in October, 2019, from Fangshan town, Lvliang city, Shanxi Province in China, GPS location: N: 38°0′15.88″E: 111°28′18.57″ and authenticated by Prof. Zhao Sanhu, Xinzhou Teachers University, China. The sea buckthorn leaves were picked from random trees and then mixed. Sea buckthorn leaves were taken to the laboratory, washed with distilled water, and then drained. The sea buckthorn leaves were divided into seven groups using the method of coning and quartering, and each group was 300 g of the total weight. Group 1 was dried in a baking box at 60°C. PF was conducted for Group 2 at 150°C for 3 min. SF was performed for Group 3; a steamer was placed on top of the boiling water, and then, spread sea buckthorn leaves were on the steamer about 3 mm thick and steamed for 2 min. BF was conducted for Group 4; we added the sea buckthorn leaves in boiling water for about 1 min, quickly removed them with a colander, and drained the water. HF was performed for Group 5 at 140°C for 5 min in a hot air oven (DHG-9240, Shanghai Yiheng Scientific Instrument Co., Ltd., Shanghai, China). MWF was done for Group 6. The microwave fixation was operated at 2,450 MHz for 2 min and the full power of the microwave is 700 W (LG-WD700 (MG-5082M), LG Electronics (Tianjin) Electric Co., Ltd., Tianjin, China). IRF was performed for Group 7 (380 × 470 × 290 mm, WS70-2, Gongyi Yingyuyuhua Instrument Co., Ltd., Gongyi, China) for 7 min. These fixed sea buckthorn leaves were rolled in the same way and then dried in an oven at 60°C. The processing method of the sea buckthorn leaf tea is presented in Table S1.

2.2. The Preparation of a Sea Buckthorn Leaf Tea Extract for Analysis. The samples of sea buckthorn leaf tea were ground with a blender (J-150A, Jingye Industry and Trade Co., Ltd., Zhejiang, China) and then sieved through a 65-mesh sieve. Sea buckthorn leaf tea powder (1 g) and 60% ethanol (20 mL) were mixed and extracted by ultrasonic water bath (300 × 240 × 150 mm, KQ400KDE, Nanjing Xuejing Biotechnology Co., Ltd., Nanjing, China) for 1 h at 65°C. The extract was centrifuged at 7000g for 5 min, and the extract was added into an evaporation bottle. The steps were repeated three times, and the extract was evaporated to dryness under reduced pressure at 50°C by rotatory evaporator (RE-52AA, Shanghai Yarong Biochemical Instrument Factory, Shanghai, China). The residue was redissolved with 60% ethanol, and the volume was fixed to 15 mL. Sea buckthorn leaf tea extract was placed in white sample tubes and then stored in the fridge at –20°C until further use.

2.3. Determination of Total Soluble Polyphenol Content.

The Folin-Ciocalteu method was used to determine the total soluble polyphenol content in the samples [38]. The sea buckthorn leaf tea powder extract of 45 μL , ethanol 360 μL , and the Folin-Ciocalteu reagent 120 μL were added in the test tube. After the tube was made to stand at room temperature for 5 min, 120 μL of 20% sodium carbonate was added. Absorbance was determined at 680 nm. Various concentrations of gallic acid (0.0071 mg/mL–0.0495 mg/mL) were used as the reference material to plot the standard curve ($y = 35.859x + 0.0832$, $R^2 = 0.9913$). Total polyphenol content was expressed as mg gallic acid equivalent (GA)/g dry weight.

2.4. Determination of Total Flavonoid Content.

The content of total flavonoids was determined using our previous method [39] with some modifications. The sea buckthorn leaf tea powder extract (0.2 mL), ethanol (4.8 mL), and 5% NaNO_2 solution (0.4 mL) were added in the test tube. The mixture was made to stand for 6 min, 5% $\text{Al}(\text{NO}_3)_3$ (0.4 mL) was added, and allowed to stand for 6 min. Then, 4% NaOH (4 mL) was added and shaken, the mixture stand for 15 min, and the absorbance was read at 510 nm. Various concentrations of rutin (0.0013 mg/mL–0.0094 mg/mL) were used as the reference material to plot the standard curve ($y = 4.039x + 0.0725$, $R^2 = 0.998$). Total flavonoid data were expressed in mg of rutin equivalents (RT)/g dry weight.

