Journal of Food Quality

Green Physical Processing Technologies for the Improvement of Food Quality

Lead Guest Editor: Daming Fan
Guest Editors: Hui-Min D. Wang, Srinivas Janaswamy, and George A. Cavender

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Editorial Board

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Editorial

Green Physical Processing Technologies for the Improvement of Food Quality

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With the rapid developments and burgeoning technologies in the food industry, the green, efficient, and fast-oriented physical processing tools are of immense interest to industrialists and academicians. They indeed reduce the environmental pollution and energy consumption. The pursuit of these novel arsenals is coupled with auxiliary strategies immensely to maintain the food quality attributes such as nutrients, texture, color, and freshness. Furthermore, they influence the food composition and structure, including the flavor, physical properties and functions, and in turn the product quality. However, there is an unmet need to clarify the intrinsic interactions between the food quality and physical processing. At this critical juncture, this special issue provides the required platform for the researchers to showcase their current efforts on the applications of physical processing technologies for improved food applications.

L. Zhang et al. investigate the influence of steaming and cooking on the phenolic content and antioxidant activity of millet and provide a desirable processing means for millet. They further optimized the processing conditions to improve the retention rate of total phenolic content (TPC). D. Tang et al. tackled the optimal conditions of heat treatment (HT) and HT coupled with preservative treatment for Ponkan fruit (*Citrus reticulata* Blanco cv. Ponkan) storage. Y. Xie et al. evaluate the effect of sodium acid sulfate (SAS) and UV-C treatment on the fresh-cut potatoes during storage. Q. Liang et al. illuminate the effect of low-frequency ultrasound on the enzymolysis of corn protein with the degree of hydrolysis (DH), conversion rate of protein (CR), and IC_{50} value of DPPH as analytical indicators. S.-Q. Tian et al. address the effect of sweeping frequency ultrasound pretreatment on the angiotensin converting enzyme (ACE) inhibitory activity of zein hydrolysates and enzymolysis thermodynamics. S. Deng et al. present the high-voltage electric field thawing characteristics of the frozen tofu. L. Chen et al. address the chemical compositions and bioactive substances of Xinjiang maca and evaluate its antifatigue activity using a mouse model. T. Li et al. present the effect of overdrying the potato starches on surimi products, which includes the change of chemical interactions and chemical composition. I. Boublenza et al. compare the physicochemical parameters, antioxidant activity, lipid composition, and sensory analyses of initial and roasted carob pod powder obtained at different roasting temperatures. L.-L. Huang et al. reveal the phenomenon that Feizixiao lychee has the higher density compared to Guiwei lychee and Nuomici lychee. Z. Yin et al. established an HPLC method for simultaneous determination of quercitrin, 3-hydroxy phloridzin, and phloridzin in *M. halliana* tea. The nutritional compositions were determined in accordance with the relevant national standards and methods. Y. Chen et al. demonstrate the antioxidant activity of five tea extracts with a potential application in cancer treatment.

We strongly believe that this special issue provides the required impetus in developing novel green and clean processing tools that are economical to address the growing human population demands for well-balanced and healthy foods.

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Research Article

Antioxidative and Antimelanoma Effects of Various Tea Extracts via a Green Extraction Method

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Tea *(Camellia sinensis)* contains high level of antioxidant elements and is a well-known beverage consumed worldwide. The purpose of this study is to compare different concentrations of green tea, black tea, oolong tea 861, oolong tea 732, and jasmine green tea. These five types of tea extracts were known to have antioxidative properties, reducing power, and metal ion chelating activity. The current study compared these five extracts in terms of their inhibiting effects on human malignant melanoma: A2058 and A375. To determine the cell viability between normal cell and malignant melanoma cells, an MTT assay was applied to evaluate the cytotoxic potential on human melanoma cells, with all tea extracts showing decreased cell viability with increasing tea extract concentrations. Cytotoxicity on HaCat (normal skin cells) showed no effect on the cell viabilities at lower concentrations of the tea extracts. These results suggest the antioxidative effect of five tea extracts that protect against oxidation and melanoma production, with green tea and jasmine green tea showing the lowest cell viability when tested against malignant melanoma cells.

1. Introduction

Antioxidative compounds derived from natural plant species may help keep human skin at a physiologically healthy state by decreasing oxidative stress [1]. By reducing oxidative stress from reactive oxygen species (ROS), antioxidative compounds can prevent ROS-induced skin damage [2]. Oxidation produces free radicals that may lead to chain reactions, which can potentially lead to cell damage. To detect the free radical scavenging ability of antioxidants, 1,1-diphenyl-2-picrylhydrazyl (DPPH) is commonly used [3]. Different types of tea extracts were produced to investigate their respective antioxidant activity. Chelating capacity involves a central metal ion attached to a large molecule, which is a ligand, and forms a ring structure. The ferric reducing potential assay is another way to analyze antioxidative properties, which involves the quantification of the reducing ability of antioxidants in reaction with a 2,4,5-tripyridyl-S-triazine Fe(III) (TPTZ) complex that forms a ferrous Fe(II)-TPTZ complex with a dark blue color [4]. A2058 is a highly invasive cell line from a 43-year-old male patient with malignant melanoma. A375 is cell line from a 54-year-old female patient with malignant melanoma. HaCaT cells are immortalized human keratinocytes, which are used for the study of epidermal homeostasis [5].

Melanoma typically starts from melanocytes, which are the cells that produce melanin. Melanoma often originates from melanocytes within moles on the skin during early stages. However, when treatments are ineffective and melanoma cells spread rapidly, metastatic melanoma occurs.

The ultraviolet (UV) rays are one of the known causes of melanoma [6]. Our skin has the ability to protect us from exterior factors, controls body temperature, and stores water and fat. However, skin cancer happens during extended exposure of sunlight, leading to cancerous benign or malignant tumors, which is now a major cause of death among patients [7].

Tea is a well-known beverage across the world. Tea polyphenols, which are antioxidative, includes epigallocatechin (EGC), epicatechin (EC), epigallocatechin gallate (EGCG), and epicatechin gallate (ECG). EGCG is the most abundant catechin within a tea leaf, which accounts for 30% of the total polyphenol in dried leaves of green tea, from subjected to steaming or pan frying to inactivate endogenous polyphenol oxidase (PPO) [8]. Green tea normally has higher antioxidant potential than oolong tea or black tea, due to its high EGCG and EGC content. Black tea also has antioxidant properties, with the ability to scavenge free radical, inhibit lipid peroxidation, and chelate metal ions [9]. Gallic acid is one of the key components of black tea, with the ability to counter cancer cells through its cytotoxic activity [10]. Oolong tea, a semifermented 84 tea, contains high amount of tea polyphenols and strongest antioxidant activity [11]. Jasmine tea is a variation of green tea, with an extra step of jasmine flower scent transfer process, as compared to typical green tea. Jasmine tea can reduce the esophageal tumor burden, but epidemiological studies are lacking [12].

2. Materials and Methods

2.1. Chemicals and Reagents. Ascorbic acid (Vitamin C), ethylenediaminetetraacetic acid (EDTA), L-3,4-dihydroxyphenylalanine (L-DOPA), dimethyl sulfoxide (DMSO), 1,1-diphenyl-2 picrylhydrazyl (DPPH), ethanol, ferrouschloride ($FeCl_2 \cdot 4H_2O$), ferric chloride ($FeCl_3$), kojic acid, methanol, potassium ferricyanide ($K_3Fe(CN)_6$), 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT), 3-tert-butyl-4-hydroxyanisole (BHA), and L-tyrosine were purchased from Sigma-Aldrich Company (St. Louis, MO, USA). Dulbecco's modified Eagle's medium (DMEM) and fetal bovine serum (FBS) were obtained from Gibco BRL (Gaithersburg, MD, USA).

2.2. Tea Extracts Process and Preparation. All five tea extracts were prepared with similar procedures. Green tea leaves were subject to panning at 280–300°C for 5-6 min [13]. Jasmine green tea leaves, black tea leaves, and oolong tea leaves were naturally dried indoors under ambient temperature (indoor wilting). Oolong tea leaves were then moved into a bamboo basket for tossing and turning to bruise the leaves. The leaves were repetitively rolled and dried by a CTC (cut, tear, curl) machine. The roasted oolong tea 732 was subject to an additional step of roasting at 100–120°C for 4 hours to produce a roasted flavor. Black tea was rolled by a CTC machine, which serves to bruise and damage the leaves, followed by oxidation of the tea polyphenols (tea fermentation) at 40°C for 2-3 hours. Final drying of all five tea leaves was at 80–120°C. All five tea leaves were extracted with hot water at 85–95°C, using 5-liter hot water per kg of tea leaf, to produce a broth of 5% solid content, which was filtered by using 10-micron PP filter bag. After vacuum concentration, the concentration was increased to 20% w/v. Maltodextrin was added to achieve an extract: maltodextrin ratio of 2 : 1 (20% tea soluble content, 10% maltodextrin w/v). The tea concentrates were then frozen at −35°C, followed by lyophilization for 72 hours (0–50 hours at below 0°C, 50–72 hours increasing to 45°C) and pulverization to produce a fine tea extract powder.

2.3. Assays of Antioxidant Effects

2.3.1. DPPH Radical Scavenging Activity Assay. The DPPH assay is an antioxidant assay to monitor the free radical scavenging ability of antioxidants, where a stable free radial shows a deep violet color [14]. DPPH solution will change into bright yellow when it encounters a free radical scavenging compound. The 1, 5, 10, 50, and 100 (μg/ml) of the tea extract were added to the DPPH solution. The reaction of DPPH with an antioxidant releases a hydrogen, which leads to a decreased absorbance value at 517 nm and a bright yellow color, corresponding to a high DPPH scavenging ability. Vitamin C was used as positive control. The formula to calculate free radical scavenging activity is as in the following equation:

$$\text{Scavenging activity (\%)} = \frac{\left(\text{OD}_{\text{control}} - \text{OD}_{\text{sample}}\right)}{\text{OD}_{\text{control}}} \times 100\%. \quad (1)$$

2.3.2. Metal Chelating Activity Assay. The chelating property of ferrous ion (Fe^{2+}) was tested. Different concentrations ranges of the tea samples were dissolved in DMSO, with the addition of 10 μL solution of $FeCl_2 \cdot 4H_2O$ (2 mM). Twenty μL of ferrozine was then added and the mixture blended for 10 minutes [15]. Upon reaction, the absorbance at 562 nm was monitored. EDTA was used as positive control. The formula to calculate metal chelating activity was similar to (1).

2.3.3. Reducing Power Assay. For assays of the reducing power ability of the extract, different concentrations ranges of each tea extract were mixed with 85 μL of 67 mM sodium phosphate buffer (pH 6.8) and 2.5 μL of 20% $K_3Fe(CN)_6$ to investigate the reducing powers of the extracts. The mixture was kept at 50°C for 20 min and centrifuged for 10 min at 3,000g. The supernatant was then mixed with 2% $FeCl_3$ and the absorbance was measured at a wavelength of 700 nm with a BHA solution as positive control [7]. High absorbance values correspond to a high capacity for metal ion reduction.

2.4. Cell Line Cultures. Human melanoma cell lines were obtained from Bioresource Collection and Research Center (Taiwan): A2058 (BCRC number 60039), A375 (BCRC number 60263), HaCat (human skin keratinocytes) were cultured in Keratinocyte-SFM (Gibco, USA) supplemented with bovine pituitary extract and human recombinant epidermal growth factor [16].

Table 1: Five different concentrations with DPPH scavenging capacity of five tea extracts.

	DPPH scavenging capacity (%)				
Extracts (μg/ml)	1	5	10	50	100
Maltodextrin	≤5.00	≤5.00	≤5.00	≤5.00	≤5.00
Green tea extract[a]	56.12 ± 0.93	86.04 ± 0.12	84.87 ± 6.49	95.07 ± 0.00	94.81 ± 0.57
Black tea extract[a]	52.83 ± 3.53	85.56 ± 0.15	84.74 ± 2.47	94.75 ± 0.07	94.62 ± 0.21
Oolong tea extract 861[a]	52.85 ± 2.47	87.35 ± 5.44	91.83 ± 0.28	94.62 ± 0.35	93.51 ± 1.84
Oolong tea extract 732[a]	46.13 ± 1.01	42.67 ± 3.30	43.02 ± 0.58	74.60 ± 0.28	66.28 ± 0.99
Jasmine green tea extract[a]	55.43 ± 1.80	90.88 ± 0.06	82.36 ± 6.10	94.49 ± 0.49	94.68 ± 0.00
Vitamin C[b]			80.82 ± 0.00		

[a]Sample consists of 33% maltodextrin. [b]Vitamin C was used as a positive control on DPPH assay at 100 μM.

2.5. MTT Assay. The cell viability was determined with an MTT assay [17]. Cells were plated at a density of 8×10^3 cells/well in a 96-well plate and incubated for 24 hours before the additions of the 1, 10, and 50 (μg/ml) of the tea extracts, after which an MTT solution was added to each well. Culture medium was then discarded and DMSO added to each well. The absorbance of the formazan salt at 595 nm was measured, and cell viability was calculated as in the following equation:

$$\text{Cell viability (\%)} = \frac{\text{OD}_{\text{sample}}}{\text{OD}_{\text{control}}} \times 100\%. \quad (2)$$

3. Results

3.1. DPPH Free Radical Scavenging Activity Assay. The DPPH assay aims to detect free radical scavenging ability of antioxidants. The absorbance at 517 nm was monitored when the DPPH radical is neutralized, as the solution turns from blue violet to light yellow in color. Lighter color indicates better antioxidant ability. Table 1 illustrates the results for five different tea extracts, with Vitamin C as the positive control and maltodextrin as the negative control. In general, the higher the tea extract concentration, the higher the percentage of DPPH scavenging capacity. However, for all five tea extracts, 50 μg/ml tends to show higher or equivalent antioxidant ability than 100 μg/ml. The comparison showed that green tea had the biggest radical scavenging effect. Oolong tea extract 861 is significantly more antioxidative than oolong tea extract 732, which may be due to their difference in processing.

3.2. Metal Chelating Activity Assay. In an oxidative environment, ferrozine forms complexes with Fe^{2+}. When the chelating materials are present, this complex construction is dislocated. When Fe^{2+} and ferrozine reacts together at 562 nm, it reflects a higher chelating ability. Five different tea extracts had Fe^{2+} scavenging activities at a concentration range of 1–100 μg/mL. Oolong tea extract had the highest value of 30 ± 2.0 at 100 μg/mL. Jasmine green tea showed the highest chelating capacity of 18 ± 0.03 at 10 μg/mL. Compared to the others, it has the lowest level of concentration needed to achieve a higher percentage of chelating capacity.

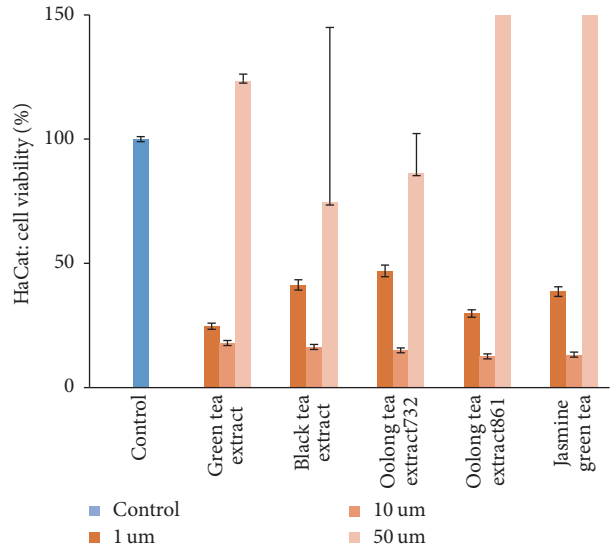

Figure 1: Effects of five extracts on cell viability of normal human cell and malignant melanoma cell according to MTT assay. Percentage of cell viability was measured in HaCat cultured with 1 μm, 10 μm, and 50 μm.

3.3. Reducing Power Assay. The ferrous ion chelating activity of tea extracts was investigated and shown in Table 3. During this assay, the reducing properties of the tea extracts were determined by monitoring their change in solution color, which turns from light yellow to blue violet when Fe(II)-TPTZ complex forms. Darker color represents better reducing power. Table 3 showed that the reducing power of black tea extract and oolong tea extract 861 was 2.83 ± 0.51 at 50 μg/mL and 2.93 ± 0.15 at 50 μg/mL, respectively. According to the results, the reducing power decreased as the concentration reaches 100 μg/mL.

3.4. MTT Assay. The effect of the tea extracts on cell development was estimated with the MTT assay, which is a colorimetric assay for assessing cell viability. This assay is based on the dehydrogenase from the cell line that changes from light yellow color of MTT's tetrazolium into a blue color of MTT formazan. From Figure 1, it can be seen that, at a concentration of 1 μm and 10 μm, the HaCat viability

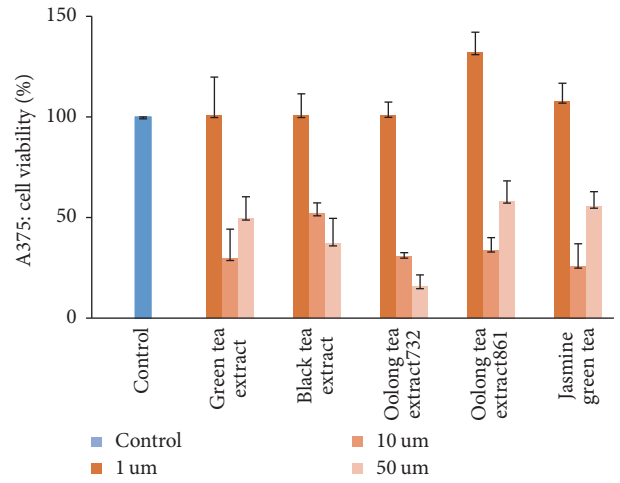

Figure 2: Effects of five extracts on cell viability of normal human cell and malignant melanoma cell according to MTT assay. Percentage of cell viability was measured in A2058 cultured with 1 μm, 10 μm, and 50 μm.

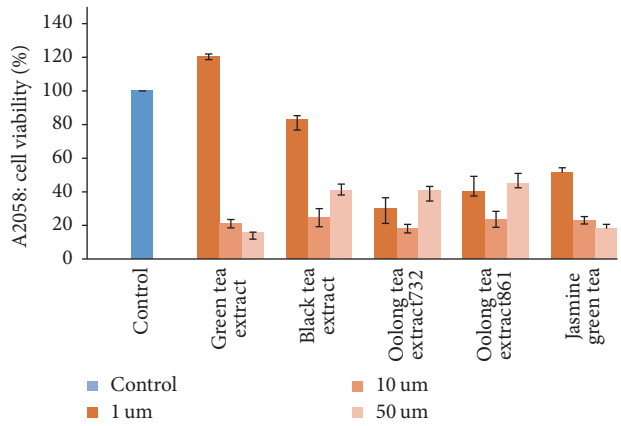

Figure 3: Effects of five extracts on cell viability of normal human cell and malignant melanoma cell according to MTT assay. Percentage of cell viability was measured in A375 cultured with 1 μm, 10 μm, and 50 μm.

showed the same pattern among the five tea extracts tested. As seen in Figure 2, the five tea extracts on A375 cell line show similar viability pattern as compared to that of the A2058 cell line in Figure 3, as both are malignant melanoma cells. The black tea extract and the oolong tea extract 732 on A375 show dose-dependent decrease in viability, while the green tea extract and the jasmine green tea extract show dose-dependent decrease in A2058 cell viability.

4. Discussion

Tea is a popular traditional beverage and is known for its physiological functions such anticancer and antioxidative power. In this study, tea extracts made from the leaves of *Camellia sinensis* were analyzed; these were green tea, black tea, oolong tea, and jasmine green tea. The current study investigated the antioxidative effect of five different tea extracts by determining the DPPH scavenging capacity, chelating activity, and reducing power. The investigation determined the antioxidative power and included Vitamin C as the positive control and maltodextrin as the negative control, to compare with different types of tea at various concentrations. It was found that high concentrations of tea extracts had a profound impact on human cell. All five types of tea extracts showed an increased percentage of DPPH scavenging capacity and chelating activity. Oolong tea extract 732 was made with an additional high temperature roasting step, as compared to the oolong tea extract 861. Thermal processing may degrade catechins, which may be the cause of the lower DPPH scavenging capacity of oolong tea extract 732.

In this study, all tea extracts at a concentration of 50 μg/mL showed the highest capacity of scavenging DPPH (Table 1). In fact, tea polyphenols are represented as the main antioxidants in teas [18]. The five tea extracts were further analyzed by investigating their chelating activity. Oolong tea extract 861 had the highest chelating value 30 ± 2.0 at the highest concentration tested (100 μg/ml) (Table 2). However, all of the tea extracts showed the highest reducing power at 50 μg/mL (Table 3). This pattern was similar to the DPPH scavenging capacity as seen in Table 1 [19, 20].

Melanoma, a malignant tumor, begins in a specific type of skin cell and activates when the abnormal cells affect part of the body and proliferates uncontrollably [21]. The cytotoxic potential of the five tea extracts on two different melanoma cells (A2058 and A375) as well as on HaCat cells as a noncancerous cell line was also investigated [22] using the MTT assay. These cell lines were treated with various concentrations (1, 10, and 50 μm) of the tea extracts for comparison. For HaCat cells, the five tea extracts at 50 μm had minor effects, and all cellular viabilities exceeded 65% after a 24-hour treatment. Thus, the tea extracts had no toxic effects on noncancerous human cells. Both A375 and A2058 are compromised, highly invasive melanoma cell lines [23]. All five tea extracts at 10 μm and 50 μm showed cytotoxic potential on both human melanoma cells tested. Nonthermal processing of tea may affect the bioactivity of the tea products, so the improvement in food quality may be achieved by adapting a green physical processing.