2.5. Determination of Isorhamnetin Content.

The isorhamnetin content of sea buckthorn leaf tea samples was determined following the manner of SPC [4] with some modifications. The sea buckthorn leaf tea extract (350 μL) and 12 mol/L HCl (350 μL) were mixed in a 10 mL screw bottle. The screw bottle was incubated at 75°C for 1 h, after cooling to the room temperature, 4.3 mL of alcohol was added in the bottle, which would be used to determine the content of isorhamnetin.

The HPLC column used for determining the content of isorhamnetin was Kromasil 100-5 C_{18} (250 \times 4.6 mm, 5 μm). The HPLC conditions are as follows: the column temperature was 25°C, the mobile phase was $\text{EtOH}/0.4\%$ phosphoric acid in water (58/42), the mobile phase flow rate was 1 mL/min, the measurement wavelength was 370 nm, and the injection volume was 10 μL . The samples were filtered through a 0.22 μm organic millipore filter before injection. Various concentrations of isorhamnetin (0.0005 mg/mL–0.002 mg/mL) were used as the reference material to draw the standard curve ($y = 3 \times 10^9x + 18186$, $R^2 = 0.9996$). The isorhamnetin standard was purchased from Shanghai Yuanye Biotech Co., Ltd.

2.6. Determination of Water Soluble Carbohydrate Content.

The water-soluble carbohydrate content of sea buckthorn leaf tea samples was measured following the anthrone-sulphuric acid method which was described by our previous method [39] with some modifications. The sea buckthorn leaf tea extract of 40 μL volatilized the solvent,

and the residue was reconstituted with 2 mL of water. Anthrone containing sulfuric acid (0.2%) of 5 mL was added to the test tube and then boiled at 100°C for 10 min. The tube was rapidly cooled to room temperature, and then, the absorbance was measured at 620 nm. Various concentrations of glucose (0.01 mg/mL–0.06 mg/mL) were used as a reference material to plot the standard curve ($y = 10.768x + 0.1324$, $R^2 = 0.9994$). Total water soluble carbohydrate content was expressed as g glucose/100 g dry weight.

2.7. Polyphenol Oxidase (PPO) Activity.

The PPO activity was analyzed by our previous method [40, 41] with some modifications. Polyvinyl poly pyrrolidone (2 g) was added to 100 mL sodium phosphate buffer (0.2 mol/L, pH 7.0), which was the extraction solution. Sea buckthorn leaf tea powder (0.5 g) was added in the tube, which was placed in an ice water bath. An extraction solution of 2 mL was added in the tube. After stirring for 1 min, the mixture was centrifuged at 16000g for 5 min at 4°C. The previous step was repeated three times. The PPO activity was determined using the supernatant obtained by three centrifugations.

Catechol (20 mg/mL, 1.5 mL), phosphate buffer (pH 7.0, 50 mmol/L, 1.5 mL), and the enzyme solution (0.2 mL) were added to the tube. Phosphate buffer was used instead of enzyme solution in the blank sample. The PPO activity was defined as 0.001 linear increases in absorbance per minute per kilogram of sea buckthorn leaf tea powder at 410 nm.

2.8. Assay of DPPH Radical Scavenging Capacity.

The DPPH radical scavenging capacity of sea buckthorn leaf tea powder extract was determined following our previous manner [39] with some modifications. Different concentrations of sea buckthorn leaf tea extract (200 μL) were added to DPPH (100 μL). After standing in the dark for 20 minutes, the absorbance was read at 517 nm. Ethanol (60%) was used instead of the extract in the blank sample. The scavenging rate of DPPH radical was calculated as follows:

$$\text{Scavenging rate (\%)} = \left[1 - \left(\frac{A_{\text{sample}}}{A_{\text{blank}}} \right) \right] \times 100\%. \quad (1)$$

The EC_{50} value is the effective concentration of DPPH free radical scavenging rate of 50%, obtained by the linear regression equation.

2.9. Assay of Hydroxyl Radical Scavenging Ability.

The hydroxyl radical scavenging capacity of sea buckthorn leaf tea powder extract was determined following the Fenton manner [42] with some modifications. Different concentrations of sea buckthorn leaf tea extract (2.2 mL) were mixed in order with sodium phosphate buffer (pH 7.4, 0.2 mol/L, 1.5 mL), 3,7-diamino-2,8-dimethyl-5-phenylphenazin-5-ium chloride (Saffron T solution) (520 $\mu\text{g}/\text{mL}$, 0.2 mL), $\text{EDTANa}_2\text{Fe(II)}$ (2.0 mmol/L, 0.7 mL), and hydrogen peroxide (H_2O_2 , 6%, v/v, 0.4 mL). After the mixture was incubated for 30 min at 37°C, the absorbance was read at 520 nm. Ethanol (60%) was used instead of the extract in the

blank sample. The scavenging rate of hydroxyl radical was calculated as follows:

$$\text{Scavenging.rate (\%)} = \left[1 - \left(\frac{A_{\text{blank}}}{A_{\text{sample}}} \right) \right] \times 100\%. \quad (2)$$

The EC_{50} value is the effective concentration of hydroxyl-free radical scavenging rate of 50%, obtained by the linear regression equation.