5. Conclusion

In summary, the experiments in this study demonstrated that the antioxidant activity was confirmed through DPPH free radical activity, chelating activity, and reducing power of the five tea extracts. Higher concentration of the tea extracts could potentially become a cancer treatment. The results suggested all five tea extracts investigated are promising natural antioxidants and potential antimelanoma agents, without toxicity against noncancerous human cells.

Conflicts of Interest

The authors have no conflicts of interest regarding the publication of this study.

Table 2: Five different concentrations with chelating activity of five tea extracts.

	Chelating activity (%)				
Extracts (μg/ml)	1	5	10	50	100
Maltodextrin	≤5.00	≤5.00	59 ± 0.01	76 ± 0.01	79 ± 0.00
Green tea extract[a]	≤10	≤10	≤10	≤10	14 ± 1.0
Black tea extract[a]	≤10	≤10	20 ± 0.03	19 ± 0.01	25 ± 3.0
Oolong tea extract 861[a]	≤10	≤10	≤10	19 ± 0.01	30 ± 2.0
Oolong tea extract 732[a]	≤10	≤10	≤10	≤10	27 ± 1.0
Jasmine green tea extract[a]	≤10	≤10	18 ± 0.03	≤10	≤10
EDTA[b]			80.76 ± 0.01		

[a]Sample consists of 33% maltodextrin. [b]EDTA was used as a positive control on metal chelating ability at 100 μM.

Table 3: Five different concentrations with reducing power of five tea extracts.

	Reducing power (OD 700)				
Extracts (μg/ml)	1	5	10	50	100
Maltodextrin	≤5.00	≤5.00	≤5.00	≤5.00	≤5.00
Green tea extract[a]	0.35 ± 0.02	0.30 ± 0.02	0.25 ± 0.01	2.38 ± 0.41	2.046 ± 0.17
Black tea extract[a]	0.26 ± 0.04	0.23 ± 0.02	0.23 ± 0.02	2.83 ± 0.51	2.71 ± 0.24
Oolong tea extract 861[a]	0.29 ± 0.06	0.25 ± 0.06	0.36 ± 0.02	2.93 ± 0.15	2.44 ± 0.20
Oolong tea extract 732[a]	0.19 ± 0.00	1.65 ± 0.01	0.23 ± 0.01	2.69 ± 0.45	2.60 ± 0.24
Jasmine green tea extract[a]	0.28 ± 0.01	0.32 ± 0.03	0.29 ± 0.02	2.73 ± 0.57	2.46 ± 0.05
BHA[b]			0.56 ± 0.03		

[a]Sample consists of 33% maltodextrin. [b]BHA was used as a positive control on reducing power at 100 μM.

Authors' Contributions

Yihui Chen, Jyun-Yin Huang, Yichi Lin, I-Fan Lin, Yi-Ru Lu, Li-Heng Liu, and Hui-Min David Wang conceived and designed the experiments; Yihui Chen performed the experiments and analyzed the data; I-Fan Lin contributed reagents, materials, and analysis tools; Jyun-Yin Huang, Yichi Lin, I-Fan Lin, Yi-Ru Lu, Li-Heng Liu, and Hui-Min David Wang wrote the paper. Yihui Chen and Jyun-Yin Huang contributed equally to this work.

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Research Article

Analysis for Difficulty during Freeze-Drying Feizixiao Lychees

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Compared to other cultivar lychees, volume density of Feizixiao lychee was higher due to serious shrinkage during freeze-drying (FD). Guiwei lychee and Nuomici lychee were used for comparison in order to illuminate the reason of the aforementioned phenomenon. Lower prefreezing temperature could not improve the volume density of Feizixiao lychee. Microstructure results show that pulp cell of Feizixiao lychee (tail) was smaller and more compact than Guiwei and Nuomici lychee pulp cell. In addition, there is a membrane around the surface of Feizixiao lychee pulp. And the microstructure of Feizixiao lychee tip pulp cell is different from tail pulp cell. Membrane and tip pulp cell are both smaller and more compact than tail pulp cell. These structure differences hinder the moisture removing of Feizixiao lychee during FD. Removing the membrane and tip pulp could not improve the volume density of Feizixiao lychee. Ultrasound treatment for 30 min could significantly enhance the volume density of Feizixiao lychee.

1. Introduction

Lychee (*Litchi chinensis* Sonn.), one of the major fruits in South China, is liked for its characteristic sweet acidic taste, excellent aroma, high nutrient value, and the attractive deep bright red color of the pericarp [1, 2]. Shenzhen Nanshan lychees, including Feizixiao, Guiwei, and Nuomici, were recognized as product of geographical indication in 2006. Lychees are a seasonal fruit. At present, lychees are commonly precooled first and then put together with ice bag during transport in order to keep the original quality of lychees. In addition, fresh-keeping agent coating and soaking are also common methods for lychee preservation [3–5]. So far, the storage life of fresh lychees barely exceeds one month even using the best preservation method according to previous studies. Therefore, the processing and preservation of lychees are very imperative.

Now, dried lychees account for 80% of all lychee processing products [6], where air dried lychees are the major. Air dried lychee pulp is brown with serious shrinkage, which limit the application of air dried lychees. Moreover, most air dried lychees are whole lychees and they need to be peeled and have their stones removed before using. Lychee pulp after air drying is tightly bound to kernel and the pulp is sticky due to high sugar content and 25–30% moisture content, which make the kernel removing difficult. In recent years, microwave was applied to dry lychees, including microwave air drying and microwave vacuum drying [1]. Microwave using could decrease the energy consumption and increase the quality of product [7]. However, these drying methods with microwave were not applied widely due to nonuniformity of microwave and other equipment problems. It is well known that freeze-drying (FD) can maintain the maximum original quality of materials [8, 9]. It is found that Feizixiao lychee showed serious shrinkage during FD but Guiwei and Nuomici lychee could be freeze dried well. This was an interesting phenomenon and deserved further research.

A successful FD process retains the volume of the material. There are many reasons which affect the volume of samples during FD, like prefreezing temperature, heating plate, sugar content of materials, and so forth. Drying temperatures below the temperature of glass transition during heating allow removal of ice within the solid. But temperatures above ice melting temperature can result in collapse [10]. The structure of materials containing sugars may collapse during dehydration if the temperature of ice within the material is higher than the collapse temperature [11]. Lychee pulp contains high sugars including sucrose, fructose, and glucose [12, 13] and the sugar content is different with lychee

cultivar. There is a close relationship among these influence factors. Sugar content decides the prefreezing temperature and prefreezing and heating plate temperature affect the drying temperature of materials during FD. Differential scanning calorimetry (DSC) can be used to measure eutectic point temperature (T_g) of materials [14]. Prefreezing temperature should be confirmed according to T_g.

Microstructure of food could reflect the macroscopic quality of food [15]. Macroscopic shrinkage of materials essentially resulted from breaking and collapse of cell structure [16–18]. Huang et al. reported that lychee pericarp consisted of three parts: outer layer with cuticle, interlayer, and inner layer. Inner layer cells are small and intact, which hinder the moisture removing during drying. It shows that cell structure of materials could significantly affect the drying process. Although peel lychee was used during FD, there may be significant differences about cell structure of lychee pulp among different cultivars. In fact, there is a film around the surface of Feizixiao lychee pulp but not Guiwei and Nuomici lychee. Moreover, there is residue in mouth after eating Feizixiao lychee pulp, especially tip pulp. But Guiwei and Nuomici lychees have no residue. These macroscopic differences may result from microstructure differences of lychee pulp with different cultivar. Therefore, it is necessary to analyze and compare the microstructure of three cultivar lychees' pulp.

In this research, authors not only clarify the difficulty of Feizixiao during FD, but also find out the effective method to resolve the problem. Ultrasound is usually used in food process to improve the quality of food, such as ultrasound-assisted freezing and ultrasound pretreatment. Ultrasound can enhance nucleation rate and crystal growth rate and improve the physicochemical properties of freezing red radish [19]. Ultrasound pretreatment can enhance the glass transition temperature of pear during FD [20]. The more the ultrasonic power application, the higher the glass transition temperature. It indicated that ultrasound pretreatment prior to freeze-drying can improve the efficiency of FD and the stability during storage of FD pear. Ultrasound pretreatment did reduce drying time by 13–17%, increased the lightness, decreased the brown pulp, and improved the rehydration properties of dried apple [21].

The objectives of this work were to analyze the difficulty of Feizixiao during FD compared with Guiwei and Nuomici lychee, to improve the FD Feizixiao lychee quality by ultrasound pretreatment.

2. Materials and Methods

2.1. Samples. Fresh lychee fruits (*Litchi chinensis* Sonn.) cv. Feizixiao, Nuomici, and Guiwei at commercially mature stage were picked from Xili orchard, Shenzhen, China (east longitude: 113°56′01.16″, north latitude: 22°36′15.26″). Mature lychee fruits that were free from visible blemish or disease were selected. Every lychee fruit weight was about 25 g. The average sizes (horizontal × vertical) of Feizixiao, Nuomici, and Guiwei lychees were 37.48 mm × 38.20 mm, 38.00 mm × 35.77 mm, and 36.91 mm × 35.64 mm. The picture of three fresh lychees was shown in Supplementary Materials. Lychees were washed, peeled, and pitted and then every lychee pulp was divided into two parts before prefreezing.

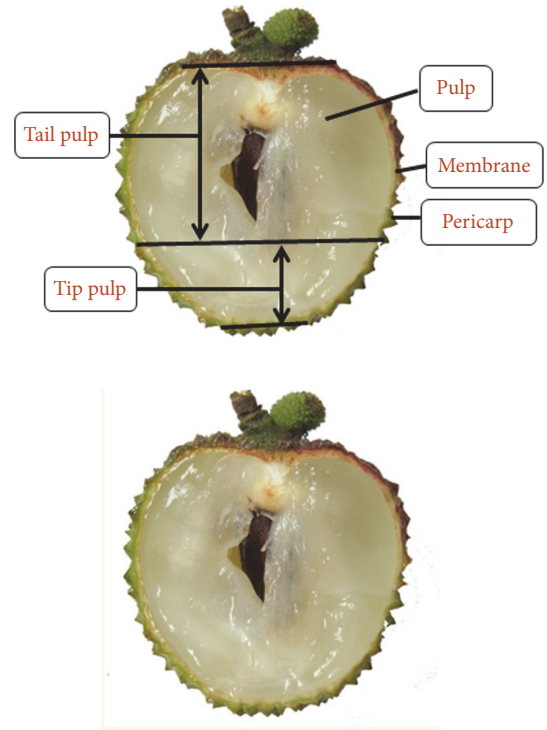

Figure 1: The schematic diagram of different part in Feizixiao lychee.

2.2. Equipment and Drying Experiment. The lab-scale vacuum freeze dryer was made by Alpha 1–4 LSC freezer (Marin Christ Company, Osterode, Germany). The minimum absolute pressure of freeze dryer is 1 Pa and the lowest temperature of cold trap is −55°C. The temperature of heating plate during the second stage was 60°C. Lychees were dried by FD until the final moisture content was less than 10% (wet basis).

Two pieces of freezing equipment were used for prefreezing in this study. One was freezer with the −30°C (Kelen business equipment Co. Ltd., Shenzhen, China) and another was −80°C ultra-low temperature freezer (EXF32086V, Thermo Co. Ltd., Waltham, MA, America).

2.3. Ultrasound Treatment. An ultrasound-microwave synergistic extraction apparatus (CW-2000, Shanghai Xintuo Analytical Instrument Co. Ltd., Shanghai, China) was applied for lychee pulp. Only ultrasound with 40 kHz and 50 W was used during experiment. The ratio of lychee pulp to water was 1 : 4 and the experiment temperature was 30°C. Different treatment time (10 min, 20 min, and 30 min) was studied. Lychees that were washed, peeled, and pitted with every lychee pulp divided into two parts were control group. For some special samples, membrane was moved by hand after being peeled and tip pulp was cut by knife after being pitted. The schematic diagram of different part in Feizixiao lychee was shown in Figure 1. The experiment was replicated three times.

Table 1: Results of three indexes for three cultivar lychees' pulp.

Cultivar	Volume density (g/cm^3)	Eutectic point (°C)	Pectin content (g/kg)
Feizixiao	0.754 ± 0.020^a	-26.80 ± 0.28^c	2.115 ± 0.083^a
Guiwei	0.495 ± 0.032^b	-16.32 ± 0.61^a	1.486 ± 0.061^c
Nuomici	0.462 ± 0.024^b	-20.30 ± 0.31^b	1.836 ± 0.091^b

Values in the same column not sharing the same superscript are significantly different ($p < 0.05$).

2.4. Microstructure. Structures of lychee pulp were studied using light microscopy (LM). The paraffin method was used [22]. Small cubes (pulp: about 4 mm^3, pericarp: about 2 mm^2) were removed from the internal zone of the samples for microscopic examination. The sample cubes were fixed in formol-aceto-alcohol (FAA, formaldehyde 5%, glacial acetic acid 5%, and 70% ethanol 90%) fixative solution for 24 hours. The ratio of fixative solution to samples was about 30 : 1. Dehydration was performed with 70%, 85%, and 95% ethanol concentrations for 2 h, respectively, and 100% ethanol concentration for 50 min. Then samples were cleared in mixed solution (ethanol : xylene = 1 : 1) and pure xylene solution for 2 h, respectively. Samples with xylene solution were put in China cups and melted paraffin was added in the cups (1 : 1). The cups were put in incubator at 40°C for 24 h. Next the cups were put in incubator at 60°C and paraffin was dumped after melting. Paraffin was added again in cups for one-hour interval and then dumped for three times. Last samples were embedded in paraffin (melting points 55°C to 57°C).

Sectioning was done with a rotary microtome (RM 2126, Shanghai Leica Instruments Ltd., China) at 10 μm thickness. Sections were stuck on microslides with gelatin adhesive. After removing the paraffin from samples with xylene for 10 min, the sections were rehydrated with a series of decreasing ethanol steps (100%, 95%, 85%, and 70%) for 10 min, respectively, and then the Heidenhain's iron-alum hematoxylin method was employed for staining. Finally, the samples were examined under a light microscope (Eclipse 50i, Shanghai Nikon Instruments Inc., China) equipped with a digital camera (DS-Fi1, Shanghai Nikon Instruments Inc., China). The histological procedures were performed in duplicate.

2.5. Pectin. Pectin was measured by carbazole spectrophotometric determination method (NY/T82.11-1988). Results of pectin were expressed as galacturonic acid (GlaA) g/100 g of initial fresh sample.

2.6. Eutectic Point. The eutectic point temperature of lychee was conducted with a differential scanning calorimeter (DSC, Q2000, TA Instruments, New Castle, DE) by Syamaladevi et al. [23] Lychee pulp was broken and then centrifuged with 8000 rpm/min at 4°C. Lychee pulp juice was used to test eutectic point temperature. Following equilibration, 15–20 mg lychee juice was sealed in aluminum pans (2 mm × 5 mm × 7 mm, 10–12 mg) and cooled from room temperature to −90°C at 5°C/min and equilibrated for 10 min. Lychee juice was scanned from −90°C to 50°C at a rate of 5°C/min (T_m). Annealing procedure was subjected to annealing at a temperature T_m for 30 min during decreasing temperature process. Then samples were cooled from T_m to −90°C at 5°C/min and equilibrated for 10 min. And samples were scanned from −90°C to 50°C at a rate of 5°C/min.

2.7. Bulk Density. Spiked millet substitution method was used to detect the volume of dried mixed chips. The granularity of spiked millet is between 0.9 and 1.1 mm [24]. The experiment was replicated three times. The bulk density ρ of the dried material is defined as

$$\rho = \frac{m}{V}, \tag{1}$$

where m is the mass of dry chips and V is total volume of dried mixed chips.

2.8. Statistical Analysis. The experimental data was analyzed using the statistical software SPSS 18 (SPSS Inc. Chicago, IL, USA) and analyses of variance were conducted by ANOVA procedure. All the measurements were carried out in triplicate. Mean values were considered significantly different when $p \leq 0.05$.

3. Results and Discussion

3.1. Effect of Prefreezing Temperature on FD Feizixiao Lychee. Volume density values of three FD lychees were shown in Table 1. It can be seen that the volume density of Feizixiao was high due to serious shrinkage. The picture of FD Feizixiao lychees was shown in Supplementary Materials. Effect of prefreezing temperature on FD process is very important. The temperature of materials should be under eutectic point temperature during prefreezing process, which will result in freeze completely. From Table 1, it can be observed that there were significant differences about eutectic point temperature among three cultivar lychees (Figure 2). And eutectic point temperature of Feizixiao lychee was lower than that of Guiwei and Nuomici lychees. Sugar content, sugar composition, and each ratio of three lychee juice could explain this phenomenon.

The results of sugar content and sugar composition of three lychees were shown in Figure 3. It can be seen that the total sugar content of Feizixiao was the highest. The higher the sugar content, the lower the eutectic point temperature of materials [25]. Moreover, lychee pulp contains three sugars: glucose, fructose, and sucrose. And each sugar ratio was different among three lychees. Sucrose content was lower and glucose and fructose content was higher in Feizixiao lychee juice. It is well known that eutectic point of Invertose is lower than sucrose [26]. Therefore, higher total sugar content and

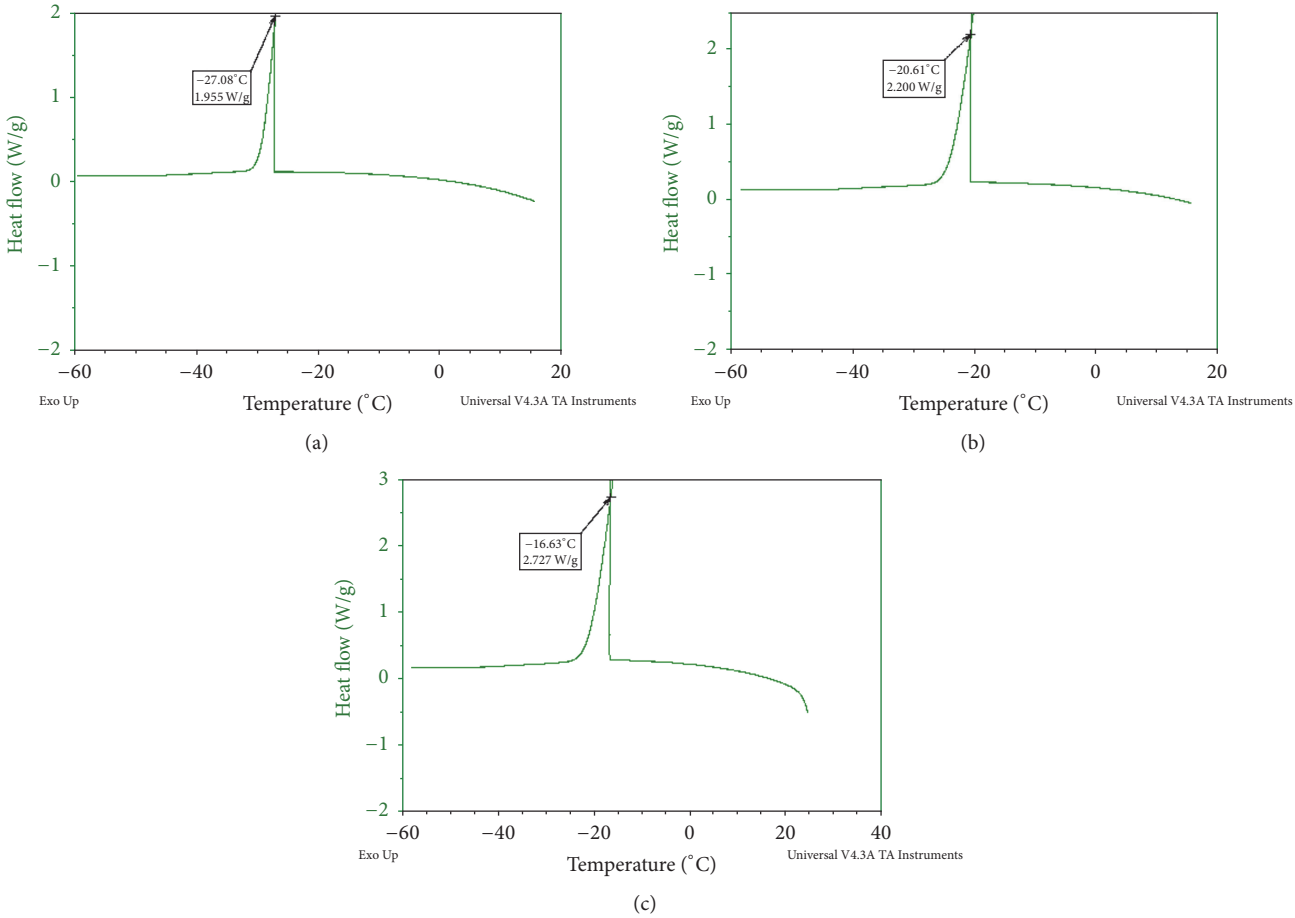

Figure 2: DSC curve for pulp clear juice of three lychees. (a) Feizixiao lychee. (b) Nuomici lychee. (c) Guiwei lychee.

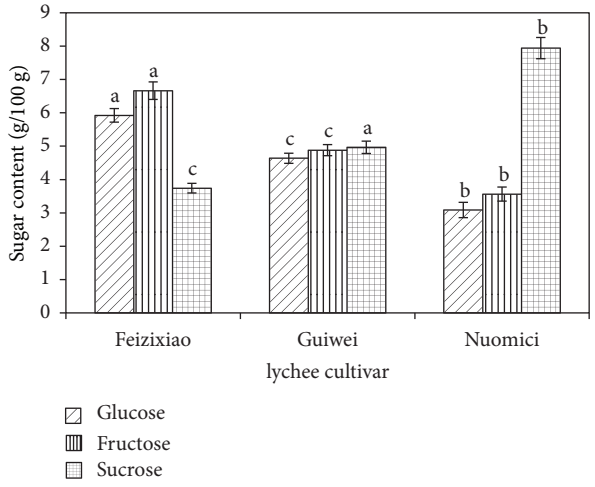

Figure 3: Sugar compositions in three cultivar lychees. ((a), (b), (c)) Values in the same column not sharing the same superscript are significantly different ($p < 0.05$).

high Invertose content were the main reason for low eutectic point temperature of Feizixiao juice.

In general, freezing temperature is 5°C–10°C below eutectic point temperature at least [27, 28]. However, the prefreezing temperature of three cultivar lychees was −30°C before this study. Guiwei and Nuomici lychees can freeze completely at this temperature. But for Feizixiao lychee, this temperature did not meet the freeze complete demand. So, the prefreezing temperature of Feizixiao should be decreased. The prefreezing temperature was −50°C in order to analyze other factors during follow-up tests.

3.2. Effect of Microstructure on FD Feizixiao Lychee. The volume density of Feizixiao lychee was also high with serious shrinkage even after −50°C prefreezing temperature. In fact, the microstructure plays an important role in determining the quality of materials [17, 18]. So, microstructure of Feizixiao lychee was tested for analysis. The micrographs of three lychee pulps were shown in Figure 4. It can be observed that the cell size of Guiwei and Nuomici lychee pulp was very close. But the cell size of Feizixiao lychee pulp was much smaller than the other two lychees. In general, lychee pulp was composed of long tubular structure including many single polygon cells. The moisture of lychee pulp is removed out by cell pore. The smaller the cell size, the higher the removing resistance of moisture [22]. This should be another reason for difficulty during freeze-drying Feizixiao lychees.

There is a translucent coating around the surface of Feizixiao lychee pulp but Guiwei and Nuomici lychees have

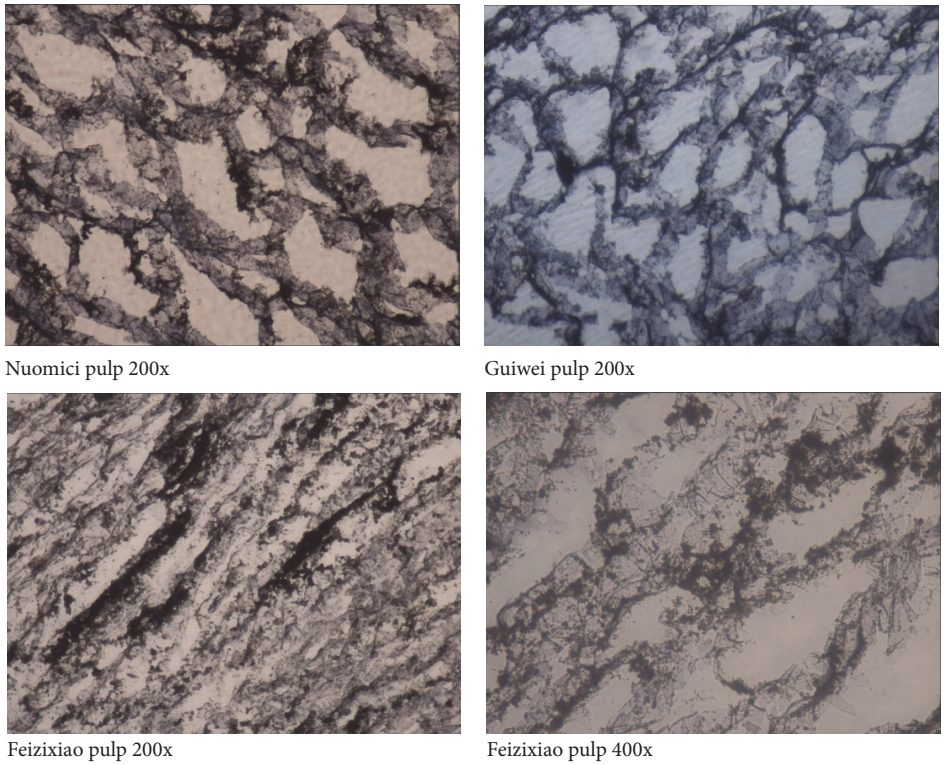

Figure 4: The light micrographs for lychee pulp of three cultivars.

not. Moreover, the color and taste of tip pulp are different from that of tail pulp. Tip pulp has slight red color and residue feeling after taste, which means that there is significant difference between tip and tail pulp of Feizixiao lychee. The micrographs of coating and tip pulp in Feizixiao lychee were shown in Figure 5. It can be seen that the cell structure of coating was small and intact, which increases the removing resistance of moisture in pulp. The tip cell was smaller and more compact than tail pulp cell. In addition, random arrangement with more folding not long tubular structure arrangement appears in tip pulp, which results in more difficult moisture removing from tip pulp. In a word, microstructure of Feizixiao pulp impedes the moisture removing during FD.

It is reported that the cell wall of potato was more resistant and stable after soaking with $CaCl_2$ [29]. It can be explained that pectin was combined with Ca^{2+} and the thickness of cell wall was increased accordingly. In this study, Feizixiao lychee pulp was soaked with 0.5% and 1% $CaCl_2$ solution. However, shrinkage of Feizixiao lychee was not improved like expected. The results of pectin content in three lychees were shown in Table 1. It can be seen that the pectin content in Feizixiao lychee was the highest. Eutectic point temperature of lychee juice will decrease with soluble pectin content increasing [25]. This was another reason for low eutectic point temperature of Feizixiao lychee juice except for sugar.

3.3. Effect of Ultrasound on FD Feizixiao Lychee. As known above, microstructure of Feizixiao lychee pulp affects the volume density of FD pulp. Ultrasound treatment can decrease the drying time and increase the quality of dried samples [21, 30, 31]. So, ultrasound was applied to treat Feizixiao lychee pulp in order to improve the appearance of FD pulp. The volume density results of Feizixiao lychees are treated by ultrasound in Figure 6. And the volume density results of Guiwei and Nuomici lychees are treated by ultrasound in Figure 7. Figure 6 shows that ultrasound could significant decrease the volume density of Feizixiao pulp. And the appearance of samples without membrane and tip pulp was much better than control group. Photos of Feizixiao lychee sample treated by ultrasound were shown in Supplementary Materials. It may be because the suitable ultrasound treatment could improve the permeability of cell wall, which results in more easy moisture removing. There was no significant difference about volume density between 20 min and 30 min treatment. This can be explained by the fact that there was similar permeability increase induced by two ultrasound treatments with different time. If the treatment time was prolonged continuously, the permeability of pulp cell wall will be improved further or decreased instead (Figure 7). However, even if the permeability of cell wall is increasing with treatment time, too much soluble solid loss resulting therefrom will appear. Therefore, the optimal ultrasound treatment time was 20 min.

The appearance of Guiwei and Nuomici lychee was improved further by ultrasound treatment. The optimal treatment time was also 20 min. It is worth noting that the volume density of Guiwei lychee pulp increased after 30 min ultrasound treatment. It may be because ultrasound treatment that is too long aggravates the breakage of cell wall.

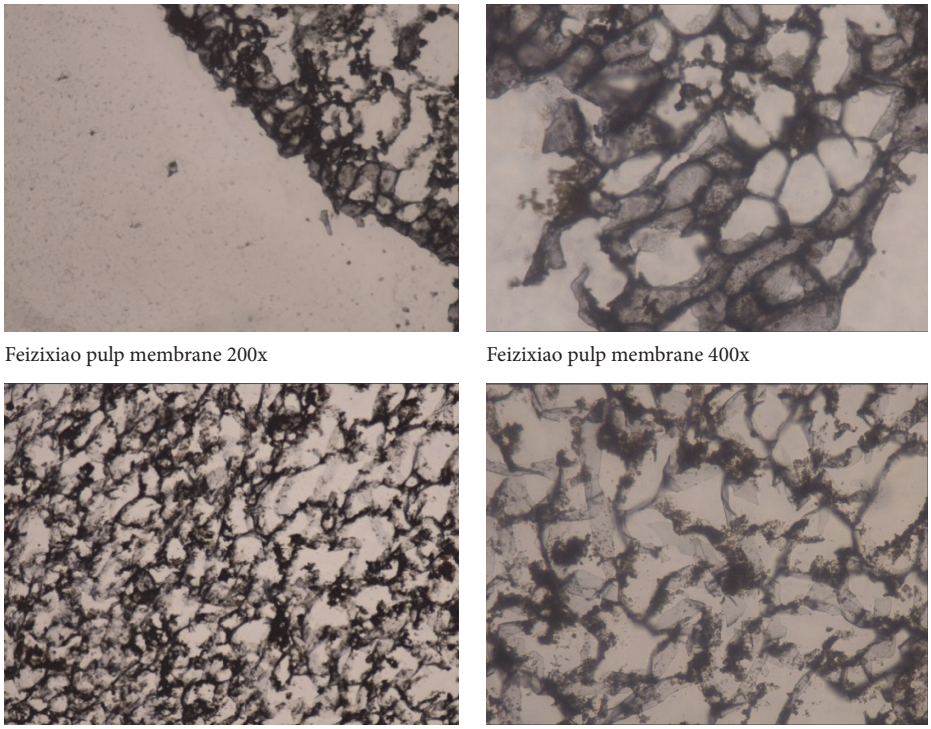

Figure 5: The light micrographs for Feizixiao lychee pulp membrane and tip.

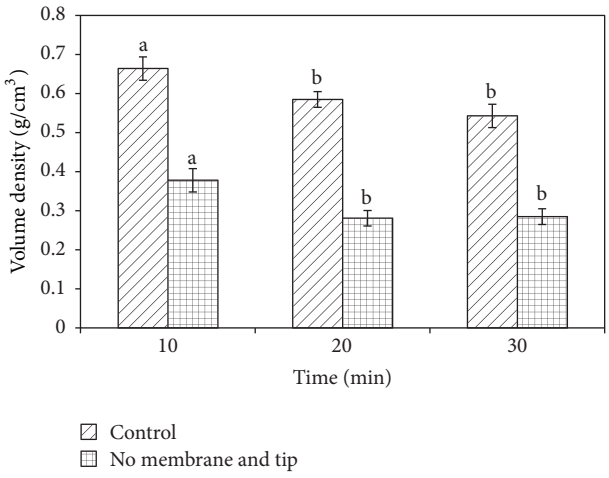

Figure 6: Effect of ultrasound on volume density of FD Feizixiao lychee. ((a), (b), (c)) Values in the same column not sharing the same superscript are significantly different ($p < 0.05$).

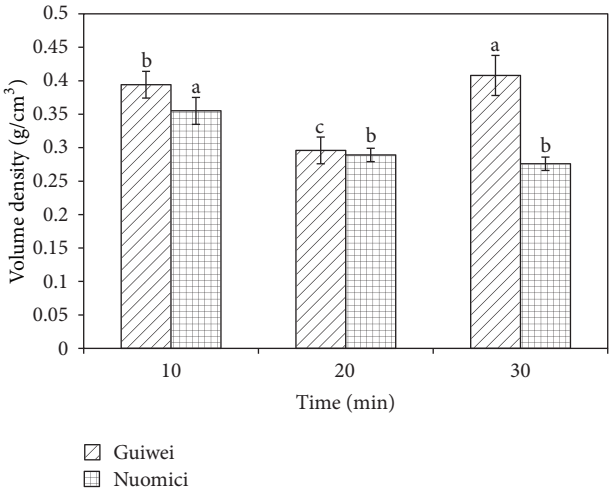

Figure 7: Effect of ultrasound on volume density of FD Guiwei and Nuomici lychee. ((a), (b), (c)) Values in the same column not sharing the same superscript are significantly different ($p < 0.05$).

Then fragment appearing and folding impede the moisture removing of lychee pulp. Photos of Nuomici and Guiwei samples treated by ultrasound for 30 min were also shown in Supplementary Materials.

4. Conclusions

Although Feizixiao lychee is one of the major lychee cultivars in Nanshan, Shenzhen, its drying characteristic is different from the other two major cultivars: Nuomici and Guiwei. It needs lower prefreezing temperature for Feizixiao lychees during freeze-drying due to higher sugar content and more Invertose content. Prefreezing temperature of Feizixiao lychee pulp should be below −32°C at least. For lychee pulp microstructure, the cell of Feizixiao lychee pulp is smaller and tighter than Guiwei and Nuomici lychees. In addition, the structure of Feizixiao lychee pulp is more complex than

Guiwei and Nuomici lychee pulp. A translucent membrane is around the surface of Feizixiao pulp. And the microstructure of tip pulp is different from the tail pulp for Feizixiao lychees. The cell of tip pulp is smaller than that of tail pulp. The membrane and smaller cell of pulp are both factors for difficulty during FD Feizixiao lychees due to hard moisture removing.

Ultrasound treatment can improve the appearance of FD Feizixiao lychee pulp, especially for samples without membrane and tip pulp. Very long treatment time could decrease the appearance of FD Feizixiao lychee pulp. Therefore, a suitable treatment time is very important. Moreover, removing the membrane and tip pulp not only is very trouble work, but also wastes materials. Therefore, new treatment methods will be quested in the future for high quality FD Feizixiao whole pulp with membrane and tip pulp.

Additional Points

Practical Applications. At present, air dried (AD) lychees are popular as traditional food in South China. The yield of AD lychees is higher than freeze-drying (FD) lychees. AD lychee has deep brown pulp with much shrinkage and poor flavour. However, the application of FD lychees increases constantly due to white color, crisp taste, and little shrinkage. For example, FD lychee powder could be added in cookies or desserts. Feizixiao lychee is one of the major lychee cultivars in China. This research could resolve the shrinkage of Feizixiao lychee during FD, which will improve the processing property and application of Feizixiao lychee.

Conflicts of Interest

The authors declare that there are no conflicts of interest regarding the publication of this paper.

Acknowledgments

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Supplementary Materials

The FD lychee photos of three cultivars were shown. It can be seen that FD Feizixiao lychees have significant shrinkage. Feizixiao lychees with ultrasound treatment for 30 min have better appearance. Feizixiao lychees with no membrane and tip have much better appearance. The samples with best appearance were Feizixiao lychees with no membrane and tip and ultrasound treatment for 30 min. Shenzhen Nanshan lychees, including Feizixiao, Guiwei, and Nuomici, were recognized as product of geographical indication in 2006. Three cultivar lychees are all main cultivars in Guangdong province. They are different from each other in appearance including color, shape, and surface structure. *(Supplementary Materials)*

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Research Article

The Composition Analysis of Maca (*Lepidium meyenii* Walp.) from Xinjiang and Its Antifatigue Activity

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Environment would affect the nutritional composition of maca, especially its secondary metabolite. The chemical compositions and function of Xinjiang maca were not very clear. The chemical compositions and bioactivity of Xinjiang maca were determined. A mouse model was also used to evaluate the antifatigue activity of Xinjiang maca as a forced swimming test was performed and certain biochemical parameters related were estimated. The results show that the Xinjiang maca is rich in protein content and amino acids, especially branched chain amino acids such as Valine and Isoleucine related to the effect of antifatigue. It also has considerable minerals ions such as Ca and Mg. Besides, bioactive ingredients such as maca amide, glucosinolate, and alkaloid of Xinjiang maca are similar to those of maca from other areas, which qualify the biological value of Xinjiang maca. The results of mice model suggest that maca has a dose-dependent antifatigue activity by decreasing blood lactic acid, as well as increasing liver glycogen content and the forced swimming time.

1. Introduction

Maca (*Lepidium meyenii* Walp.), a biennial herbaceous plant of the family brassicae, which is cultivated mainly in the central Andes of Peru at elevations of 3500–4500 m above sea level, has been used as both a food and a traditional medicine in the region for over 2000 years [1]. Domestic and foreign experts researched on the nutritional compositions and secondary metabolites of maca, finding that it not only contains rich protein, amino acids, fat, and minerals ion [2] but also contains a variety of secondary metabolites: maca ene, alkaloid (including maca amide), glucosinolate, and other components [3–5]. These secondary metabolites are considered closely related to the health effects of maca. Recent research shows that the logical ability of maca includes improving fertility, improving sexual performance, antiproliferative function, improving growth rate, antipost-menopausal osteoporosis, and ability in vitality and stress tolerance [6]. Most studies have been conducted to examine its biological activity on enhancing sexual performance or fertility. Only few reports show the antifatigue effect of maca powder [7] or maca extract [8]. Maca mainly grows at a cold but humid climate since its hardy, strong adaptability, so it is suitable to plant it in the high altitude region for 2700–3200 m above sea level in some areas of western China [9]. In 2004, Yunnan Huize introduced maca from the United States and the cultivation experiment was a success [10]. In China, maca is currently mainly cultivated in the Yunnan region, where its cultivation has formed a certain scale [11]. In addition to Yunnan, Pamirs in China Xinjiang is also suitable to cultivate maca for its geographical location and climate where maca has also been introduced recently. However, only a few reports were carried out to analyze the chemical composition and biology activity of yellow maca cultivated at Pamirs in China Xinjiang [12]. Its nutritional value and quality evaluation are not clear and deserve further evaluation. In addition, those foods in which maca is the main raw material, such as maca candy and maca granules, are popular in the market. In recent years, the unique overall effect of maca has been widespread in the world of health food industry, especially after China introduced maca as a kind of new resource food in 2011 [13]. Recently, many kinds of health

products with maca as the main raw material on the market rapidly expand and have been getting more and more concerns. However, their declared antifatigue effect has not been confirmed by actual data and it is difficult to guarantee their quality.

Only a few researches related to the chemical composition and biology activity of Xinjiang maca although it has been planted widely in Xinjiang. In this paper, the contents of essential nutritional compositions of maca cultivated in Xinjiang are investigated. The contents of basic chemical compositions of water content, oil content, protein content, amino acid content, and mineral content were analyzed. Besides, the biological activities of some secondary metabolites were investigated, such as alkaloids, maca amide, and glucosinolates. The quality of Xinjiang maca can be valued in the level of composition ingredients by comparison with the data from other reports about the compositions of maca. Animal models were also made to test the antifatigue effect of Xinjiang maca, in order to provide theoretical support for further development of health care products made of Xinjiang maca.

2. Materials and Methods

2.1. Materials. The dried yellow maca root was collected from Pamir in Xinjiang Province at altitude of 3000 m provided by Tangshiyi Biotechnology Co., Ltd. The dried maca was grounded into fine powder (75 μm) using a crusher. The roots were stored in polyethylene bags and frozen at −20°C until their use.

2.2. Chemicals and Instruments. Reagents used for chromatography were of HPLC grade (Fisher, Pittsburgh, USA). Other reagents used were of analytical grade. A crusher (Ningbo Shunhui Electric Appliance Co. Ltd., Zhejiang, China) was used for crushing the maca tubers to powders. A UV 2600 spectrophotometer was used in all absorbance measurements (Techcomp Ltd., Shanghai, China). A fridge (Haier Co. Ltd., Shandong, China) was used for storing the samples until tested. An H1850 Centrifuge (Xiangyi Centrifuge Instrument Co., LTD., China) was used for centrifugation.

2.3. Analysis Method of Compositions

2.3.1. Analysis Method of Chemical Compositions. Moisture content was determined by the Association of Official Analytical Chemists 925.10 method [14]. The crude protein content was established in a Kjeldahl apparatus, following the AOAC 920.87 method [14]. The factor $N \times 6.25$ was used to convert nitrogen into crude protein. The crude fat content was determined by an SOX 406 automatic fat analyzer (Hanon Instruments, Shandong, China). Petroleum ether was used as solvent and the operating temperature was 70°C. The crude ash content was determined by the AOAC 923.03 method [14]. Amino acids were determined using a Mikrotechna AAA 881 automatic amino acid analyzer according to the method described by Moore and Stein [15]. Hydrolysis of the samples was performed in the presence of 6 M HCl at 110°C for 24 h under nitrogen atmosphere. To estimate the content of minerals ion, maca samples were digested by concentrated nitric acid and perchloric acid (4 : 1, v/v). Minerals ion (K, Na, Mg, Ca, Zn, Fe, Cu, and Mn) were measured by using an atomic absorption spectrophotometer (Shimadzu Instruments, Inc., AAF-7000F, Kyoto, Japan) following the recommendations of the Association of Official Analytical Chemists [14].

2.3.2. Analysis Method of Bioactive Ingredients. The content of total alkaloids in maca was determined by acidic dye colorimetry as described by Gan et al. [16]. Bromothymol blue was used for chromogenic agent and nuciferine was used as standard to draft the standard curve. The content of maca amide is determined by the method of HPLC-MS [17]. N-benzyl hexadecanamide was used as standard. The content of glucosinolates was estimated by the HPLC-MS method [18]. Benzyl glucosinolate was used as standard.

2.4. Antifatigue Effects In Vivo of Maca

2.4.1. Reagents and Kits. The diagnostic kits for blood lactic acid, tissue glycogen, and serum urea nitrogen were purchased from Jiancheng Bioengineering Institute (Nanjing, China). Other commercial chemicals used in the experiments were of analytical grade and were purchased from Guoyao Chemical Reagent Factory (Shanghai, China).

2.4.2. Experiment Animals. 160 male Kunming mice, weighing 18–22 g at the beginning of the study, were purchased from Shanghai SLAC Laboratory Animal Co., Ltd. (Shanghai, China), License Number: SCXK (Shanghai) 2012-0002, certificate number: 2013001805390. They were fed under controlled environmental conditions of temperature (22 ± 2°C) and a 12-h light/dark cycle and maintained on a standard rodent diet and tap water unless otherwise stated. All animals received professional humane care in compliance with the guidelines of the Experimental Animal Management and Animal Welfare Ethics Committee of Jiangnan University (Wuxi, China). The number of animal experimental ethical inspection is JN Number 20140417-0529(30).