2.10. Assay of Nitrite Scavenging Ability. The nitrite scavenging capacity of sea buckthorn leaf tea powder extract was determined using the Griess reagent [43] with some modifications. Different concentrations of sea buckthorn leaf tea extract (90 μL) were mixed in order with of nitrite sodium (NaNO_2) (50 mg/L, 50 μL), citrate buffer (pH = 3.0, 0.1 mol/L, 250 μL), and water (110 μL). The mixtures were incubated at 37°C for 30 min, cooled to room temperature, and then 0.4% sulfanilic acid (1 mL) was added. After 5 min, 0.2% N-(1-naphthyl) ethylenediamine dihydrochloride (500 μL) and distilled water (3 mL) were added to the mixture. After the mixture was placed for 15 min at room temperature, absorbance was read at 538 nm. Ethanol (60%) was used instead of the extract in the blank sample. The scavenging rate of nitrite was calculated as follows:

$$\text{Scavenging.rate (\%)} = \left[1 - \left(\frac{A_{\text{sample}}}{A_{\text{blank}}} \right) \right] \times 100\%. \quad (3)$$

The EC_{50} value is the effective concentration of nitrite scavenging rate of 50%, obtained by the linear regression equation.

2.11. Assay of Reducing Power. The reducing power of sea buckthorn leaf tea powder extract was determined following our previous manner [39] with some modifications. The sea buckthorn leaf tea extract (1 mL) was added to phosphate buffer (0.2 mol/L, pH 6.6, 2.5 mL) and potassium ferricyanide (1%, 2.5 mL), and then, it was placed in a constant temperature water bath at 50°C. After 20 minutes, it was rapidly cooled, and trichloroacetic acid (TCA) solution (10%, 2.5 mL) was immediately added to terminate the reaction. After centrifugation at 1000g for 10 min, the supernatant of 1 mL was taken out, and then, 1 mL of distilled water and ferric chloride (0.1%, 0.1 mL) were added. After 10 min, the absorbance was measured at 700 nm. Ascorbic acid was used as a reference material to draw the standard curve. The reducing power was expressed as μmol ascorbic acid equivalents per g of dry weight.

2.12. Assay of α -Glucosidase Inhibitory Activity. The α -glucosidase inhibitory ability of sea buckthorn leaf tea was determined according to Miao et al. [44] with some modifications. The α -glucosidase from yeast (70 $\mu\text{g}/\text{mg}$) was purchased from Shanghai Yuanye Biotechnology Co., Ltd. (Shanghai, China). Different concentrations of the sea buckthorn leaf tea extract (100 μL) was added to the tube; then, 60 μL α -glucosidase solution (5 $\mu\text{g}/\text{mL}$) was added, and

350 μL phosphate buffer (0.1 mol/L, pH = 6.8) was added at the end. After reaction at 37°C for 10 min, 10 μL 10 mM *p*-nitrophenyl- α -d-glucopyranoside (*p*NPG) in 0.1 mol/L phosphate buffer (pH = 6.8) solutions was added. After reaction at 37°C for 30 min, 500 μL sodium carbonate (0.5 mol/L) was added, and the absorbance was measured at 405 nm. Ultrapure water was used instead of the extract in the blank sample. The α -glucosidase inhibitory ratio was calculated as follows:

$$\text{Inhibitory.ratio (\%)} = \left[1 - \left(\frac{A_{\text{sample}}}{A_{\text{blank}}} \right) \right] \times 100\%. \quad (4)$$

The IC_{50} value is the effective concentration of α -glucosidase inhibitory rate of 50%, obtained by the linear regression equation.

2.13. Assay of Lipase Inhibitory Activity. The lipase inhibitory ability of sea buckthorn leaf tea was determined according to Mcdougall et al. [45] with some revision. Lipase from porcine pancreas (BR, 30 $\mu\text{g}/\text{mg}$, Shanghai Yuanye Biological Co., Ltd., Shanghai, China) was dissolved in ultrapure water at a concentration of 5 $\text{mg}\cdot\text{mL}^{-1}$, centrifuged at 7000g for 5 min, and then, the supernatant was used. The substrate was 0.8 mg/mL *p*-nitrophenyl laurate (*p*NP laurate) of 450 μL , which dissolved in 5 mmol/L sodium acetate (pH 5.0) containing 1% Triton X-100. The 400 μL Tris buffer (pH 8.2) of 0.1 mol/L was mixed in order with 100 μL porcine pancreases, and 100 μL samples which contained the same amount of total polyphenols or total flavonoids, and 450 μL substrates was added and the mixtures were allowed to react for 60 min at 37°C; then, the absorbance was measured at 405 nm. Ultrapure water was used instead of the extract in the blank sample. The lipase inhibitory ratio was calculated as follows:

$$\text{Inhibitory.ratio (\%)} = \left[1 - \left(\frac{A_{\text{sample}}}{A_{\text{blank}}} \right) \right] \times 100\%. \quad (5)$$

2.14. Statistical Analysis. All experimental results were the mean value of three sets of experimental data, and significant differences ($p \leq 0.05$) were compared using S-N-K and LSD *post-hoc* tests. The data were statistically analyzed using the IBM SPSS Statistics 20.

3. Results and Discussion

3.1. Moisture, Total Polyphenol, Total Flavonoids, Isorhamnetin, Water-soluble Carbohydrate, and Polyphenol Oxidase (PPO) Activities. The contents of moisture, PPO activities, total polyphenol, total flavonoid, isorhamnetin, and water-soluble carbohydrate of sea buckthorn leaf tea are presented in Table 1. The moisture content of sea buckthorn leaf tea ranged from 5.73% to 6.18%, which were lower than the moisture content (7%) of green tea [46].

The fixed samples (5.43–6.93 AU/kg·min) had lower PPO activities compared with the sea buckthorn leaf (23.11 AU/kg·min). The PPO activities of the SF sample and

TABLE 1: Moisture, polyphenol oxidase (PPO), total soluble polyphenol, total flavonoid, isorhamnetin, and water-soluble carbohydrate of sea buckthorn leaf tea.

Sample	Moisture (%)	PPO (AU/kg • min)	Total soluble polyphenol (mg GA/g)	Total flavone (mg RE/g)	Isorhamnetin (mg/g)	Water soluble carbohydrate (%)
SBL	6.09 ± 0.00 ^{cd}	23.11 ± 1.24 ^c	76.34 ± 0.87 ^a	38.27 ± 0.17 ^a	2.40 ± 0.02 ^f	1.79 ± 0.03 ^a
PF	6.16 ± 0.02 ^d	5.47 ± 0.19 ^a	102.82 ± 0.03 ^d	48.10 ± 0.08 ^c	0.73 ± 0.01 ^d	3.31 ± 0.26 ^e
SF	5.73 ± 0.03 ^a	6.81 ± 0.33 ^b	95.45 ± 0.37 ^c	61.09 ± 0.03 ^e	0.65 ± 0.01 ^c	3.13 ± 0.12 ^d
BF	6.18 ± 0.02 ^d	5.93 ± 0.01 ^a	93.19 ± 0.79 ^b	46.07 ± 0.06 ^b	0.34 ± 0.01 ^a	1.95 ± 0.03 ^b
HF	6.10 ± 0.01 ^{cd}	6.93 ± 0.19 ^b	93.11 ± 0.79 ^b	54.26 ± 0.07 ^d	1.01 ± 0.01 ^e	2.81 ± 0.01 ^c
MWF	5.90 ± 0.00 ^b	5.43 ± 0.01 ^a	135.18 ± 0.80 ^f	79.20 ± 0.08 ^f	0.69 ± 0.02 ^c	4.39 ± 0.04 ^g
IRF	5.99 ± 0.02 ^{bc}	5.60 ± 0.02 ^a	115.37 ± 0.75 ^e	92.48 ± 0.31 ^g	0.53 ± 0.02 ^b	4.24 ± 0.01 ^f

Values are means ± SD. Means with different letters in the same column differ significantly ($p \leq 0.05$). SBL: sea buckthorn leaf, PF: pan-firing fixation, SF: steaming fixation, BF: boiling water fixation, HF: hot air fixation, MWF: microwave fixation, and IRF: infrared fixation.

HF sample were slightly higher than that of other fixation samples. The results indicated that fixed was effective in inhibiting PPO activity. Turkmen et al. [47] reported that fresh leaves are immediately steamed at 95–100°C for 35–45 seconds had a particularly good inactivation effect on polyphenol oxidase. Megias–Perez et al. [27] reported that the fixation can inhibit the PPO enzymes.