2.4.3. Experiment Design. After one week of adaptation, the mice were randomly divided into four groups (40 mice in each group) as follows: (i) control (C) group: the mice were allowed free access to a standard rodent diet and treated with distilled water; (ii) low-dose Maca-treated (LMT) group: the mice were allowed free access to a standard rodent diet and treated with 40 mg/kg.bw of maca; the maca tuber powder was dissolved in distilled water; (iii) moderate-dose Maca-treated (MMT) group: the mice were allowed free access to a standard rodent diet and treated with 400 mg/kg.bw of maca; (iv) high-dose Maca-treated (HMT) group: the mice were allowed free access to a standard rodent diet and treated with 1200 mg/kg body weight of maca. All treatment groups were administrated with the same volume at 0.2 mL/10 g.bw·day by gavage using a feeding needle, once a day for 30 consecutive days. All mice were provided with free access to standard rodent pellet food, which contains crude protein (18%), crude fat (4%), crude fiber (5.0%), Ca (1–1.8%), P (0.6–1.2%),

moisture (10%), and ash (8%). The body weights of mice were measured weekly.

2.4.4. Forced Swimming Test. The forced swimming test was analyzed according to the method describe by Zhang et al. [19] with some modification. After the last treatment, 10 mice of each dose group were used for the forced swimming test. The 40 mice were allowed to rest for 30 min and then weighted and loaded with a tin wire (5% of body weight) attached to the tail. The forced swimming capacity of mice was carried out in an acrylic plastic pool (50 cm × 50 cm × 40 cm) with 30-cm-deep water at 25 ± 0.5°C. The water was stirred to keep the mice limbs moving. The mice were determined to be exhausted when they failed to return to the surface of water to breathe within a 7-s period; then the forced swimming time was immediately recorded.

2.4.5. Blood Lactic Acid Assay. Blood lactic acid was analyzed according to the method describe by Zhang et al. [19] with some modification. After the last treatment, 10 mice of each dose group were used for the blood lactic acid assay. The mice were allowed to rest for 30 min, and then forced to swim without loads in the swimming pool as described in forced swimming test for 10 min while the water temperature was changed to 30 ± 0.5°C. Fifty microliters of blood sample was collected by inner canthus bleeding method before, immediately after, and 20 min resting after swimming, respectively. Blood samples of the mice were collected in heparinized tubes. The concentration of blood lactic acid was determined by the kits purchased from Nanjing Jiancheng Bioengineering Institute (Nanjing, China). The accumulation of blood lactic acid (the area of blood lactic acid under the curve) was calculated according to technical standards for testing and assessment of health food [20] using the following equation:

$$\text{The accumulation of blood lactic acid} = \frac{1}{2}(a+b)\times 10 + \frac{1}{2}(b+c)\times 20. \quad (1)$$

In the equation, a is the blood lactic acid concentration of mice before swimming; b is the blood lactic acid concentration of mice immediately after swimming; c is the blood lactic acid concentration of mice 20 min (resting) after swimming.

2.4.6. Serum Urea Nitrogen Analysis. After the last treatment, 10 mice of each dose group were used for the serum urea nitrogen analysis. After swimming for 90 min without loads (as described in blood lactic acid test), the blood samples were collected through removing the eyeball and then centrifuged at 3500 ×g, 4°C for 15 min before analysis. The concentration of serum urea nitrogen was determined by the serum urea nitrogen kits.

2.4.7. Liver Glycogen Analysis. After the last treatment, the other 40 mice were allowed to rest for 30 min and then sacrificed by decapitation under anesthesia with sodium pentobarbital (40 mg/kg.bw, ip) to collect livers. The livers were washed with 0.9% saline and blotted by a filter paper. The liver sample (~100 mg) was accurately weighted. The content of liver glycogen was determined according to the recommended procedures provided by the kits purchased from Nanjing Jiancheng Bioengineering Institute (Nanjing, China).

2.5. Data Analysis. The tests in this paper were duplicated for each sample and mean values of the duplicated tests are presented. Comparisons were carried out on software of SPSS for Windows (version 19.0, SPSS Inc. 2015). All animal experimental data were expressed as the mean ± SD. The data were subjected to one-way analysis of variance. $P < 0.05$ was considered to be statistically significant.

3. Results and Discussion

3.1. Compositions of Xinjiang Maca

3.1.1. Chemical Compositions. It is showing in Table 1 that the water content of the maca cultivated in Xinjiang is 7.01%, which is slightly lower than the data reported by Dini et al. (10.40%) (1997), as well as Yang et al. (10.40%) [21], while it is close to that reported by Yu and Jin (7.64%) [22]. Protein is the most important nutrient for human since it is related to organisms running and life activities, so that protein content is an important indicator of the nutritional value of foods. As it shows from Table 1, the protein content of yellow maca cultivated in Xinjiang is 13.42%, which is higher than protein content of 10.2% reported by Dini et al. [2], 9.1% reported by Yang et al. [21], and 8.87% reported by Yu et al. (2004). It means that Xinjiang maca is an abundant resource of nutrition as a new kind of introduced food. The lipid content of 1.42% is lower when compared to the results of 2.2% by Dini et al. [2] and 2.0% by Yu et al. (2004), but it is close to result of 1.38% reported by Yang et al. [21]. This variation may be attributed to the planting environment. The ash content of 3.41% in our report is similar to 3.08% reported by Du et al. [23].

3.1.2. Amino Acid Composition. The amino acid composition (Table 2) shows that Xinjiang maca has a high content of essential amino acids, reaching 27.2%. Compared with the essential amino acid pattern of FAO-WHO in 1973 [24], the contents of methionine, phenylalanine, and leucine are, respectively, 8.5 mg/g protein, 31.9 mg/g protein, and 46.4 mg/g protein, which are lower than essential amino acid pattern. The contents of Threonine, Valine, Isoleucine, and Lysine are, respectively, 32.7 mg/g protein, 65.0 mg/g protein, 36.6 mg/g protein, and 50.4 mg/g protein, which are close to those in the pattern. The Valine content of 65.0 mg/g protein is much higher than that of 50 mg/g protein in the pattern. Valine is closely connected with the antifatigue activity of maca. Besides, Isoleucine and Leucine can also contribute to the antifatigue function of maca. As a result, maca cultivated in Xinjiang shows an excellent profile, confirmed by the high content in essential amino acids.

3.1.3. Mineral Composition. Mineral compositions of yellow maca cultivated in Xinjiang are shown in Table 3. The three elements including Ca, Mg, and Zn are closely related to the

Table 1: Compositions of yellow maca cultivated in Xinjiang.

Compositions	Water content/%	Protein content/%	Oil content/%	Ash content/%	Maca amide (mg/g maca)	Glucosinolate (mg/g maca)	Alkaloid (mg/g maca)
Maca	7.01 ± 0.04	13.42 ± 0.57	1.42 ± 0.16	3.41 ± 0.02	0.17 ± 0.01	1.24 ± 0.04	0.20 ± 0.05

Data in this table are all expressed by wet basis content.

Table 2: Amino acid composition of yellow maca cultivated in Xinjiang.

Amino acids	Content mg/g protein	Essential amino acid pattern	Chemical score
Essential amino acid			
Threonine*	32.7	40	82
Valine*	65.0	50	130
Methionine*	8.5	35	24
Phenylalanine*	31.9	60	53
Isoleucine*	36.6	40	92
Leucine*	46.4	70	66
Lysine*	50.4	55	92
Nonessential amino acid contents			
Aspartate		82.8	
Glutamate		138.9	
Serine		25.4	
Histidine		27.5	
Glycine		43.1	
Arginine		202.3	
Alanine		39.4	
Tyrosine		22.8	
Cysteine		2.5	
Proline		0.5	

(a) Amino acid with "∗" means one of the essential amino acids; (b) Chemical score = 100∗ (per gram of the amino acid contents in detected protein/per gram the amino acid contents in the essential amino acid pattern).

antifatigue activity of maca. The Xinjiang maca is especially rich in the content of Mg, Ca, and K. Although the content of Cu is slightly lower and the content of Ca is higher, the other mineral contents of the Xinjiang maca are found to be in consistency with the results of earlier investigation by Dini et al. [2].

3.1.4. Biological Active Ingredients. Alkaloid is one of the most important bioactive components and it is related to many healthy effects of maca. From Table 1, we can find that the content of alkaloid in Xinjiang maca is 0.20%. This result is similar to the data 0.22% reported by Gan et al. [16]. As the unique alkaloid only found in maca, maca amide was reported to have influence on increasing libido [25]. The content of maca amide is 0.17 mg/g maca (0.0017%), which is in accord with the range of 0.0016%–0.013% in other reports [26]. Glucosinolates are secondary metabolites with negative ion hydrophilic which contain sulphur and nitrogen in plant. There are changeable side chains (R) and a sulpho β-D-glucofuranoses in them [27]. The decomposition products of them and themselves were considered to have lots of biological activity, such as the ability to combat pathogens and cancer. The content of glucosinolate was shown in Table 1. The benzyl glucosinolate content of Xinjiang maca is 1.24 mg/g maca, which also means that the proportion of glucosinolate is 0.124% in maca. This is lower than the result of 0.2% reported by Li et al. [5].

3.2. Mice Experimental Results

3.2.1. Effect of Xinjiang Maca on Forced Swimming Time. Fatigue is one of the most frequent physiological reactions. Tan et al. [28] have described the complex mechanism of fatigue as follows: it is caused by the depletion of energy sources, including decrease in glycemic levels and liver glycogen consumption. The side effects of fatigue include the accumulation of blood lactic acid, the disorder of internal environment, and metabolic control disorders of nervous system [28]. In our report, the forced swimming test was performed and certain biochemical parameters related were estimated.

The forced swimming test is a common experimental exercise model to evaluate the antifatigue activity [29]. The maximum swimming time is directly related to the ability of fatigue, so prolonged swimming times in forced swimming test indicate an increasing ability of antifatigue [30].

The effects of Xinjiang maca on forced swimming time are shown in Figure 1. As we can see from Figure 1, forced swimming times of mice in MMT and HMT groups are significantly longer ($P < 0.05$) than that in control group and increase by 54.3% and 77.3%, respectively. Forced swimming time in LMT group is 17.7% longer than that in the control group but the difference is not significant. This may be attributed to the lower dose of LHT group, which is only 10% of the dose in MMT and 3.33% of that in HMT, respectively. Wen et al. [31] found that the forced swimming time of mice with 5.0 g/kg BW Yunnan maca significantly extended and reached 324 sec compared with the control, which was 211 sec. These results indicated that Xinjiang maca has significant antifatigue activity when the dose is enough and is capable of elevating the exercise tolerance in mice.

3.2.2. Effect of Xinjiang Maca on Blood Lactic Acid Content. During intense exercises, the muscle produces a considerable amount of lactic acid when it obtains sufficient energy from anaerobic glycolysis, and the increased concentration of lactic acid brings about a reduction in the pH value in muscle tissue and blood, which could induce various biochemical and physiological side effects and lead to fatigue [32]. Therefore,

Table 3: Mineral ion composition of yellow maca cultivated in Xinjiang (mg/kg).

Mineral ion	Zn	Fe	Cu	Mn	K	Na	Mg	Ca
Content	30.7 ± 1.3	82.4 ± 0.8	5.9 ± 0.6	11.2 ± 0.6	11700.0 ± 141.4	188.0 ± 24.9	847.5 ± 15.3	13700.0 ± 282.8

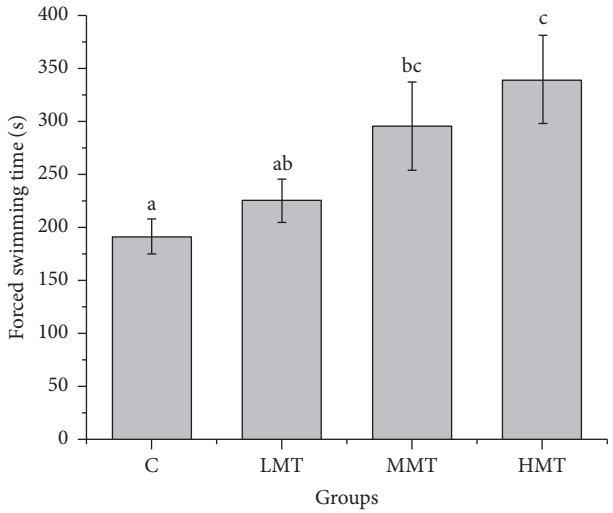

Figure 1: Effect of Xinjiang maca on forced swimming time (C, control; LMT, low-dose maca-treated group; MMT, medium-dose maca-treated group; HMT, high-dose maca-treated group. Each value represents the mean ± SD (n = 10). Different letters indicate significant differences among groups ($P < 0.05$)).

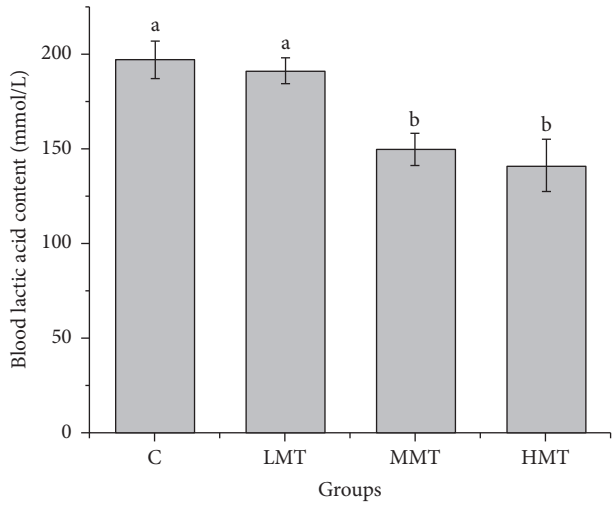

Figure 2: Effect of Xinjiang maca on blood lactic content (C, control; LMT, low-dose maca-treated group; MMT, medium-dose maca-treated group; HMT, high-dose maca-treated group. Each value represents the mean ± SD (n = 10). Different letters indicate significant differences among groups ($P < 0.05$)).

blood lactic acid content is a sensitive index of fatigue status. The accumulation of blood lactic acid content can be an integrated indicator for investigating the changes of blood lactic acid content.

The effect of Xinjiang maca on blood lactic acid content is shown in Figure 2. We can find that after a 10-min swimming and 20-min rest, the accumulations in MMT and HMT groups were significantly lower than that in the control group ($P < 0.05$), decreased by 23.9% and 28.2%, respectively. This result was similar to those of Zhang et al. [33] and Gao et al. [34], which used Yunnan maca and Peru maca, respectively. The accumulation of blood lactic acid content in LMT group is also lower than the control group, but it is not significant. These results indicate that Xinjiang maca effectively delays the increase in blood lactic acid content, reduced the catabolism of protein for energy, and increased the adaptive capacity to exercise load. However, the dose of Xinjiang maca should be taken into consideration to reach the antifatigue activity.

3.2.3. Effect of Xinjiang Maca on Serum Urea Nitrogen Content. Urea is formed in the liver as the end product of protein metabolism. Protein and amino acids have a stronger catabolic metabolism when the body cannot acquire enough energy produced by carbohydrates and fat catabolic metabolism after an intense exercise, during which urea nitrogen increases [35]. Thus, serum urea nitrogen content is another sensitive index of fatigue status.

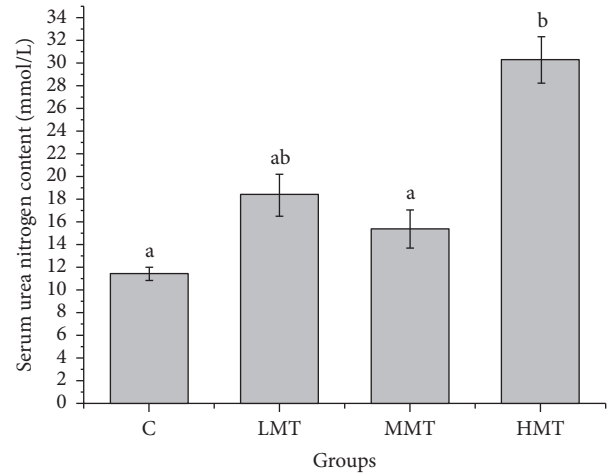

Figure 3: Effect of Xinjiang maca on serum urea nitrogen content (C, control; LMT, low-dose maca-treated group; MMT, medium-dose maca-treated group; HMT, high-dose maca-treated group. Each value represents the mean ± SD (n = 10). Different letters indicate significant differences among groups ($P < 0.05$)).

As shown by Figure 3, the serum urea nitrogen contents of LMT and MMT groups are not significantly higher than that of control group. This results show that LMT and MMT group can not significantly increase the serum urea nitrogen content. The serum urea nitrogen content of HMT group

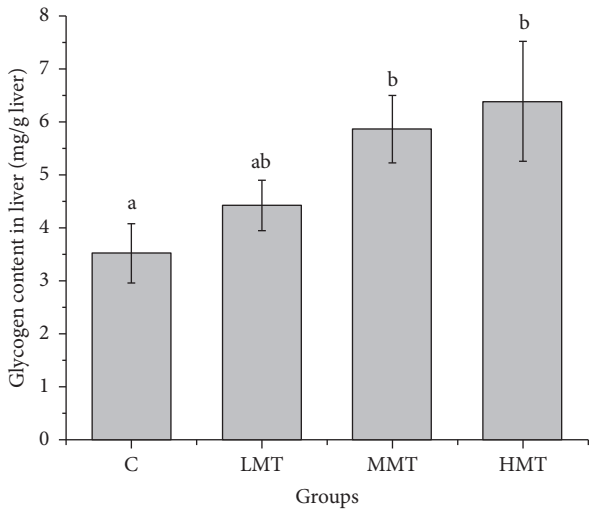

Figure 4: Effect of Xinjiang maca on glycogen content in liver (C, control; LMT, low-dose maca-treated group; MMT, medium-dose maca-treated group; HMT, high-dose maca-treated group. Each value represents the mean ± SD ($n = 10$). Different letters indicate significant differences among groups ($P < 0.05$)).

is significantly higher than that of the control group, which indicates that too much intake of maca may increase the burden of protein metabolism of body so that the serum urea nitrogen content of the dealing group was higher than the control group. As a consequence, the dose of maca for 1200 mg/kg bw in HMT group can do harm to the mice. However, Zhang et al. [33] found that Yunnan maca with the dosage of 0.4 g/kg BW and 1.2 g/kg BW could significantly decrease the serum urea nitrogen content of mice compared with the control. Gao et al. [34] found that Peru maca during the experimental dosage has no obvious effect on the serum urea nitrogen content of mice compared with the control. The different results may be related to the different planting region and chemical composition.

3.2.4. Effect of Xinjiang Maca on Glycogen Content in Liver. Energy for exercise is derived initially from the breakdown of glycogen in muscle. After intense exercise, it may be depleted, and at later stages the energy will be derived from hepatic glycogen [36]. Therefore, the depletion of glycogen stores may be a significant factor in the development of fatigue.

The effects of Xinjiang maca on glycogen content in liver are shown in Figure 4. The liver glycogen content of MMT and HMT groups was significantly higher ($P < 0.05$) than that of control group, increased by 66.3% and 80.9%, respectively. The glycogen content in liver in the LMT group is also lower, but not significantly ($P > 0.05$). These results indicated that Xinjiang maca may contribute to the activation of energy metabolism which could delay physical fatigue by increasing the storage of glycogen in liver. Also, as mentioned before, the intake of maca must reach a certain dose as to show the activity of antifatigue. Similarly, Zhang et al. [33] also found that Yunnan maca with the dosage of 0.4 g/kg BW and 1.2 g/kg BW could significantly increase the glycogen content in liver of mice compared with the control.

4. Conclusions

Xinjiang maca is rich in protein content and amino acids, especially branched chain amino acids such as Valine and Isoleucine related to the activity of antifatigue. It also has considerable minerals ion contents such as Ca and Mg which is related to antifatigue activity. Besides, biological active ingredient contents such as maca amide, glucosinolate, and alkaloid of Xinjiang maca are similar to those in maca from other areas, which indicates it can provide similar health benefits like maca from other areas. The results show that the dried powder of Xinjiang maca has a reasonable nutritional structure and is a kind of high value quality food. The results of mice experiment showed that intake of maca for 400 mg/kg bw and 1200 mg/kg bw can decrease blood lactic acid content as well as increase liver glycogen content and forced swimming time. However, maca intake of 1200 mg/kg bw can significantly increase the serum urea nitrogen content as to do harm to the body. So Xinjiang maca has a dose-dependent antifatigue effect by decreasing blood lactic acid as well as increasing liver glycogen content and forced swimming time. The intake dose of maca for 400 mg/kg bw can bring out significant antifatigue activity.

Conflicts of Interest

All authors declare that there are no conflicts of interest regarding the publication of this paper.

Acknowledgments

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Research Article

Improvement of the Quality of Surimi Products with Overdrying Potato Starches

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This study investigated the effect of overdrying potato starches on surimi products. The chemical composition of protein and chemical interactions, gel solubility, and protein conformation of the mixture of surimi gel protein, respectively, with 8% native potato starch and with 8% overdrying potato starch were investigated. The results show that the starch increased the insoluble protein content. In terms of the chemical interactions, the overdrying potato starch increased the amount of hydrogen bond and nondisulfide covalent bond and decreased the amount of ionic bond, which might stabilize the network structure of protein gel. The analysis of Raman Spectroscopy shows that more α-helices turn into random coin structure after the starch was added, which is conducive to higher strength and a better water retention ability of the surimi product.

1. Introduction

Starch is an important ingredient in surimi seafood products since it would affect textural and physical characteristics of surimi fish protein gels. For instance, it can improve surimi gel strength, modify texture, reduce cost [1], and improve freeze-thaw stability [2]. The starch could replace a portion of the fish protein while maintaining desired gel properties due to its water-holding ability [3].

For the mechanism of improving the quality of surimi gel with the addition of starch, there are three theory models which have been widely recognized: the cavity model raised by Couso et al. [4] at 1998; the filling extrusion model raised by Yang and Park [5]; and the pack and bundle effect model raised by Kong et al. [6]. In general, these three models were from the physical point of view, explaining the relationship between the starch and gel properties of surimi. However, the influence of starch on surimi gel protein function from the chemical perspective has not been reported yet.