The total soluble polyphenol contents of fixed samples (93.11–135.18 mg GA/g) were higher than the SBL sample (76.34 mg GA/g), which was consistent with studies by Ma et al. [11]. The total polyphenol content of the MWF sample was the highest, followed by IRF, PF, SF, HF, and BF. This phenomenon may be due to the PPO accelerated the oxidation or breakdown of polyphenols. The total flavonoid contents of fixation samples (46.07–92.48 mg RE/g) were higher than the SBL sample (38.27 mg RE/g), which was consistent with studies by Ma et al. [11]. The total flavonoid content of the IRF sample was the highest, followed by MWF, SF, HF PF, and BF. Guo et al. [48] reported that PPO was responsible for the decline of flavonoids content during oxidation. The results of the BF sample may be due to the total soluble polyphenol and the flavonoids being dissolved in water during fixation in the boiling water [49, 50]. The results of MWF and IRF samples may be due to oxidation or polymerization of phenols caused by heat treatment, and the MWF and IRF can quickly reach high temperature and quickly weaken the PPO activity [51].

The isorhamnetin content of the SBL sample (2.40 mg/g) was higher than those fixed samples (0.34–1.01 mg/g). The content of isorhamnetin in the HF sample was the highest, followed by PF, MWF, SF, IRF, and BF. Ma et al. [11] reported that the glycosides of isorhamnetin were the main flavanol glycosides in the sea buckthorn leaves. Guo et al. [48] reported that polyphenol oxidase plays a major role in the transformation of flavonoid glycosides. Based on the report, we speculate that isorhamnetin glycoside in samples may be hydrolyzed to isorhamnetin under the action of PPO, which was the highest in the SBL sample.

The water-soluble carbohydrate content of fixed samples (1.95–4.39%) was higher than the SBL sample (1.79%). The water-soluble carbohydrate content of the MWF sample was the highest, followed by IRF, PF, SF, HF, and BF. The water-soluble carbohydrate includes soluble polysaccharides and

soluble monosaccharides [52]. For no or relatively poor fixation samples, the enzyme of the sample was activated or partly activated, which facilitated the hydrolysis of proteins into amino acids. Those amino acids would reaction with reducing sugars [53].

3.2. Antioxidant Capacities of the Sea Buckthorn Leaf Tea.

The results of antioxidant capacities for sea buckthorn leaf tea are shown in Table 2. The SF sample (EC_{50} values of DPPH radical scavenging capacity was 103.94 $\mu\text{g}/\text{mL}$) had the strongest DPPH radical scavenging activity, followed by PF, IRF, and MWF (EC_{50} values of DPPH radical scavenging capacity were 132.70–137.59 $\mu\text{g}/\text{mL}$), in which there was no significant difference among those three samples, and the last three were HF sample (EC_{50} value of DPPH radical scavenging capacity was 144.62 $\mu\text{g}/\text{mL}$), BF sample, and SBL sample in order. The IRF sample (EC_{50} values of hydroxyl radical scavenging capacity was 515.85 $\mu\text{g}/\text{mL}$) had the strongest hydroxyl radical scavenging activity, followed by MWF, SF, BF, PF, and HF. The MWF sample (EC_{50} values of nitrite scavenging capacity was 794.97 $\mu\text{g}/\text{mL}$) had the strongest nitrite scavenging activity, followed by SF, PF, BF, IRF, and HF. The reducing powers of the IRF (153.38 $\mu\text{mol AC}/\text{g dw}$) was the highest, followed by MWF, SF, SBL, PF, HF, and BF. Given the different mechanisms of the models used to analyze the antioxidant capacity, the order of the antioxidant capacity of samples under each model is different. Phan et al. [54] reported that different bioactive ingredients play the antioxidant role independently or cooperatively.

3.3. Alpha-Glucosidase Inhibitory Activity of the Sea Buckthorn Leaf Tea.

The α -glucosidase inhibitory activity of sea buckthorn leaf tea is presented in Table 2. The IC_{50} value of α -glucosidase inhibitory activity (77.17 $\mu\text{g}/\text{mL}$) of the IRF sample was the lowest, which had the strongest α -glucosidase inhibitory activity. The α -glucosidase inhibitory activity declined gradually in the order of PF, SF, HF, BF, SBL, and MWF. Kim et al. [55] reported that phenolic fraction from sea buckthorn may have an inhibitory effect on α -glucosidase. Qu et al. [52] reported that polysaccharides show inhibitory effects on α -glucosidase

TABLE 2: Antioxidant capacity, alpha-glucosidase inhibitory activity, and lipase inhibitory activity of sea buckthorn leaf tea.

Sample	DPPH radical scavenging activity (EC_{50} , $\mu\text{g/mL}$)	Hydroxyl radical scavenging ability (EC_{50} , $\mu\text{g/mL}$)	Nitrite scavenging ability (EC_{50} , $\mu\text{g/mL}$)	Reducing power ($\mu\text{mol AC/g dw}$)	α -glucosidase inhibitory activity (IC_{50} , $\mu\text{g/mL}$)	Lipase inhibitory activity (%)	
						IRTF	IRTP
SBL	229.23 \pm 0.16 ^c	706.21 \pm 3.23 ^f	3065.6 \pm 89.02 ^g	106.06 \pm 0.48 ^d	372.62 \pm 20.30 ^f	59.38 \pm 0.01 ^b	55.06 \pm 0.00 ^a
PF	132.70 \pm 0.42 ^b	627.50 \pm 0.19 ^e	975.89 \pm 29.30 ^c	102.66 \pm 0.26 ^c	112.73 \pm 4.01 ^b	92.15 \pm 0.21 ^g	71.56 \pm 0.79 ^d
SF	103.94 \pm 0.00 ^a	573.58 \pm 2.71 ^c	916.0 \pm 20.01 ^b	120.39 \pm 0.15 ^e	165.11 \pm 6.59 ^c	83.72 \pm 0.28 ^f	69.50 \pm 0.14 ^c
BF	165.34 \pm 1.40 ^d	596.09 \pm 0.43 ^d	1078.8 \pm 0.44 ^d	90.55 \pm 0.47 ^a	244.37 \pm 12.61 ^e	73.88 \pm 0.52 ^d	63.30 \pm 1.00 ^b
HF	144.62 \pm 0.38 ^c	700.27 \pm 0.48 ^f	1793.7 \pm 11.20 ^f	97.14 \pm 0.56 ^b	195.68 \pm 14.38 ^d	75.53 \pm 0.54 ^e	71.06 \pm 0.08 ^{c,d}
MWF	137.59 \pm 0.46 ^b	549.42 \pm 7.91 ^b	794.97 \pm 13.22 ^a	133.83 \pm 0.53 ^f	577.56 \pm 0.83 ^g	62.02 \pm 0.02 ^c	75.66 \pm 0.93 ^f
IRF	137.11 \pm 0.72 ^b	515.85 \pm 0.49 ^a	1133.3 \pm 0.68 ^e	153.38 \pm 0.04 ^g	77.17 \pm 3.31 ^a	56.27 \pm 0.69 ^a	55.62 \pm 0.17 ^a

Values are means \pm SD. Means with different letters in the same column differ significantly ($p \leq 0.05$). SBL: sea buckthorn leaf; PF: pan-firing fixation; SF: steaming fixation; BF: boiling water fixation; HF: hot air fixation; MWF: microwave fixation; IRF: infrared fixation.

TABLE 3: Correlation coefficients for the relationship of isorhamnetin, water-soluble carbohydrate (WSC), total flavonoids (TF), total soluble polyphenols (TP), DPPH scavenging activity (DPPHEC₅₀), hydroxyl radical scavenging ability (OH•EC₅₀), nitrite scavenging ability (NO₂•EC₅₀), reducing power (RP), polyphenol oxidase activity (PPO), α -glucosidase inhibitory activity (α GIC₅₀), and lipase inhibitory activity (IRTF and IRTP) of sea buckthorn leaf tea.

Parameter	Isorhamnetin	WSC	TF	TP	DPPHEC ₅₀	OH•EC ₅₀	NO ₂ •EC ₅₀	RP	PPO	α GIC ₅₀	IRTF	IRTP
Isorhamnetin	1.000											
WSC	0.228	1.000										
TF	-0.106	0.859*	1.000									
TP	-0.032	0.808	0.567	1.000								
DPPHEC ₅₀	-0.279	-0.673	-0.811*	-0.268	1.000							
OH•EC ₅₀	0.722	-0.463	-0.747	-0.495	0.384	1.000						
NO ₂ •EC ₅₀	0.611	-0.552	-0.658	-0.758	0.285	0.916*	1.000					
RP	-0.150	0.831*	0.998**	0.536	-0.800	-0.771	-0.664	1.000				
PPO	-0.364	-0.444	-0.319	-0.701	0.669	0.060	-0.207	-0.586	1.000			
α GIC ₅₀	0.041	0.167	-0.115	0.633	0.091	-0.043	-0.417	-0.147	0.736	1.000		
IRTF	0.928*	0.040	-0.197	-0.324	-0.164	0.791	0.806	-0.227	-0.519	-0.306	1.000	
IRTP	0.606	0.337	-0.074	0.537	-0.149	0.327	-0.031	-0.127	0.332	0.815*	0.280	1.000

* $p \leq 0.05$ and ** $p \leq 0.01$.

activity. The relationship was not found in our study. For the result that the MWF sample had the weakest α -glucosidase inhibitory activity, we speculated that may be due to polysaccharides which are not the main component of water-soluble carbohydrates.