In this study, compared with pure minced surimi gel, the effects of starch on surimi gel protein were investigated under the addition of 8% native and overdrying potato starch, in order to provide a theoretical basis for the mechanism of interaction between starch and protein in surimi.

2. Materials and Methods

2.1. Materials. Native and overdrying potato starches were obtained from France ROQUETTE. AA grade of silver carp surimi was purchased from Hong hu Hong ye Aquatic Food Co., Ltd. Polyvinyl chloride (PVC) plastic casing were purchased from Longhai Ri sheng plastic color printing packaging Co. Ltd.

2.2. Samples Preparation. Frozen surimi was thawed at 4°C overnight and then diced and chopped for 3 min. 3% salt was added to the surimi and it was cut for 2 min until the surimi paste was fully decentralized. Then 8% potato starches were added and the mixture was mixed at a low speed for 3 min until the slurry was uniform (adjust the final moisture content to 78%). The final intestinal samples were made according to

simulated industrial formula. 8% fat, 5% ice egg white, 4% soy protein, 0.6% sugar, and 0.6% of the MSG were added to the slurry, and then the mixture was mixed for 2 min. This fish paste was chopped and mixed through the exhaust into a diameter of 30 mm plastic casing, made of approximately 20 cm in length of intestine. The intestines were placed at 40°C water bath for 30 min, the gelation; then at 90°C for 30 min; then in ice water to cool the samples rapidly. The samples were stored at 4°C overnight.

2.3. Determination of Protein Composition. According to Parker's method [7], the composition of protein was determined as follows.

Determination of water soluble protein content: 100 mL low phosphate buffer (0.05 mol/L KCl-0.01 mol/L NaH_2PO_4-0.03 mol/L Na_2HPO_4) was added to 10 g of chopped surimi gel samples. The mixture was mixed and homogenized for 2 min and then stirred for 3 h and centrifuged at 4°C, 5000 r/min for 10 min. Protein content in the supernatant was measured by BCA method.

Determination of salt soluble protein content: 100 mL high phosphate buffer solution (0.5 mol/L KCl-0.01 mol/L NaH_2PO_4-0.03 mol/L Na_2HPO_4) was added to 10 g chopped surimi gel samples. The mixture was mixed and homogenized for 2 min and then stirred for 3 h and centrifuged at 4°C, 5000 r/min for 10 min. Protein content in the supernatant was measured by BCA method.

Determination of insoluble protein content: the measured total protein in surimi gel (crude protein) was subtracted by the content of water soluble and salt soluble protein content.

2.4. Determination of Chemical Forces. 4 g minced fish sausage sample was, respectively, added to 20 mL of 0.05 mol/L NaCl (S1), 0.6 mol/L NaCl (S2), 0.6 mol/L NaCl + 1.5 mol/L urea (S3), 0.6 mol/L NaCl + 8 mol/L urea (S4), and 0.6 mol/L NaCl + 8 mol/L urea + 0.5 mol/L beta mercaptoethanol (S5). The mixture was mixed and homogenized for 2 min. The homogeneous liquid was centrifuged 20000g for 15 min [8]. The protein in the supernatant was determined by Lowry method. The differences in protein dissolved in S2 and S1 solutions said the contribution of ionic bond, the differences in S3 and S2 solutions indicated a hydrogen bond contribution, the differences dissolved in S4 and S3 solutions indicated the contribution of hydrophobic interactions, and the difference of protein content dissolved in S4 and S5 solutions indicated disulfide bond contribution [9]. All results were expressed as the percentage of protein content accounting for the total amount of protein.

2.5. Determination of Solubility of Gel. 20 mL 20 mmol/L was added to 1 g chopped surimi gel sample, pH 8.0 in Tris-HCl buffer solution, and mixed and homogenized for 2 min. The buffer contains 1% (w/v) SDS, 8 mol/Lurea and 2% (v/v) β-mercaptoethanol. The mixed solution was heated at 100°C for 2 min and centrifuged at 10,000g for 30 min after stirring at room temperature for 4 h. After centrifugation, 50% (m/v) trichloroacetic acid (TCA) was added in 10 mL supernatant and the protein was precipitated. Mixing liquid was stored at 4°C for 18 h and then centrifuged at 10,000g for 30 min. Precipitation dissolved in 30 mol/L NaOH 0.5 mL after washing with 10% (m/v) concentration of TCA. Lowry method was used to determine protein content. The dissolution rate was expressed as the percentage of total protein content of the protein in the solvent [10]. Total protein content was measured by the amount of protein dissolved in 0.5 mol/L NaOH of surimi gel.

2.6. FT-Raman Spectroscopic Analysis. The sliced gel samples were stick to a layer of foil glass slide and placed on the object loading table of Raman Spectroscopy. The scanning range was from 300 to 3800 cm^{-1} [11]. Income spectrum for baseline correction with a benzene ring in $1003 \pm 1\,cm^{-1}$ ring vibration (due to the little microenvironmental impact) is the normalized spectra within standard [12]. Peak Fit software was used for original spectrum curve fitting of amide I band, and the overlap in different bands was completely resolved. The percent of the protein secondary structure was calculated by the integral area.

2.7. Statistical Analysis. All measurements were conducted at least three times. The least significant difference (LSD) at 5% was applied to define the significant difference. All analyses were performed using SPSS software v 19.0.

3. Results and Discussion

3.1. Protein Composition. Due to the presence of starch in the starch-surimi system, the total protein (TP) contents would be different among samples of CON (control, i.e., pure surimi gel), NPS (surimi gel with native potato starch), and LMPS (surimi gel with overdrying potato starch). Therefore, the total protein contents of surimi gel samples were first determined. The water soluble protein (WSP), salt soluble protein (SSP), and insoluble protein content (ISP) are expressed as the fraction accounting for the total amount of protein (Table 1). It showed that potato starch had a significant effect on surimi gel protein composition ($P < 0.05$). Soluble and salt soluble protein contents of LMPS were significantly lower than those of CON and NPS, while the insoluble protein content of LMPS was significantly higher. It is of note that the surimi gel formation is essentially a process of salt soluble protein crosslink and then gradually converted into the insoluble protein [11]. Therefore, we proposed that the presence of overdrying potato starch could promote the crosslink formation of salt soluble protein in the surimi gel, thus enhancing the quality of the gel.

3.2. Chemical Forces. The network structure in the surimi gel system is mainly maintained through the interactions among and within protein molecules, such as chemical bonds, ionic bonds, hydrogen bond, hydrophobic interaction, and covalent bonding [13] (Table 2). It can be seen from Table 2 that the potato starch has a significant effect on the formation of chemical interactions in the surimi gel proteins ($P < 0.05$).

Table 1: Protein composition of surimi gel samples.

Sample	CON	8% NPS	8% LMPS
TP (%)	12.20 ± 0.03	10.92 ± 0.09	10.88 ± 0.05
WSP (%)	5.09 ± 0.00	4.63 ± 0.04	4.03 ± 0.02
SSP (%)	5.57 ± 0.03	4.75 ± 0.00	4.36 ± 0.01
ISP (%)	89.34 ± 0.00	90.62 ± 0.02	91.61 ± 0.02

CON (control, i.e., pure surimi gel), NPS (surimi gel with native potato starch), and LMPS (surimi gel with low-moisture potato starch).

Table 2: Chemical forces of surimi gel samples.

Sample	CON	8% NPS	8% LMPS
Ionic bond (%)	1.14 ± 0.03	0.99 ± 0.02	0.91 ± 0.01
Hydrogen bond (%)	1.79 ± 0.03	2.19 ± 0.02	2.93 ± 0.03
Hydrophobic interaction (%)	47.84 ± 0.04	28.68 ± 0.00	28.51 ± 0.05
Disulfide bond (%)	27.62 ± 0.07	12.27 ± 0.02	18.47 ± 0.06

CON (control, i.e., pure surimi gel), NPS (surimi gel with native potato starch), and LMPS (surimi gel with low-moisture potato starch).

There is a large amount of ionic bonds in the frozen surimi, thus salt ions are generally needed to break the ionic bond in order to disperse the protein, and then the dispersed protein could form the gel with elastic structure after heat treatment [14]. We found that the ionic bond was reduced in our starch-surimi gel systems, which may be because the presence of the starch blocks the formation of ionic bond between and within proteins. It could be concluded that the potato starch is beneficial to the elasticity of the surimi gel. Hydrogen bonds in surimi gel play an important role in the stability of the bound water and increase the strength of surimi gel during the cooling process [13]. Table 2 shows that the potato starch especially the overdrying starch significantly increased the amount of hydrogen bonds in the surimi protein ($P < 0.05$).

The hydrophobic sites in surimi protein would be exposed in the water environment after heat treatment. In order to maintain the stability of the thermodynamic system, the hydrophobic interaction is enhanced, resulting in the aggregation of protein to form a gel network [13]. In this study, the hydrophobic interactions between proteins significantly decreased ($P < 0.05$) after the adding of potato starch. The interaction between the starch and the surrounding water may change the water status around the proteins, thus affecting the hydrophobic interactions within the surimi gel systems.

When the heating temperature is higher than 40°C, disulfide bonds are thought to be the main covalent bonds that can promote the formation of protein gel [15]. Compared with the pure surimi system, the amount of disulfide bonds was significantly reduced in native starch-surimi system ($P < 0.05$). However, the amount of disulfide bonds after adding the overdrying potato starch was higher than that in the native starch-surimi system. It indicates that such negative effect on the formation of disulfide bonds induced by the potato starch might be affected by the structure of starch granules.

3.3. Solubility of Gel. The solvent containing SDS, urea, and beta mercaptoethanol is usually used to dissolve nondisulfide covalent bonds in protein [16]. Therefore, the solubility of surimi gel refers to the formation of nondisulfide covalent bond that is also involved in one of the main chemical interactions to form surimi gel network structure [11]. As seen in Figure 1, the presence of starch especially overdrying potato starch significantly reduced the surimi gel dissolution rate ($P < 0.05$), indicating that overdrying starch significantly increased the amount of nondisulfide covalent bonds. Presumably, starch granules absorbed water in the heating process; therefore the contents of protein within the continuous phase and endogenous glutamine transfer enzyme (TGase) were increased.

3.4. Protein Conformation. Raman Spectroscopy (Figure 2) can be used to investigate intermolecular interactions among protein molecules: it could provide relative intensity information about amino acid side chains, peptides, and the vibration frequency of the polysaccharide backbone.

The Raman spectrum band within 1600~1700 cm^{-1} is called the amide I band, which gives information about the protein secondary structure. Specifically, the spectra of the bands of 1650~1660, 1665~1680, and 1660~1665 cm^{-1} ranges are, respectively, corresponding to α-helix, β-sheet, and random coil of the protein. The band peaks of CON, NPS, and LMPS were located in 1653 cm^{-1} and 1667 cm^{-1}, in 1654 cm^{-1} and 1662 cm^{-1}, and in 1656 cm^{-1} and 1664 cm^{-1}. It can be seen that the addition of two kinds of starch makes a blue shift of the characteristic peaks of the helix structure, indicating a decrease of α-helix [17]. Liu et al. claimed the reason why the amount of α-helix in silver carp surimi decreased with the formation of the gel structure was the transition of α-helix into random coil structure [11] (Table 3). Table 3 shows the quantitative analysis of the protein secondary structure in the amide I band, which reports that the overdrying potato starch has a significant influence on the transition of α-helix into random coil structure: more α-helices were transformed into random coils, promoting the aggregation and interaction of

Table 3: Secondary protein structure content of Amide I.

Sample	Content (%)			
	Alpha helix	Beta folding	Beta turn	Random coil
CON	30.7 ± 0.6^{a}	34.4 ± 0.9^{a}	9.4 ± 0.3^{a}	25.5 ± 0.1^{c}
8% NPS	28.4 ± 0.1^{b}	35.6 ± 0.5^{a}	8.5 ± 0.6^{b}	27.5 ± 0.4^{b}
8% LMPS	25.4 ± 0.7^{c}	25.0 ± 1.1^{b}	7.9 ± 0.4^{b}	41.7 ± 0.8^{a}

CON (control, i.e., pure surimi gel), NPS (surimi gel with native potato starch), and LMPS (surimi gel with low-moisture potato starch). Values with different letters within a column indicate statistically significant differences ($P > 0.05$).

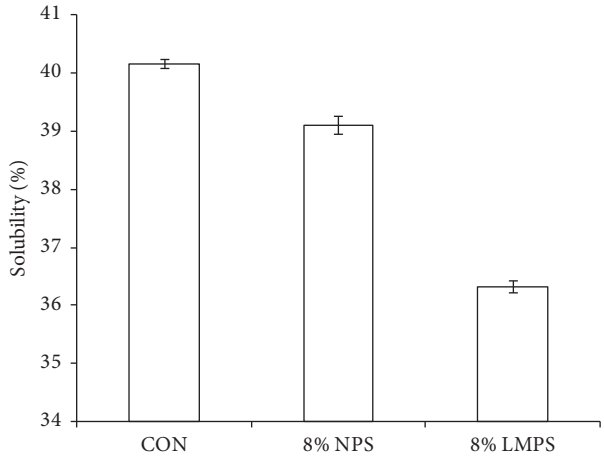

Figure 1: The solubility of surimi gel. CON (control, i.e., pure surimi gel), NPS (surimi gel with native potato starch), and LMPS (surimi gel with low-moisture potato starch).

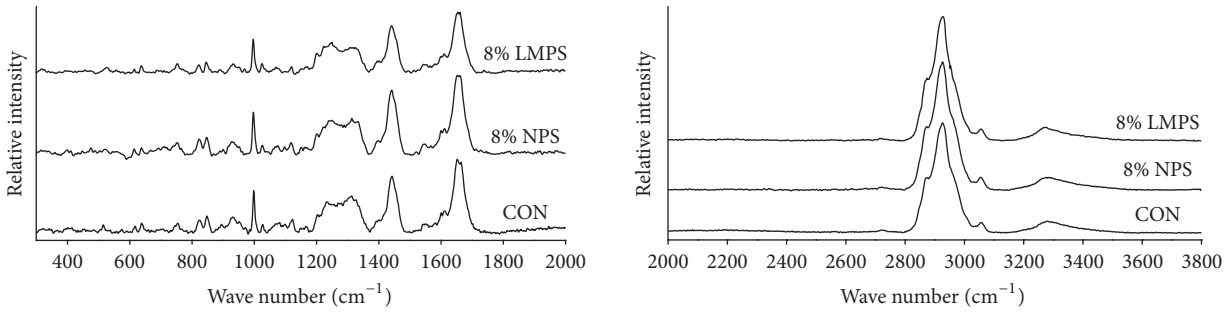

Figure 2: The Raman spectra of surimi gel. CON (control, i.e., pure surimi gel), NPS (surimi gel with native potato starch), and LMPS (surimi gel with low-moisture potato starch).

protein molecules; therefore the starch seems to render the network of surimi gel more stable.

In terms of the range of 3100~3500 cm^{-1}, the Raman spectrum peak refers to the stretching movement of O-H, which is related to the intermolecular vibration of water molecules bonded with hydrogen bond [18]. In this study, the O-H stretching band peaks of CON, NPS, and LMPS were located in 3280, 3277, and 3274 cm^{-1}, and the corresponding strength values were 0.76, 0.84, and 0.89, respectively. The decrease in the wave number of these peaks implies a stronger hydrogen bond with water, and a higher strength value reveals a larger amount of bound water molecules [12, 19]. Hence, potato starch could improve water binding and retention ability of the surimi gel, which is conducive to the formation of the gel matrix during the heating process. The effect of overdrying potato starch seems more notable.

4. Conclusions

The overdrying potato starch is proved to improve properties of surimi gel. It promoted the formation of insoluble protein, decreased the amount of ionic bonds, and increased that of nondisulfide covalent bonds and hydrophobic interaction, which would facilitate the formation of gel network structure. The data about protein conformation also confirms these positive effects of potato starch on the water binding and retention ability of surimi gel. Currently, because of the complex food system and pretreatment, it is difficult to

use small interactions such as π-π stack to explain the improvement of the properties of surimi gel.

Additional Points

Practical Applications. The overdrying potato starch can improve properties of surimi gel, as it promoted the formation of insoluble protein, decreased the amount of ionic bonds, and increased that of nondisulfide covalent bonds and hydrophobic interaction. Thus, potato starch, especially overdrying potato starch, can be used to improve the quality of surimi products.

Conflicts of Interest

The authors declare that they have no conflicts of interest.

Acknowledgments

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Research Article

Analysis of Chemical Constituents Changing in Physical Process and Nutritional Components of *Malus halliana* Koehne Tea

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This study aimed to establish a HPLC method for simultaneous determination of the changing of quercitrin, 3-hydroxyphloridzin, and phloridzin in physical process of *M. halliana* tea. Meanwhile, the nutritional compositions were determined, using anthrone-sulfuric acid colorimetry and direct titration determination of total sugar and reducing sugar, respectively, in order to provide theoretical basis for quality control and tea production. The results showed that the regression equations for quercitrin, 3-hydroxyphloridzin, and phloridzin were linear in the range of 0.0972–12.15 μg (r = 0.999 8), 0.0932~11.65 μg (r = 0.999 1), and 0.9~112.5 μg (r = 0.999 6), respectively. The average recoveries ranged from 98.19% to 99.35%. The contents of crude protein and the crude fat were measured by spectrophotometric detection and soxhlet extraction detection, respectively. The contents of total sugar, reducing sugar, the fat, and protein were 6.8 g/100 g, 8.5 mg/100 g, 2.399 g/100 g, and 4.362 g/100 g, respectively, in *M. halliana* tea.

1. Introduction

Malus halliana (M. halliana) Koehne, belonging to the family Rosaceae, is abundant and widely distributed in Jiangsu, Zhejiang, Anhui, Shanxi, Sichuan, and Yunnan provinces in China, which grows in the jungle of slopes or stream sides, commonly cultivated for ornamental industry [1]. *Chinese Materia Medica* records that its taste is light, bitter, and flat, and it can regulate the menstrual function and blood and treat metrorrhagia [2]. In folk medicine, it has been used as traditional Chinese herbal medicine in the treatment of traumatic injury, fractures, and hemorrhage [3]. In recent years, more studies have focused on gardening cuttings, cultivation, breeding, and so on [4, 5]. The *M. halliana*-derived healthy drink has been developed [6].

Drinking tea not only can add some of the necessary trace elements in the human body, but also has a pharmacological function and health effects on the human body. Modern medicine researches have shown that it not only can prevent thrombosis, reduce blood viscosity, increase high-density lipoprotein, and reduce the body's capillary permeability and brittleness, but also possesses certain preventive effects on cardiovascular and cerebrovascular diseases and antiaging and increases immunity [7]. Drinking tea can make people excited, has diuretic impact, results in sterilization, has anti-inflammatory, cardiac, and other effects, enhances memory, and prevents diabetes [8, 9].

Our group has conducted a series of related researches in early stage, including chemical composition [3, 10, 11], pharmacological activity [12–15], and content determination [15]. Flavonoids are characteristic constituents in *M. halliana*, and pharmacological investigations indicate that it possesses antioxidant [12], hepatoprotective [13], α-glucosidase inhibitory [14], tyrosinase-activating [15], and antimicrobial [16] activities.

To date, only *M. hupehensis* is used as drinking tea known as Ning Qing tea, which is widely used as summer cool and refreshing drink in Three Gorges region of China [17]. The main component was phloridzin [18], and there has been a patent to disclose a preparation method and application as antidiabetic, hypolipidemic, antioxidant, and antitumor medicine of *M. hupehensis* tea extract (ZL 200710053716.8).

Our previous research has found that phloridzin is also the main ingredient of *M. halliana* leaves [11]. While the natural resource of *M. halliana* was rich, no studies on the quality control and the analysis of the nutritional compositions of *M. halliana* tea are available in the literatures. Our study was undertaken to adopt the traditional tea processing technology to develop the *M. halliana* tea, and the contents of quercitrin, 3-hydroxyphloridzin, and phloridzin in *M. halliana* tea were measured using high-performance liquid chromatography (HPLC). In the separation and purification of the *M. halliana* tea, we found that quercitrin, 3-hydroxyphloridzin, and phloridzin contained in the tea were more than other flavonoids, for example, juglanin, avicularin, afzelin, and 5,7-dihydroxychromone. In addition, the literature reviews found that the activities of these three compounds in *Malus* genus were more reported. In comprehensive analysis, we used these three compounds as the object of study. The contrast analysis of the changes in the same compositions of *M. halliana* tea before and after processing was also carried out. Meanwhile, the main nutritive components of *M. halliana* tea were studied. Further studies on quality control standards and nutritive components of *M. halliana* tea are essential for providing theoretical basis for quality control and tea production.

2. Materials and Methods

2.1. Material and Reagents. Quercitrin, 3-hydroxyphloridzin, and phloridzin with purity greater than 98% were made by our laboratory. Acetonitrile (HPLC grade) was purchased from Avantor Performance Materials, Inc. (USA). Methanol (HPLC grade) was purchased from Tianjin Shield Fine Chemicals Co., Ltd. The water was Wahaha pure water.

The series of glucose standard solutions (10~100 μg/mL): the glucose (Xiangshui Tianyi Huagong Co., Ltd., Jiangsu) was weighed via drying at constant temperature using a constant temperature incubator (LZJS, Shenzhen in the South China Sea Masson Technology Industrial Co., Ltd.) at 105°C. Metered volume was filled with water to 1000 mL and then shaken well and 1, 2, 4, 6, 8, and 10 mL were sucked from it, respectively. Then they were transferred into six volumetric flasks (100 mL), diluted with water to volume, and shaken well, respectively. They were stored at 0–4°C refrigerator for two weeks.