3.4. Lipase Inhibitory Activity of the Sea Buckthorn Leaf Tea.

The lipase inhibitory activity of sea buckthorn leaf tea was evaluated in two ways, one of which was that the samples contained the same amount of total flavonoids (IRTF), and another was the samples contained the same amount of total soluble polyphenol (IRTP). The results are presented in Table 3. The IRTF of most fixed samples (62.02–92.15%) were stronger than that of the SBL sample (59.38%). In the fixed samples, the IRTF of PF was the highest, followed by SF (83.72%), HF (75.53%), BF (73.88%), MWF (62.02%), and IRF (56.27%) which had the lowest IRTF. IRTF was positively correlated with the contents of isorhamnetin ($r = 0.928$, $p \leq 0.05$, Table 3). The IRTP of fixed samples (55.62–75.66%) were stronger than that of the SBL sample (55.06%). In the fixed samples, the IRTP of MWF was the highest, followed by PF (71.56%), HF (71.06%), SF (69.50%), BF (63.30%), and IRF. IRTP was positively correlated with the contents of isorhamnetin ($r = 0.606$, $p \leq 0.05$, Table 3). The results show that isorhamnetin is responsible for lipase inhibitory activity. The literature reported that isorhamnetin possessed lipase inhibitory activities [56, 57].

3.5. Correlations between Compounds and Antioxidant Capacities. The relationship between components and antioxidant capacities is shown in Table 3.

There was a negative correlation between the DPPH radical scavenging capacity (EC₅₀) with the total flavonoids content and the water-soluble carbohydrate content ($r = -0.811$, $p \leq 0.05$ and $r = -0.673$, $p \leq 0.05$, respectively, Table 3). The hydroxyl radical scavenging capacity (EC₅₀) and content of the total flavonoids had a negative correlation ($r = -0.747$, $p \leq 0.05$, Table 3). The negative correlation existed between the nitrite scavenging activity and content of

the total soluble polyphenols, total flavonoids, and water-soluble carbohydrates with their related coefficients of -0.758 , -0.658 , and -0.552 , respectively. Reducing power was positively correlated with the contents of total flavonoids, water-soluble carbohydrate, and total soluble phenolic ($r = 0.998$, $p \leq 0.01$, $r = 0.831$, $p \leq 0.05$, and $r = 0.536$, $p \leq 0.05$, respectively, Table 3). Those results suggested that flavonoid, total soluble polyphenols, and the water-soluble carbohydrate had a greater contribution to the antioxidant capacity. The literature [58, 59] reported that the phenolic content and the flavonoids content are related to antioxidant capacity. The results suggested that the flavones play a major role in hydroxyl radical scavenging activity.

The results suggested that the soluble polyphenols play a major role in the nitrite scavenging activity.

3.6. Principal Component Analysis (PCA). Principal component analysis (PCA) gives the score results based on the correlation matrix of the data as shown in Figures 1(a)–1(c). The variance explained by principal components 1, 2, and 3 were 43.7%, 21.1%, and 19.0%, respectively. From Figure 1(a), principal component 1 (PC₁) was mainly related to reducing power, total flavonoid content, EC₅₀ of HO•, water-soluble carbohydrate (WSC), total soluble polyphenol content, and IRTF, and the related coefficient was 0.757 to 0.969. PPO and α -glucosidase inhibitory activity were related to principal components 2 (PC₂). Principal components 3 (PC₃) was mainly affected by IRTP, EC₅₀ of DPPH, and isorhamnetin content. It can be seen from the PC scoring chart in Figure 1(b) that there were two categories. Figure 1(c) showed the comprehensive principal component value, which was calculated according to the principal component comprehensive model. The first category included (Figure 1(c)) the IRF sample, MWF sample, and SF sample. The second group included the PF sample, BF sample, and HF sample, with a negative score (Figure 1(c)). This result suggested that the IRF, MWF, and SF had a similar effect on the principal components, while they were significant different from the PF, BF, and HF.