2.2. Plant. Air-dried leaves were collected from Jinming district, Henan University (Kaifeng, Henan province of China). Among them, the first batch of samples was collected on March 11 in 2016, the second batch was collected on March 16 in 2016, the third batch was collected on March 21 in 2016. The plant was identified as *M. halliana* of family Rosaceae by Professor Changqin Li (Henan University, Kaifeng, China). The voucher specimens were deposited in the Institute of Natural Products, Henan University (number 20160312).

2.3. Preparation of M. halliana Tea. March *M. halliana* leaves were used. The *M. halliana* tea was made by de-enzyming, rolling, drying, and the same processes. Specific tea was processed as described in the patent [19].

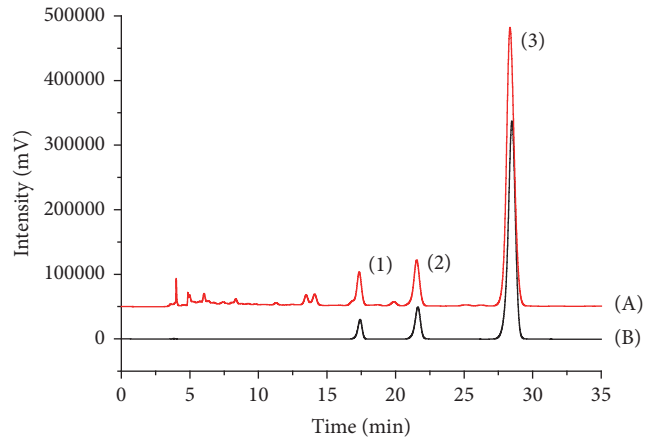

Figure 1: HPLC chromatograms of sample solution (A) and standard solution (B); quercitrin (1); 3-hydroxyphloridzin (2); and phloridzin (3).

2.4. Analysis of Chemical Components

2.4.1. Preparation of Standard Solution and Test Sample Solution. The three standard solutions of quercitrin, 3-hydroxyphloridzin, and phloridzin were prepared in methanol at the concentrations of 486, 466, and 4500 μg·mL^{-1} and stored at 4°C, respectively.

M. halliana leaves or tea (0.02 g, 50 mesh) were mixed with 1 mL of methanol solution in centrifuge tube. The mixture of solid and liquid was centrifuged at 5000 r·min^{-1} for 5 min after being extracted by ultrasonic (50 min, 500 W). Then, the extract was filtrated through a 0.22 μm microporous membrane. The subsequent filtrate was taken as the test sample solution.

2.4.2. Development of the HPLC. LC-20AT high-performance liquid chromatography (Shimadzu) was used and the system was equipped with a degasser, a quaternary gradient low pressure pump, the CTO-20AC column oven, a SPD-M20A UV-detector, and a SIL-20AC autosampler. The data were acquired and processed using LC-solution chromatography data processing system.

Two chromatographic separation steps were all performed on a Agilent TC-C_{18} column (4.6 mm × 250 mm, 5 μm) at a column temperature of 25°C. The flow rate of mobile phase was 0.8 mL·min^{-1}. The UV detection wavelength was set at 270 nm. All the injection volumes were 10 μL. The mobile phase consisted of acetonitrile-1% phosphoric acid (20 : 80, v/v). The HPLC chromatograms of the standard solution and the extract of sample were shown in Figure 1.

2.5. Analysis of Nutritional Components

2.5.1. Determination of Crude Protein Content. 0.5010 g (40 mesh) of *M. halliana* tea powder was accurately weighed. The extraction method of crude protein was determined according to the method as described in Chinese National Standard (GB 5009.5-2016) and ultraviolet spectrophotometric method was used to measure the protein content. At the

same time, blank experiment was carried out with the same method to calculate the total nitrogen content. The protein content formula is as follows: protein content = total nitrogen × 6.25.

2.5.2. Determination of Crude Fat Content. 5.001 g of *M. halliana* tea powder was accurately weighed. Crude fat was measured by the soxhlet extraction method as described in Chinese National Standard (GB 5009.6-2016) previously. The fat content formula is as follows: fat content = $(m_1 - m_0)/m_2 \times 100$, where m_1 refers to quality of absorption bottle and crude fat (g); m_0 represents the quality of absorption bottle (g); m_2 is the quality of sample (g).

2.5.3. The Content Determination of Reducing Sugar. M. halliana tea powder was accurately weighed as 4.0036 g. The reducing sugar extraction method was determined according to the method described in Chinese National Standard (GB 5009.7-2016) and we used direct titration method for calibration. The reducing sugar formula is as follows: the reducing sugar content (%) = $[m_1/(m \times V/250 \times 1000)] \times 100$.

m_1 is quality of the reducing sugar (mg); V is consumption volume of sample solution in calibration (mL); m is quality of sample (g).

2.5.4. The Content Determination of Total Soluble Sugar. 0.1 g of *M. halliana* tea powder was accurately weighed and extracted. The total soluble sugar content was determined according to the method described previously [20]. Meanwhile, the method for determining the total soluble sugar was the same as that used for determining the reducing sugar content. The total soluble sugar formula is as follows: the mass percentage of total sugar = $(c \times f \times V_{\text{Sample total}}/10^6 \times m) \times 10$ (c is sugar concentration of standard curve (μg/mL); f is dilution ratio; $V_{\text{Sample total}}$ is total volume of sample (mL); m is quality of sample (g)).

3. Results and Discussion

3.1. Analysis of Chemical Components

3.1.1. Linearity. The stock standard solutions (0.2, 1, 5, 10, 15, 20, and 25 μL) were accurately injected to chromatographic instrument for the construction of calibration curves, respectively, and the corresponding sample sizes of the injected quercitrin were 0.0972, 0.486, 2.43, 4.86, 7.29, 9.72, and 12.15 μg, respectively; the corresponding sample sizes of the injected 3-hydroxyphloridzin were 0.0932, 0.466, 2.33, 4.66, 6.99, 9.32, and 11.65 μg, respectively; the corresponding sample sizes of the injected phloridzin were 0.9, 4.5, 22.5, 45.00, 67.5, 90.00, and 112.5 μg, respectively. The calibration curves were constructed by plotting the peak areas versus the injected sample quality (μg) of each compound. The results were presented in Table 1. The r values were in the range from 0.9991 to 0.9998, which indicated that the methods displayed good linearity.

3.1.2. Precision. 10 μL of each the stock standard solution was accurately injected to chromatographic instrument. The stock solutions were analyzed in six replicates within one day for determining the precision of the developed assay. The relative standard deviations (RSDs) of peak areas for the three compounds were 0.35%, 0.35%, and 0.79%, respectively. The results indicated that the methods were precise for quantitative analysis of phloridzin, 3-hydroxyphloridzin, and quercitrin.

3.1.3. Stability. The sample solutions were prepared under the optimum extraction conditions and placed at room temperature, and then 10 μL of sample solution was injected into chromatographic instrument at 0, 3, 6, 9, 12, and 24 h, respectively. The RSDs of peak areas for the three compounds were 0.25%, 0.45%, and 0.41%, respectively. The results indicated that the sample solution was basically stable at room temperature within 24 h.

3.1.4. Repeatability. Six test sample solutions were processed under the optimum extraction conditions, and 10 μL of each solution was then injected to chromatographic instrument for analyzing. The RSDs of peak areas for the three compounds were 0.33%, 2.38%, and 1.24%, respectively, indicating that the analytical methods have the acceptable level of repeatability.

3.1.5. Recovery. Nine batches of leaves and flowers samples of *M. halliana* were prepared and divided into three groups, respectively. Then three standard substances at three different amounts were added to the leaves and flowers samples. The spiked samples were prepared according to the optimum extraction conditions. All the calculated recovery values of the analytes ranged from 98.19 to 99.35% and the RSDs were 1.6%, 1.4%, and 1.9%, respectively. The results demonstrated that the methods were reasonable and feasible.

3.1.6. Sample Analysis. The mass fractions of phloridzin, quercitrin, and 3-hydroxyphloridzin in original leaves and tea were presented in Table 2 ($n = 3$), respectively.

3.2. Analysis of Nutritional Components

3.2.1. The Content Determination of Crude Protein. According to the above experimental method, the standard curve of crude protein was drawn. We took extract of *M. halliana* tea and measured its crude protein content. The field experiments were carried out with six replications and then the data were expressed as mean.

The absorbance of *M. halliana* tea was 0.0136, when it was substituted into the standard curve and the crude protein content of *M. halliana* tea was 4.362 g/100 g. Protein played an important role in the body, which provided power and energy for the protein metabolism and maintained the normal operation of all kinds of tissues. It also can strengthen the body resistance, hypoxia, and fatigue resistance and have high nutritional value, which is the important nutritional protein for old body [21]. The crude protein content of *M. halliana* tea was 4.362 g/100 g which explained that it had more amino acids, which could promote the formation of aroma in tea. And the stated amino acids colloid in tea soup, which played an important role in keeping clear of the

Table 1: Regression equations and linear ranges for three compounds.

Compound	Regressive equation	r	Linear range/μg
Phloridzin	$Y = 1165813.0631X + 976247.0924$	0.9996	0.9~112.5
Quercitrin	$Y = 1306866.7475X + 58713.4808$	0.9998	0.0972~12.15
3-Hydroxyphloridzin	$Y = 654528.6618X + 41738.1201$	0.9991	0.0932~11.65

Here Y is the peak area; X refers to injection amount (μg).

Table 2: The contents of three flavonoids in *M. halliana* tea and original leaves for different periods (mg/g).

Compound	Period					
	I		II		III	
	Tea	Original leaves	Tea	Original leaves	Tea	Original leaves
Phloridzin	448.118	382.687	299.486	301.121	504.680	251.456
Quercitrin	9.919	4.237	14.877	7.077	7.412	7.182
3-Hydroxyphloridzin	143.630	112.306	110.055	91.911	137.408	84.023

beverage and the stability of the colloidal solution. It also had offset function of the nervous system excitement caused by caffeine. So *M. halliana* tea can enhance the body resistance, hypoxia, and fatigue resistance and have high nutritional value.

3.2.2. The Content Determination of Crude Fat. According to the method and formula, the crude fat of *M. halliana* tea was 2.399 g/100 g. The lipid composition in tea played a positive role in the formation of the tea aroma, which could accelerate the absorption of fat-soluble vitamins and prevent vitamin deficiency disease. The fat content in tea was less confirmed by medical research, but it had function of improving human immunity, preventing cancer, and so on. Therefore, the special population who had liver disease, hypertension, hyperlipidemia, and pancreatic function not congruent also easily drinks this in addition to the ordinary people.

3.2.3. Determination of Reducing Sugar Content. The cupric tartrate solution consumption of *M. halliana* tea was 9.98. According to the formula, the reducing sugar of *M. halliana* tea was 8.5 mg/100 g.

3.2.4. Determination of Total Soluble Sugar Content. According to the above method, the standard glucose was diluted into six concentrations with gradient dilutions; then the series of glucose standard solution concentrations were taken as the abscissa and absorbance was taken as the ordinate to make a standard curve and calculate the regression equation; the equation was $y = 0.003 \times X + 0.005$ ($R^2 = 0.999$).

The absorbance of total soluble sugar was taken into the equation to draw standard curve for the total sugar contents of sample, and all the field experiment results were carried out with six replications and then the data were expressed as mean. According to the method and formula, the total soluble sugar of *M. halliana* tea was 6.8 g/100 g.

The contents of total sugar and reducing sugar, which are taken as the criteria for evaluating the plant nutrition, are the important components in food flavor and nutrition. Tea polysaccharide had antidiabetic, hypolipidemic, antioxidant, and immunoregulation function [22]. From the results of total soluble sugar and reducing sugar, *M. halliana* tea can be developed as a health care food in the prevention and treatment of those diseases.

4. Conclusions

The contents of glycosides, being made up of sugar and nonsaccharide components, are higher than those in other kinds of medicinal materials such as root, peel, flower, and fruit. Glycosides as the active ingredients coexisted with a variety of enzymes in many traditional Chinese medicines. Thus, they can be hydrolyzed by those enzymes. Therefore, Chinese traditional medicine containing glycosides can inhibit enzyme activity and protect glycosides by frying, cooking, and other methods, which can effectively control the glycosidase solution [23].

Figure 2 showed that the three flavonoids contents of all the whole *M. halliana* tea were higher than that of the original leaves, the phloridzin contents of which were the highest. The possible reason was that glycosides digestion ability is reduced and glycosides component content of *M. halliana* tea after stir-frying increased, or there might be some components by dissolution and decomposition and they might turn into new compositions. Different *M. halliana* teas came from different original leaf source, and the contents of 3-hydroxyphloridzin and phloridzin presented a decreasing and then increasing trend, and the content of quercitrin displayed an increasing and then reducing trend. Compared with our group previously related research [15], we found that the significant difference in the contents of three flavonoids existed in different harvest periods.

Phloridzin as the main ingredient in *M. halliana* [11], which had antidiabetics, improving memory, antioxidant, anticancer, estrogenic, and antiestrogenic activities [24–26], was a competitive inhibitor of glucose transport [27]. Phloridzin has extensive application and helps in development in food, new drugs, and natural health food because of the low toxicity characteristic. *M. halliana* is one of China's unique

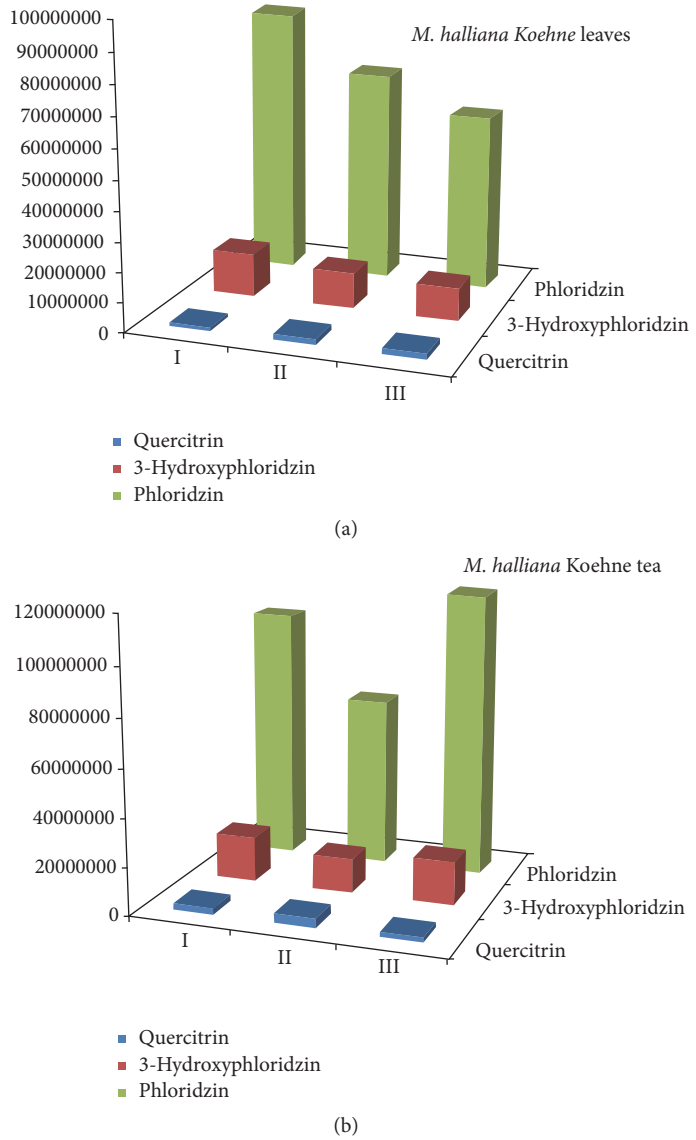

Figure 2: The contents of three flavonoids in *M. halliana* tea and original leaves for different periods.

plants and cultivated in various regions, but mainly as ornamental plant. Because of its rich resources, wide distribution, and easiness of breeding, cutting, and grafting, it brings favorable conditions for mass production. The processing tea of *M. halliana* was similar to green tea production process and it could be harvested in summer and autumn. Our study developed *M. halliana* tea as a health care drinking and established a HPLC method for simultaneous determination of quercitrin, 3-hydroxyphloridzin, and phloridzin; meanwhile, the analysis of the same compositions' change of *M. halliana* tea before and after processing was contrasted.

Through the analysis of the main nutrients of *M. halliana* tea, we found that diversity of nutrients, containing the total sugar, reducing sugar, crude protein, crude fat, and other nutrients, is the tea nutrition characteristic. Therefore, the nutrient contents of *M. halliana* tea are quite high, and some nutrients are quite rich, which is helpful to comprehensive understanding of the benefits of drinking tea. This study showed that *M. halliana* tea had certain development in food and nutrition health care value. We believed that it will become very popular after comprehensive analysis of the main nutrition compositions and efficacy components of *M. halliana* tea.

Conflicts of Interest

The authors declare that there are no conflicts of interest.

Authors' Contributions

Zhenhua Yin and Yong Zhang contributed equally to this work.

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Research Article

Influence of Roasting on Sensory, Antioxidant, Aromas, and Physicochemical Properties of Carob Pod Powder (*Ceratonia siliqua* L.)

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The main objective of this research was to compare physicochemical parameters, antioxidant activity, lipid composition, and sensory analysis of initial and roasted carob pod powder (*Ceratonia siliqua* L.) obtained at different roasting temperatures. The roasted products became darker and the average moisture content, water activity, oil content, and sweetness values decreased at higher temperatures. Total polyphenol content and antioxidant activity increased with increasing roasted temperature. Oleic acid, linoleic acid, and palmitic acid were the main fatty acids present in carob oil. Results showed that the roasted carob pod powders are sweeter, have more caramel-like taste, and have more cacao-like aroma at lower roasting temperatures but have more astringent taste, coffee-like aroma, and roasted aroma at higher roasting temperatures.

1. Introduction

Ceratonia siliqua L. (carob) is an evergreen tree belonging to the Caesalpinioideae subfamily of Leguminosae family [1–6]. The scientific name of carob tree derives from Greek *keras*, horn and Latin *siliqua*, indicating the hardness and shape of the pod. It is also known as St. John's bread with reference to its presumed use by St. John the Baptist [7]. The centre of origin of *C. siliqua* carob pod powder was recognized in the eastern Mediterranean region (Turkey and Syria). The Greeks introduced carob in some European countries, like Greece and Italy, and the Arabs spread it along the North African coast and north into Spain and Portugal [8].

Carob was spread in recent times to other Mediterranean-like regions such as California, Arizona, Mexico, Chile, and Argentina by Spaniards, to parts of Australia by Mediterranean emigrants and to South Africa and India [9, 10]. Carob has been used for over 4000 years as feed and food, especially in times of food shortage, mainly due to its sugary pulp [11–13]. Carob kibbles are traditionally used to make a boiled juice product, named "pekmez," which is mainly consumed in Turkey [14–17]. About 8 percent of the high cholesterol sufferers know and use carob in Turkey. They usually mash the carob kibbles and eat it either with yogurt or raw, in order to lower their cholesterol [18]. In the Middle East, carob is usually well known among people as purgative, antidiarrheal, and antiulcer. It is also used to treat mouth inflammation and tonic in this area (Jaradat) [19]. Lev and Amar [20] reported that, in Kingdom of Jordan, the carob kibble is known as stomach strengthener and phlegm clearer; the carob jam is also used for tongue sores and stomachache treatments and the seed is usually used as purgative and toothache calmative. Some researchers have also reported carob uses for its therapeutic virtues as diuretic, antidiarrheal, antitussive, and warts remover [21–23]. Today, the world carob fruit production is estimated at 315000 tons per year [24]. In Algeria, the most carob trees are wild and grow in north and northwest of the country. The annual production of

carob attains 3000 tons per year [10]. Local farmers manually do the harvesting of the whole fruits at the end of the summer from August to October.

Ripe carob pod is brown and contains 10–20% of carob kernels or seeds and 80–90% of carob kibbles [4, 9, 25, 26]. The seeds, surrounded by a brown coat, contain a white and translucent endosperm (also called carob gum, locust bean gum (LBG), or E410) and the yellow germ which is recovered as the byproduct of the seed processing [26, 27]. Locust bean gum is widely used in the food industry as thickening and stabilizing agent in food preparations because of its ability to form viscous solution at relatively low concentration. It is also used in the cosmetic, pharmaceutical, textile, paper, petroleum, paint, oil drilling, and construction industries [10, 13, 27, 28]. Carob germ flour is used as dietetic human food or as a potential ingredient in cereal-derived foods for celiac people [29]. Carob kibbles can be used raw, usually for animal feeding, or roasted, in food industry [10, 11, 14]. They are also used to extract sugars for making syrup or bioethanol [17, 30–32]. The biological activities of the fruit of carob tree are mainly related to the inositol and the polyphenols present in the kibble, galactomannan in the endosperm, and protein content in the germ of kernel of this fruit [10, 26, 27, 33].

In recent years, carob pulp which is a byproduct is becoming more popular for its organoleptic properties, aroma, color, and taste, and also for its dietary quality. Having a cocoa-like aroma of roasted carob, carob pulp is mainly roasted and grinded to powder to be used as cocoa substitute. Unlike cocoa powder, carob is free from the two stimulants caffeine and theobromine [14, 34]. Besides this, carob contains a high amount of sugar, mostly sucrose, and it is also rich in dietary fibers and poor in protein and fat; therefore it has less energy value than cocoa [24, 35]. Carob powder contains daily nutritional amount of potassium, calcium, magnesium, and iron [15, 36]. It is reported that carob comprises a high amount of pinitol, which has beneficial effects on human health, such as the regulation of blood glucose levels and reduction of hyperlipidemia and inflammation [37, 38]. Containing a large amount of bioactive compounds, carob kibble has shown good antioxidant activities [2, 10, 39, 40], anticancer and antiproliferation effects [41], antidiabetic effect [42, 43], cholesterol lowering effect [44, 45], and antimicrobial effects [23] and it has positive effects on cardiovascular diseases [42, 46].