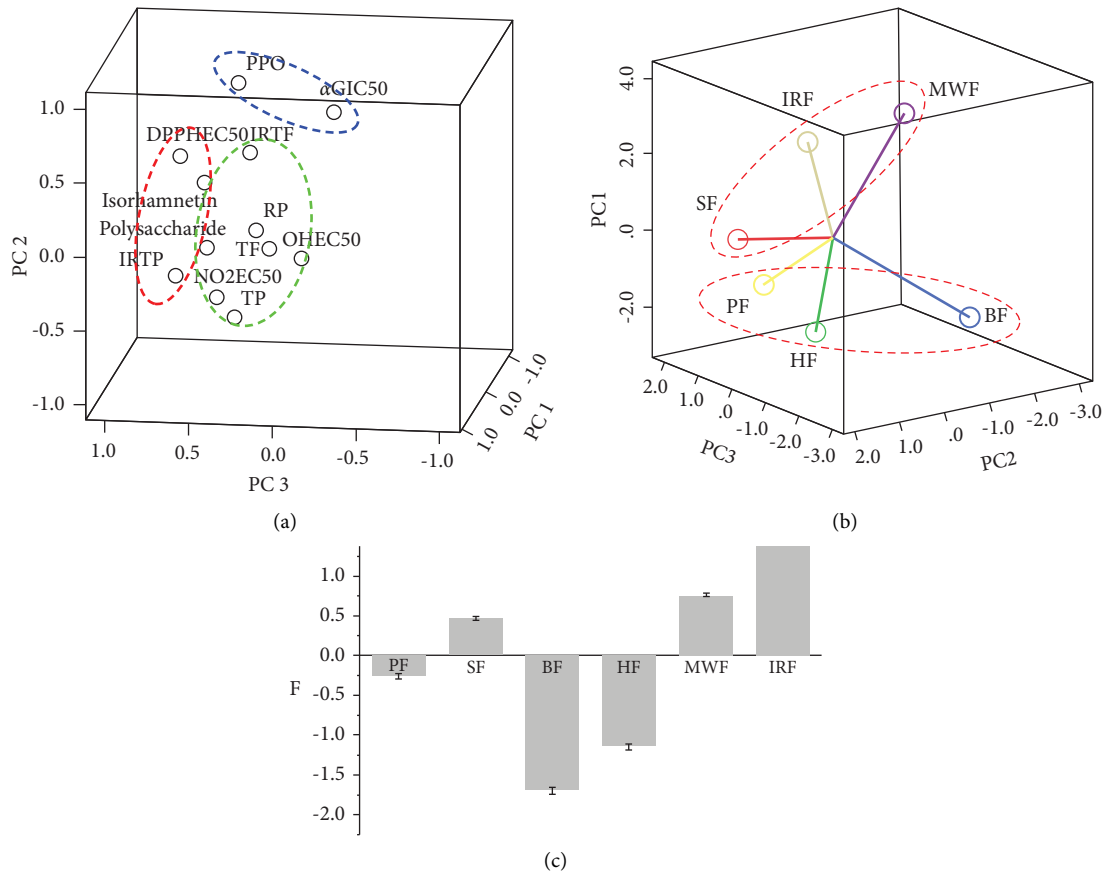


FIGURE 1: Principal component analysis (PCA) of sea buckthorn leaf tea prepared by different fixation methods. (a) Scores scatter plot of PCA, (b) loadings plot of PCA, and (c) scores of histogram of PCA.

4. Conclusions

In conclusion, significant differences in main components, α -glucosidase inhibitory activity, lipase inhibitory activity, and antioxidant capacity were observed among the different fixed sea buckthorn leaf tea. Among the fixed methods tested, IRF, MWF, and SF were better than others for fixing sea buckthorn leaf tea, which had higher contents of most of the main components, antioxidant capacity, α -glucosidase, and lipase inhibitory activity. Combined with PCA analysis, the score of IRF, MWF, and SF was higher. The IRF and MWF were the best modern methods to replace traditional methods, while the MWF had the advantages of short time and high efficiency compared with the IRF. Therefore, the MWF was the proper method for sea buckthorn leaf tea fixed. The results of this study also can be referential for fixed methods and consumption of sea buckthorn leaf tea. [60].

Data Availability

The data that support the findings of this study are available from the corresponding author, upon reasonable request.

Conflicts of Interest

The authors declare that they have no conflicts of interest.

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Supplementary Materials

Supplementary data to Section 2.1 (Table S1) of this article can be found online. (*Supplementary Materials*)

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