The objective of this work is valorization of carob pulp, which is a byproduct of processing carob to get seeds for carob gum, into roasted carob powders which could be used directly or as a raw material for producing aromas. This global study aims to compare physicochemical parameters, antioxidant activity, lipid composition, sensory analysis of initial and roasted carob powders obtained at different roasted temperatures. This study will permit investigating the possible synergistic effects of the mentioned parameters on final roasted carob powder.

2. Materials and Methods

2.1. Sample. Ripe carob pods were harvested from Tlemcen region, west Algeria. The fruits were cleaned, mixed, and crushed and the seeds were removed. Then, the carob kibbles were roasted at different time/temperature conditions in an artisanal roaster (Figure 1). Grinding roasted carob in grinder was followed by sieving powders at granulation less than 100 μ, which was the ultimate operation for preparing samples to be analyzed. The initial grinded carob pulp, C0, was just dried at 110°C. The roasted carobs C1, C2, and C3 have been roasted consecutively at temperatures 110°C, 130°C, and 150°C.

2.2. Moisture Analysis. To determine the moisture, an aluminum cup was filled with about 1 g of extract and placed in an infrared balance (Sartorius MA 150, France), which is based on the thermogravimetric principle. In the beginning, the initial weight of the sample was recorded and afterwards, the sample was dried using an infrared lamp. An integrated balance measured the weight of the aluminum cup, continuously. The overall weight loss was interpreted as the corresponding humidity.

2.3. Water Activity Analysis. The aw value was measured using the VSA (Vapor Sorption Analyser, AQUALAB, France). 300 mg of powder was placed in an inox cup, which was then introduced into the device, and the aw was determined by using a chilled-mirror dew point sensor. The dew point is defined as the temperature at which the air is saturated with water and begins to form droplets. The mirror was cooled until the dew was formed, which was detected by a photodetector. A temperature sensor was used to determine the dew point, with which the device was then able to deduce the water activity of the sample.

2.4. pH Evaluation. After calibration of the pH meter Hanna HI2002, the pH samples of different powders were measured in triplicate. The concentration of each solution was at 10% m/v.

2.5. Color Assessment. The color of the products was measured using the Chromameter Konica Minolta CR-410 $L^*a^*b^*$ system, which is a simplified mathematical approximation to a uniform color space, composed of perceived color differences. Any color represented in the rectangular coordinate system of axes L^*, a^*, and b^* can alternatively be expressed in terms of polar coordinates with the perceived lightness L^* and the psychometric correlate of chroma (C^*) as shown in the equation [47, 48]: $C^* = (a^{*2} + b^{*2})^{1/2}$. Three measurements were made for each powder preparation and the mean value was reported. The results were expressed in C^*. Detailed analysis has been explained and detailed in our previous publication (Pingret et al. 2011) [47].

2.6. Total Sugars Analysis and Sucrose, Glucose, and Fructose Analysis. The phenol-sulphuric acid method known by Dubois method was used to evaluate total sugars of unroasted carob and different roasted powders. Five grams of each powder was mixed with 50 mL of water with Ultra-Turrax and then passed to centrifugation. The supernatant was transferred into tubes by PIPETMAN to avoid any floating particles and diluted at 1/500 to obtain the adequate values

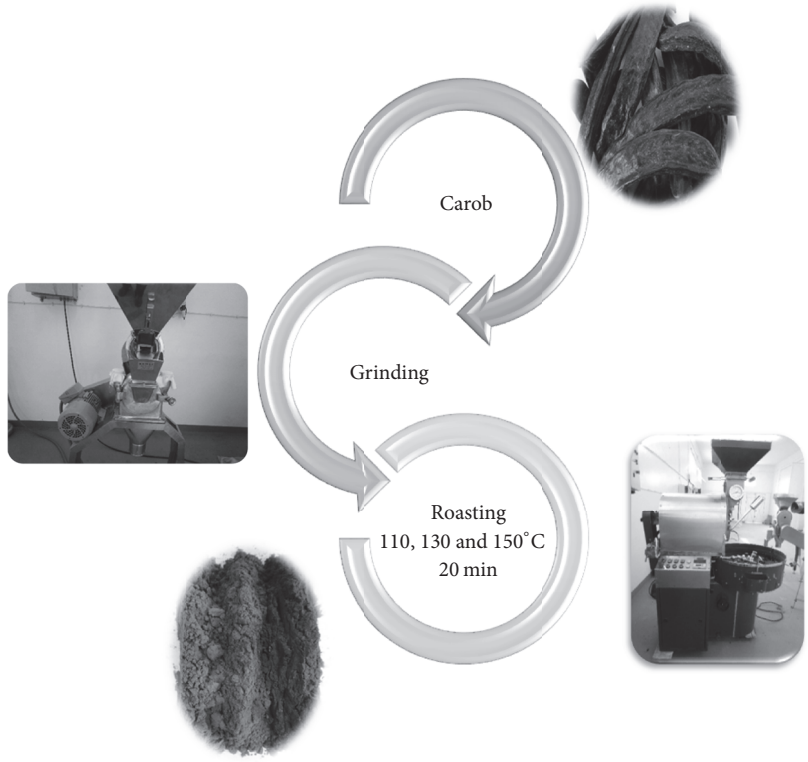

Figure 1: Roasting process of carob pulp.

comparing to the standards of glucose values. 2 mL of diluted solution was put in tube containing 1 mL of phenol 5% and then, 5 mL of concentrate sulphuric acid was added and left in water bath at 25–30°C for 20 min. These tubes were immediately cooled at 20°C with tap water. The absorbance was measured at 485 nm with spectrophotometer Jenway 700 against the blanks. Phenol correction was done against a solution containing 2 mL of water added by 1 ml of phenol 5% and 5 mL of sulphuric acid. The glucose assay was also prepared with the known concentrations from 0,025 g/L to 0,1 g/L. A standard curve of corresponding absorbance was drawn.

An enzymatic method was used to determine biochemically different sugars, like sucrose, glucose, and fructose with enzymatic kits (Biosentec, France).

The concentration of D-glucose/D-fructose/sucrose in the sample, used in the assay procedure, had to be between 0.05 and 0.8 g/L, wavelength 340 nm, optical path 1 cm, and temperature 20–37∘C.

2.7. Total Ash Content Assay. Three grams of each sample was weighted in crucibles which has to be preashed and was put in muffle furnace at 550°C for 5 hours. After incineration, the crucibles were left in desiccator for 30 mn and then weighed. The content of total ash is calculated as percentage relative to dry matter.

2.8. Polyphenol Content Assay. Five grams of each powder was mixed with 2 times 50 mL of methanol/water (80/20) with Ultra-Turrax for 7 min and then passed to centrifugation. The supernatant was transferred into the flasks and completed at 100 mL. The total phenolic content was evaluated by using the Folin-Ciocalteu method. Twenty microliters of the extract was mixed with 100 μL 1 : 10 diluted Folin-Ciocalteu reagent and 80 μL sodium carbonate solution (75 g/L) in wells of 96-well microplate. After 1 h of remaining in the darkness and at room temperature, the absorbance was measured at 740 nm in the microplate reader. Gallic acid monohydrate (1.05–21 mg/100 mL) was used as the standard for the calibration and the construction of a linear regression line and water as blank; the total phenolic content was calculated as gallic acid equivalents in mg/L or g/100 g of matrix. Polyphenols quantification was performed in triplicate.

2.9. DPPH Radical Scavenging. The radical scavenging activity of extract was evaluated by a modified version of the method, proposed by Brand and Williams, converted into micro method. More specifically, a stock methanolic solution (10 mg/mL) of each extract was diluted to prepare the samples, ranging from 20 to 0.625 μg/mL and then, 50 μL of each sample was pipetted into 96-well plates in triplicate and was assessed in every well 50 μL of DPPH solution (0.5 mM in methanol). Plates were placed in dark for 40 min at room temperature and then the absorbance was measured at 510 nm. The results were plotted as the percentage of remaining DPPH (% I DPPH) against the concentration (μg/mL) of the added samples.

$$\%\ \text{I DPPH} = \left[\frac{A\ \text{blank} - A\ \text{sample}}{A\ \text{blank}}\right] * 100, \quad (1)$$

where A sample = absorbance of the sample and A blank = absorbance of the blank.

Results are expressed as inhibitory concentration (IC50) which corresponds to extract concentration (μg/mL) or g/100 g of matrix, required to quench 50% of the initial DPPH radicals under the given experimental conditions.

2.10. Conventional Soxhlet Extraction (CSE). For CSE, 100 g of carob was placed in the extraction chamber of a Soxhlet apparatus (125 mL capacity). The cellulose thimble was plugged with cotton in order to avoid transfer of sample particles in the distillation flask. The Soxhlet apparatus, fitted with a condenser, was placed on a 2000 mL boiling flask, containing 1000 mL of solvent. Extraction was performed using a solid to liquid ratio of 1 to 10 (m/v), for 8 hours. After extraction, the extract was concentrated until being dried by solvent evaporation under vacuum (Laborota 4001, Heidolph, Germany) and finally, it was conserved at 4°C before analysis. Detailed analysis has been explained and detailed in our previous publication (Meullemiestre et al. 2016) [49].

2.11. Lipid Analysis Composition. Lipid classes in oil extract were determined by high-performance thin-layer chromatography (HPTLC), using two different development chromatography methods to separate polar and neutral classes. Lipids were quantified by a CAMAG 3 TLC scanning densitometer (CAMAG, Muttenz, Switzerland) with identification of the classes against the known polar and neutral lipid standards. Lipid classes of each carob extract were identified and quantified against those of corresponding lipid standards. Detailed analysis has been explained and detailed in our previous publication Meullemiestre et al. 2016 [49].

2.12. Preparation of Fatty Acids Methyl Esters (FAMEs). FAMEs were prepared from the lipid extract, using acid-catalyzed transmethylation. 1 mL of methanolic sulphuric acid (5%) solution was added to a specific amount of extracted carob oil. Triheptadecanoin (C17:0 TAG) was used as internal standard. Detailed analysis has been explained and detailed in our previous publication (Meullemiestre et al.) 2016 [49].

2.13. FAMEs Analysis. Fatty acids methyl esters were separated, identified, and quantified by gas chromatography, coupled with flame ionization detector (GC-FID). The instrument Agilent (Kyoto, Japan) was equipped with a BD-EN14103 capillary column 30 m × 320 μm × 0.25 μm (Agilent). FAMEs were identified by retention time and comparison with purified FAME standards (Sigma Co., USA). Detailed analysis has been explained and detailed in our previous publication (Meullemiestre et al.) 2016 [49].

2.14. Sensory Analysis. Sensory analyses were conducted by a panel consisting of 18 graduate students from the University of Avignon, France. The subjects were seated in sensory booths with appropriate ventilation and lighting. The samples were presented to each panelist on white polystyrene plates. Subjects were instructed to place the stimuli on the tongue and rub the tongue against the palate. Tap water was supplied to the panelists for rinsing between samples. The following attributes were evaluated for the three products: roasted aroma, cacao aroma, sweet taste, astringent taste, and caramelised taste. For overall quality, the scale range was from 0 to 10. On this scale, a score of 0 represented the weakest attribute and a score of 10 represented the strongest one. Detailed analysis has been explained and detailed in our previous publication (Pingret et al. 2011) [47].

Table 1: Physicochemical characterization of initial and roasted carob pod powder.

	C0	C1	C2	C3
Moisture (%)	9 ± 0.8	6.3 ± 0.6	4.3 ± 0.8	3.5 ± 0.7
Aw	0.6 ± 0.05	0.2 ± 0.05	0.155 ± 0.05	0.14 ± 0.05
pH	5.6 ± 0.1	5.5 ± 0.1	5.4 ± 0.1	5.5 ± 0.1
Total sugars (%)	43.4 ± 0.5	36 ± 0.5	27.3 ± 0.4	15.4 ± 0.5
Sucrose (%)	27.6 ± 0.2	23.3 ± 0.2	10.5 ± 0.2	6.2 ± 0.2
Glucose (%)	4.1 ± 0.2	2.6 ± 0.2	2.5 ± 0.2	1.6 ± 0.2
Fructose (%)	5.9 ± 0.2	4.0 ± 0.2	3.1 ± 0.2	1.8 ± 0.2
Ash	3,22 ± 0,3	3,78 ± 0,3	4,1 ± 0,3	4,92 ± 0,3
Coloration C^*	22.6 ± 0.5	23.1 ± 0.5	20.7 ± 0.5	16.9 ± 0.5

3. Results and Discussion

By roasting the carob pulp under different conditions of roasting time-roasting temperature combinations, the obtained product can have different specifications such as modulated color, aroma, and taste. Studying the effects of these parameters and the physicochemical ones allows the food industries to control the process for obtaining the best products.

Roasting has been done at different temperatures for the same processing time (20 min) to obtain different products in terms of color, aroma, and taste. The initial grinded carob pulp, C0, was just dried at 110°C to obtain the first product, C1, which has a lighter color and the specific initial carob odour. At 130°C, carob sugars undergo the Maillard reaction and the caramelisation, producing a new product, C2, which is obviously different from the unroasted one. Roasting at high temperature (150°C), the last product, C3, was completely dark as a result of producing brown pigments. The overall experimental plan is shown in Figure 2.

3.1. Comparison of Physiochemical Properties of Carob at Different Roasting Temperatures. Different parameters as moisture, water activity, total sugars, and different sugars as sucrose, glucose, and fructose have decreased when the temperature of roasting increases. The results are shown in Table 1.

The average moisture content of nonroasted carob powder samples was determined as 9% which reduced to 6.3%, 4.3%, and 3.5% after 20 min of roasting at 110°C, 130°C, and 150°C, respectively. These values are intermediate between carob moisture values of Sahin et al. 2009 [50] and the values reported by Vitali Cepo et al. 2014 [51]. This can be due to

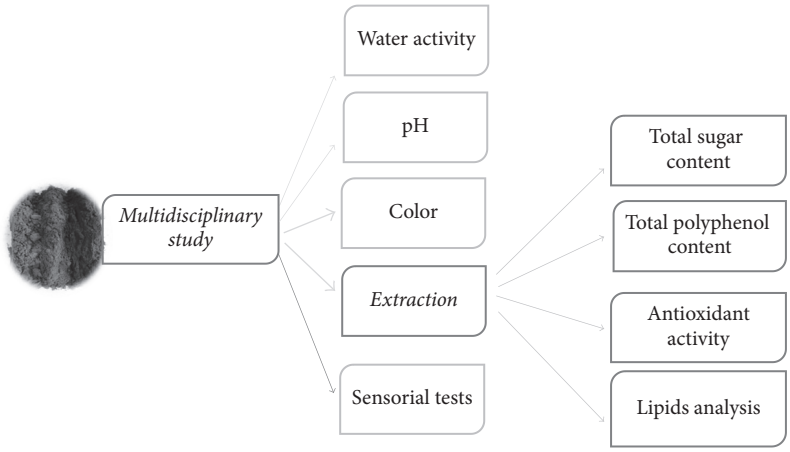

Figure 2: Multidisciplinary study of roasting carob pulp.

the fruit variety and also to ripening stage when harvested. C1 has been dried and the water activity has decreased so much regarding C0. C2 and C3 have approximated values of moisture and water activity due to the maximal evaporation of water. Reduction in the moisture content allows an easier milling and can extend the shelf-life of the carob powder as explained by Iipumbu [7]. In Table 1, it was also observed that water activity decreased as the roasting temperature increased as revealed by Yousif and Alghzawi 2000 [14]. The aw value (water activity), important parameter in browning reactions, gives information about chemical, physical, and microbiological product quality. Sahin et al., 2009 [50], have reported that pH decreases gradually with increasing temperature after 20 mn. pH value of the different samples decreases slowly when increasing roasting because of the caramelisation reaction releasing acidity, making the solution sour, and also the Maillard reaction products (MRP) [52].

Maillard reaction requires temperatures superior to 50°C and it is favoured when pH is around 4–7 while caramelisation proceeds at temperatures superior to 120°C and pH between 3 and 9 [52]. Unroasted carob undergoes drying with Maillard reaction at pH 5.5 at 110°C to obtain C1, when C2 and C3 have both caramelisation and Maillard reaction.

Carob is sweet product due to its content of total sugars; mostly sucrose, glucose, and fructose decrease more and more with the temperature and the time of roasting. Roasted carob C3 is less sweet than the natural carob. The ratio of individual sugars to total sugar in carob was similar in the three-roasted carob. The sweetness values decreased as the roasting temperature increased. Heating sucrose in concentrated solution at high temperature leads to hydrolysis and production of fructose and glucose. These components participate in different reactions of caramelisation to form stable 5-hydroxymethylfurfural (HMF) [53]. The Maillard reaction is triggered between glucose and an amino acid and a multitude of reactions causing the formation of MRPs as furfurals, hydroxymethylfurfural (HMF), and final products, melanoidins (brown nitrogenous pigments) [54]. At 110°C, C0 is dried and the reaction of Maillard begins at the first five minutes. At 130°C and 150°C, raw carob undergoes Maillard and caramelisation reaction causing brown pigments giving specific color to each product. Little increase of minerals content in the four products is due to the calculation of the content to the dry matter.

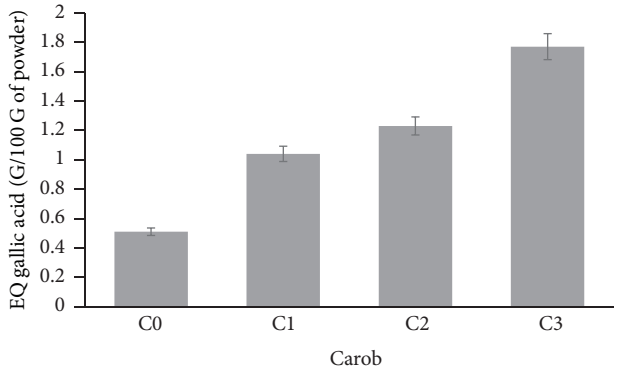

Figure 3: Total polyphenol content (TPC).

3.2. Comparison of Antioxidant Properties of Carob at Different Roasting Temperatures. Phenolic compounds are of high interest as alternative for synthetic antioxidant to prevent lipid peroxidation in food products. Total polyphenol content (TPC) extracted from carob powder by Ultra-Turrax (2 ∗ 50 mL, 80% methanol, 2 ∗ 7 min) is shown in Figure 3.

The obtained TPC increased by increasing the roasting temperature of 110°C, 130°C, and 150°C, respectively. C0 contains 0.51% a percentage in concordance with Sahin et al. 2009 [50]. Phenolic content, obtained from soluble fraction, increases with increasing temperature as revealed by Vitali Cepo et al. 2014 [51] clearly indicating that, during roasting, polyphenols are released from certain polymers making them available for absorption. They also have shown that, after prolonged roasting longer than 15 min at 150°C or 30 mn at 130°C, the increase of the total polyphenolic compounds in soluble fraction and antioxidant activity is stopped. C1, C2, and C3 have been roasted at 110°C, 130°C, and 150°C,

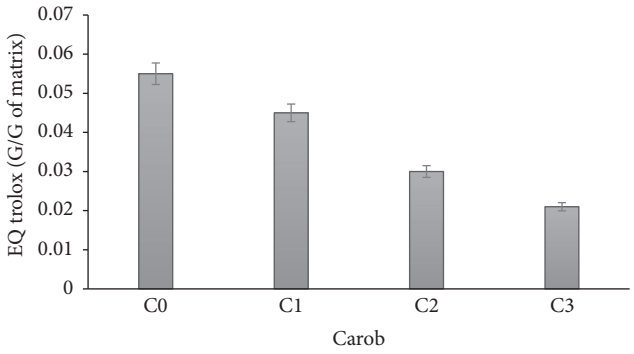

Figure 4: IC50 values determined by the DPPH assay for carob extracts obtained by Ultra-Turrax.

respectively, during 20 mn; it could be the necessary time for obtaining the higher amount of polyphenols.

The antioxidant potential of the extracts is shown in Figure 4. The DPPH tests provided information about the activities of the compounds with stable free radicals; DPPH effect was assumed to be due to their hydrogen donating ability. Higher IC50 values signify less antioxidant activity and vice versa.

Comparing TPC to AA (antioxidant activity) data obtained from the extracts, it can be observed that AA was proportional to TPC. A high antioxidant activity is expected to be caused by its high content in polyphenols. Sahin et al., 2009 [50], have investigated chemical changes of total phenolic content, total antioxidant activity, and browning index on different roasted carob powders; they reported that, during the roasting process, important chemical reactions including sugar caramelisation and Maillard reaction took place, which cause significant changes in product quality. Many studies have focused on the properties of Maillard reaction products (MRPs), particularly on the antioxidant activity of MRPs in food products.

Folin-Ciocalteu reagent detects all phenolic groups present in sample, containing the naturally occurring phenolic and also the newly formed compound during roasting process. MRPs with phenolic type structure can also be determined by the Folin method. The increase in the TPC of the carob powders could be explained by the formation of MRPs with phenolic type structures during the process. The increase in the antioxidant activity of carob with increasing roasting degree was attributed to Maillard reaction products (MRPs) formed during roasting of carob like roasting coffee [55].

Since no standardized method is available to evaluate the antioxidant capacity of plant extracts and numerous methods have been employed to estimate the antioxidant potential, in the present study, DPPH radical scavenging effect was used to assess the antioxidant activity of carob powder before and after roasting.

The antioxidant activity (AA) of the extracts is due to the presence of phenolic compounds [55]. The antioxidant activities of the extracts at different roasting temperatures were measured. According to the results, the extracts of the high roasting temperature, C3, had a higher antioxidant activity and a higher quantity of polyphenols. Maillard reaction and caramelisation products improve antioxidant activity due to the liberation of HMF and also for the formation of melanoidins [50]. Therefore, these results are in concordance with the results of Sahin et al. 2009 [50] and prove that antioxidant activity of the samples increases as the roasting temperature goes up. Time of roasting is an important parameter for controlling the antioxidant activity as shown by Sahin et al. [50, 51] which concluded that roasting carob at 130°C for 30 min can be proposed as the procedure of choice for obtaining carob powder with high antioxidant activity.

3.3. Comparison of Color of Carob at Different Roasting Temperatures. The different roasted carobs were also compared by their physical characteristics as color (Table 1). Heating sugars and sugar rich food causes reactions inducing color and flavour. C0 has lighter color than C1, and from 130°C, the color became brown and then darker brown than the initial color as presented in Figure 5. In their investigation, Vitali Cepo et al., 2014 [51], have revealed that the highest formation of brown pigments was noticed during the first five minutes of thermal treatment at different applied temperature.

3.4. Quantitative and Qualitative Analysis of Lipids Classes. Roasted and unroasted carob were extracted with hexane for 8 hours by Soxhlet extraction. The extracts were centrifuged and evaporated. The obtained oil was analyzed by high-performance thin-layer chromatography (HPTLC) to gain the lipid classes. For obtaining fatty acid profiles, the gas chromatography coupled with a flame ionization detector (GC/FID) was used after transmethylation.

Carob oils were classified into two categories according to their polarities: neutral lipids and polar lipids. Carob oils tend to accumulate neutral lipids, including monoacylglycerol (MAG), diacylglycerol (DAG), triacylglycerol (TAG), free fatty acids (FFA), and alkyl chain. Using the high-performance thin-layer chromatography (HPTLC), neutral lipids of the extracted carob oils were separated and quantified. For all extracted carob oils, the distribution of the different lipid classes was obtained by the external calibration. To quantify the percentage of the lipid classes, four standards were used, including monoglycerides (MAG), diacylglycerol (DAG), triglycerides (TAG), and free fatty acids (FFA) (C18), and deposited on the HPTLC plate. As shown in Figure 6, MAG and TAG were not found in all extracts. DAG and FFA are the major components which is due to drastic conditions of drying and roasting which induce oxidation and hydrolysis of TAG to DAG and MAG [56].

As shown in Figure 6, lipid yields decrease as the roasting temperature increases; it is due to the oxidation induced by the high temperature and the formation of products when reacting with amino acids or proteins causing brown pigments, similar to melanoidins [7, 57]. Fatty acid profiles (Figure 6) show that oleic acid (C18:1), linoleic acid (C18:2n6), and palmitic acid (C16:0) were mainly present in carob oil and represented at least 90% of the total extract (about 50% of C18:1, 20% of C18:2n6, and 20% of C16:0). It was also observed that palmitoleic acid (C16:1) and stearic acid were in

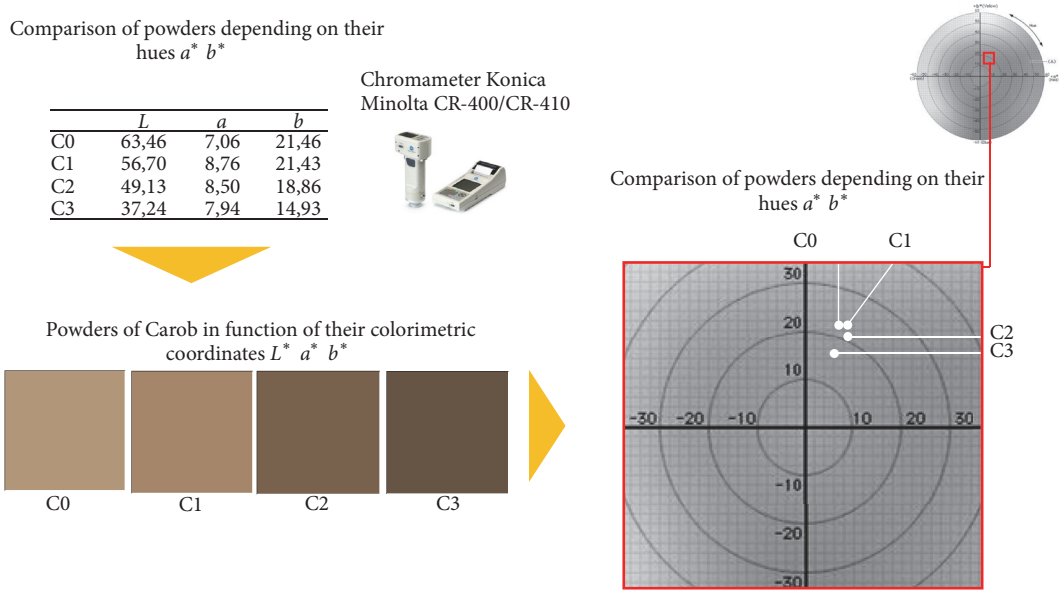

Figure 5: Color assessment of initial and roasted carob pod powders.

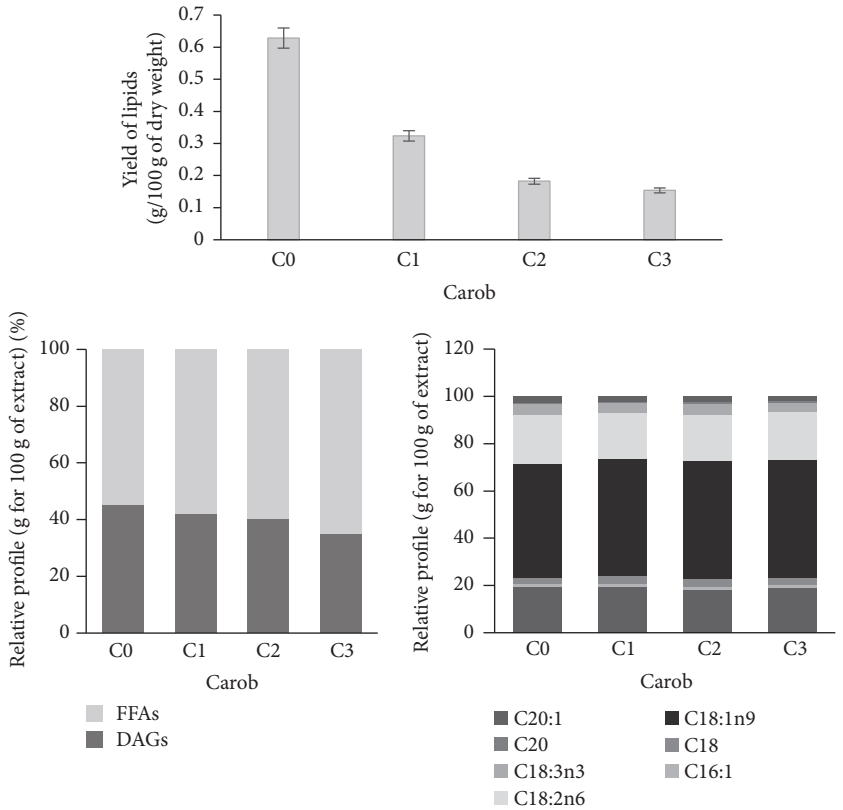

Figure 6: Lipid composition.

minor amounts. The detailed composition for each extract is reported in Figure 6. C0 contains 0,8% of lipids, a very good amount of fat compared to cocoa. By roasting, C1, C2, and C3 have less fat, 0,4%; carob powder can be the best dietary substitute of cocoa [10, 14].

3.5. Sensorial Tests. Roasted carob has potentialities to be a dietary replacement of cocoa due to its lower content in fat and its good content in dietary fiber [10]. Sensorial tests can predict the consumer feedback about an unknown product cocoa replacement.

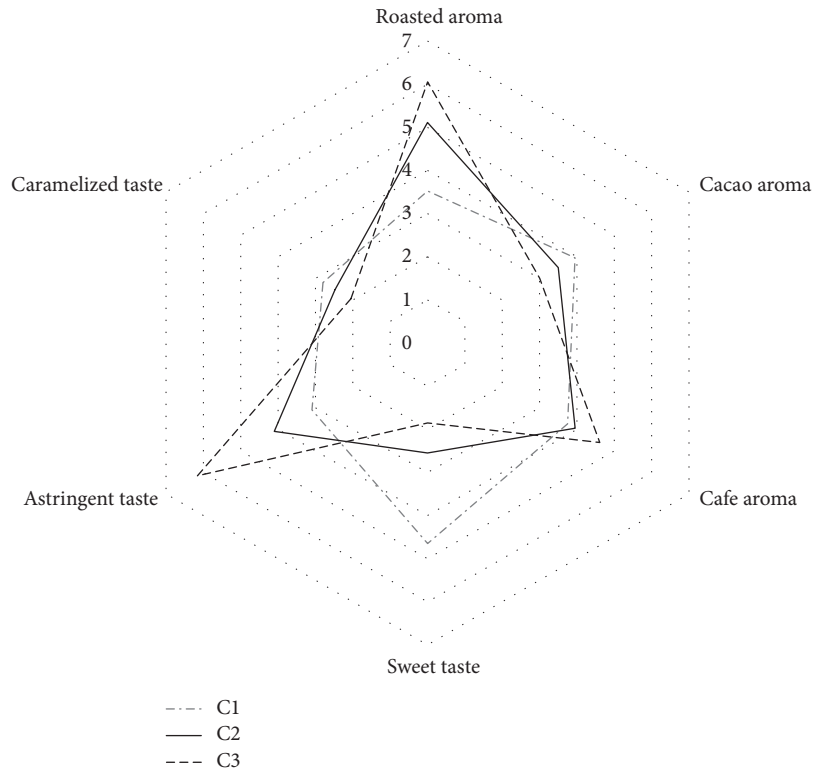

Figure 7: Sensorial analysis.

The results of sensorial tests for the three carobs are correlated with their physical characteristics in Figure 7. The panelists found C1 to be sweeter due to its contents in sugars and mainly in sucrose which can induce reducing the added processing sugars in food industry, have more caramel-like taste, and have more cacao-like aroma than the other ones [14]. They also recognized the most astringent taste, coffee-like aroma, and roasted aroma in C3. They found that C2 has astringent taste and roasted aroma.

Berna et al. [33] recognized that the undesirable smell produced by isobutyric acid in carob can be reduced through roasting process and losing that compound and the higher loss of isobutyric acid will bring a higher sensory quality of the carob powder, which is a factor to consider when establishing the roasting time. The volatile fraction of carob bean pulp during a roasting process has been also analyzed by Cantalejo 1997 [58] and 137 components were identified. 91.4% of the identified compounds in raw carob contained acids, alcohols, and aldehydes; during the roasting process, the amount of these compounds decreased about 51.2% of the total compounds.

4. Conclusion

The effect of temperature on physicochemical parameters, antioxidant activity, lipid composition, and sensory analysis of unroasted and roasted carob powders were compared in this study. The initial carob powder (C0) was dried at 110°C (C1), 130°C (C2), and 150°C (C3) for the same processing time (20 min). The pH of the roasted products decrease just a little but the color of the products became darker, the average moisture content of C0 was reduced from 9% to 6.3% and 4.3% and 3.5%, and the water activity and the sweetness values decreased as the roasting temperature was increased. Total polyphenol content and the antioxidant activity which is due to the presence of the phenolic compounds, Maillard reaction, and caramelisation products increased by increasing the roasting temperature. Carob oil analysis showed that lipid yields decreased at higher roasting temperatures. They tended to accumulate neutral lipids, including monoacylglycerol, diacylglycerol, triacylglycerol, free fatty acids, and alkyl chain. Diacylglycerol and free fatty acids were the major components of all the extracted carob oils, with a decrease in the percentage of diacylglycerol and an increase in the percentage of free fatty acids as the roasting temperature increased. Fatty acid profiles showed that oleic acid, linoleic acid, and palmitic acid were mainly present in carob oil and represented at least 90% of the total extract. The panelists found C1 to be sweeter, have more caramel-like taste, and have more cacao-like aroma than the other ones and the most astringent taste, coffee-like aroma, and roasted aroma in C3. Roasted carob powder could be used as food ingredient in different kinds of food and also as dietary supplement.

Additional Points

Practical Applications. Carob pulp which was byproduct with little value is becoming a real raw material for food ingredients for its organoleptic properties, aroma, color, and taste, and also for its dietary quality. Having a cocoa-like

aroma of roasted carob, carob pulp is mainly roasted and grinded to powder to be used as cocoa substitute. This study shows that by adding a food process such as roasting in the whole chain we could change a byproduct to raw material with high value for producing food ingredients. It is a real success story that could be applied for other products.

Conflicts of Interest

The authors declare that there are no conflicts of interest regarding the publication of this paper.

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Research Article

Effects of Layering Milling Technology on Distribution of Green Wheat Main Physicochemical Parameters

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With layered milling flour technology, the efficiency of the nutrient distribution and hardness was demonstrated with the green and normal wheat separation milling. The results showed that the total content of amino acid in green wheat was 8.3%–13.0% higher compared to the common wheat. Comparing the main nutriments Se, Fe, and Ca between green wheat and common wheat, the results showed that different milling treatment methods are capable of separating the different wheat flour from endosperms, bran, and aleurone layers. Micro- and physicochemical characterization of different wheat flour and layers by means of microscopy techniques and images analysis provided relevant qualitative and quantitative information, which can be useful for the study of the microstructure of green and normal wheat products and also for its processing and utilization.

1. Introduction

Wheat is one of the top cereals of the world with a global annual production of 676 million tons. More than one-fifth of human population feeds on wheat products [1]. The availability of amino acids and mineral substance from wheat has been considered to be the main contributor to the development of wheat breeding [2]. Recently, with the accelerated pace of working and improving of people's living standards, the nutrition, color, and flavor from the main food have been found to increase nutritional value during daily diet processing [3]. Development of Nanyang green wheat has taken place in the last two decades in China [4]. In slow industrialization process, the key is the advanced stages of product development and commercialization of products [5].

Color reactions are the most obvious chemical changes because of their different chemical composition during cooking and are likely to have a direct correlation with other important chemical processes, such as viscosity increment, flavor present, and starch breakdown [6–8]. Furthermore, color of wheat influences man's sensory perceptions of the product. Several researches have been interested in studying purple wheats [9–11]. The composition of the antioxidation fraction has been found to be characterized with anthocyanin from cyanidin 3-O-glucoside and peonidin 3-O-glucoside in purple-grained wheats; however delphinidin 3-O-rutinoside and delphinidin 3-O-glucoside prevail in blue wheats [12, 13].

These bioactive substances are mainly located in the outer membranes of green-grained wheat. The bran fraction contains distinctly higher concentrations of nutrient components than those in common flour [14]. However, conventional wheat flour milling methods, which remove most of the germ and bran, reduce the amounts of total phenolics and microelement in wheat products [15]. Wheat pigment distribution has been assessed pointing out that green pigments of green wheat were located in the aleurone layer [16]. In spite of the interest of producers in obtaining whole-wheat flour specialties with potentially functional properties, marginal attention has been paid to evaluate the performance of green wheat during milling [17, 18]. A single study regarded the evaluation of milling effect on the nutritive properties of green bran and explored some of the physical properties of green wheat and their dependence on moisture content, which can help out in the design of handling, processing, and packaging machinery for green wheat production [19].

Recently, much attention has been focused on green wheat varieties. Moreover, green-grained wheat has been held to have high free radical scavenging ability and polyphenols content. Green wheat varieties have higher protein content than normal wheat and antioxidant activity, being due to the

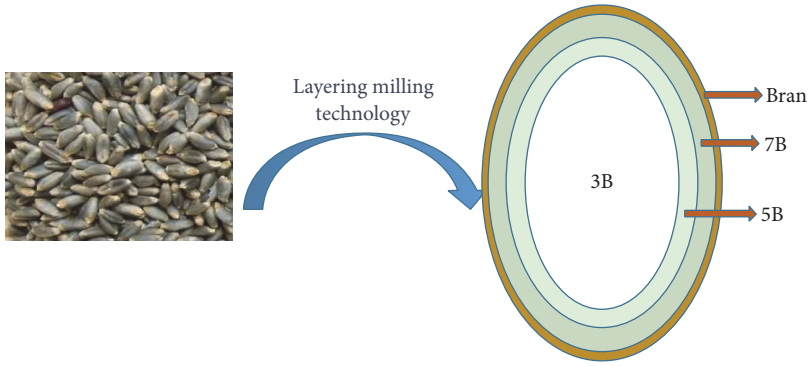


Figure 1: The model of layering milling technology on wheat flour.

presence of abundant phenolic acid and vitamin C [10, 13]. However, green wheat bran has high antioxidant activity, which is positively correlated with pigmentation. The relationships between green wheat and nutrient qualities have been reported by Li et al. [20]. Meanwhile, few scientific studies have illustrated green wheats were different from white wheat or red wheat in protein content, starch types, and mineral component [21]. In this study, to clarify the effect of green wheat, milling methods, and layers types on microelement and amino acids contents and its activities, green wheat variety (BW3201) and one normal wheat variety (YN19) were milled to different degrees (yielding whole-wheat flour, partially debranned grain flour, and refined flour). Wet gluten, microelement, and amino acids of the different flours were analyzed.

2. Materials and Methods

2.1. Materials. Two different wheat (*Triticum aestivum* L.) varieties were collected from the 2014 harvest in Henan province. Green wheat 3104 (GW 3104) was provided by Woerkang Agricultural Technology Co., Ltd., at Nanyang, and Yannong 19 (YN 19) as normal wheat was provided by Mengxiang Food Co., Ltd., at Ruzhou.

2.2. Flour Milling. The wheat seeds were cleaned and stored at room temperature and damaged seeds were removed. The clean samples were processed in experimental mills according to the manufacturers unaltered flows: three milling procedures were used, yielding first-break flour (3B), second-break flour (5B), and third-break flour (7B) [21]. JMFB70×30 (Grain Reserves Corporation, Chengdu, China) with three break systems and three reduction systems was used for first-break flour. LRMM-8040-3-D (Buhler, Wuxi, China) with three break systems and three reduction systems was used for 5B flour and 7B flour. LFS-30 (Buhler, Wuxi, China) was assembled at 3B flour system. To prepare 3B flour, whole-wheat kernels were first stripped of the bran layer using a grain polisher (TYT200, Tianyang Machinery Co. Ltd., Shandong, China) and then milled. Figure 1 shows the model of layering milling technology on wheat flour.

2.3. Determination of Flour Yield. An electric sieve shaking method was used for flour yield determination. The preweighed sample (10 g) was placed on the top sieve of the sieve shaker (LFS 30, Buhler, Wuxi, China). After 5 min of shaking, the material on each sieve was weighed and recorded.

2.4. Grain Analysis

2.4.1. The Wheat Quality Analysis. Thousand grain weight (TGW) was evaluated counting 200 kernels. Determination of hardness index (HI) in this study was determined using the Single Kernel Characterization System (Model 4100, Perten Instruments, Sweden). The model measures the single wheat weight, diameter, moisture, and force needed to crush kernels.

2.4.2. Chemical Analyses of the Wheat. Lipid contents of the wheat samples were determined using the accelerated solvent extraction method [22]. Protein content was determined by Kjeldahl determination. A 0.5 g of green wheat and normal samples was mixed with 4 mL of concentrated sulfuric acid in the 100 mL round-bottom flask, and then the mixed liquid was heated to 440°C with a conventional convective-conductive heating system until it boiled. However, the heating time was no longer than 3–5 min. Ash contents of the wheat samples were determined by employing standard methods of analysis [23]. Wet gluten contents of the wheat samples were obtained by washing Brabender-milled wheat flour according to Wellner method with minor modification [24]. Thirty grams of wheat flour was mixed with 18.6 mL of water for 30 min. The dough was washed with deionized water until no starch was left. Measurements were carried out in triplicate for the physical quality chemical analyses and the values were averaged.

2.5. Analysis of Amino Acids. Amino acids analysis of the wheat was performed according to Du et al.'s method [25]. The gluten flour (100 mg) was hydrolyzed with 10 ml 5 M NaOH for 20 h at 110°C. The mixture was transferred and dissolved in deionized water in a 50 ml volumetric flask. However, the solution was filtered through a 0.45 μm of nylon syringe filter (Filtrex Technology, Singapore). The amount of each amino acid was determined with an automatic amino acid analyzer (Biochrom 30+, Cambridge, UK). Amino acids were postcolumn derivatized with ninhydrin

Table 1: Flour yield of different wheat variety by milling methods.

Variety	3B (%)	5B (%)	7B (%)
GW 3104	65.16 ± 2.23	4.15 ± 0.17	2.24 ± 0.11
YN 19	65.77 ± 2.85	3.26 ± 0.21	2.62 ± 0.17

reagent (0–50 mL/h) and detected by absorbance at 570 nm and 440 nm. The amino acids and standard solution were analyzed under the same conditions, and all of the above measurements were carried out in triplicate.

2.6. Determination of Microelement. Determination of Ca, Fe, and Se was carried out with an Agilent 240FS atomic absorption spectrometer (Agilent Technologies, Santa Clara, CA, USA) equipped with flame atomization (a mixture of air (13.5 L/min) and acetylene (2.9 L/min)). However, the chromium hollow cathode lamp (Cathodeon) operated at a current of 10 mA. The solutions were stored in high-impact polystyrene bottles (Nalgene®, Rochester, USA). All the plastic bottles were cleaned and soaked in 10% (v/v) HNO_3 at least 24 h and rinsed abundantly in ultrapure water before use. Calibration solutions of calcium, ferrum, and selenium were obtained by diluting the standard samples provided by Sigma. The wheat flour samples were also analyzed by Agilent 240FS at the lines 422.7 nm (Ca), 248.3 nm (Fe), and 196.1 nm (Se). The emission mode was set at 766.5 nm for K.

2.7. Scanning Electron Microscopy. The inner surfaces and edges of pieces from different wheat tissues were observed using a KYKY-2800B scanning electron microscope (KYKY Technology Development Ltd., Beijing, China). The flour and bran samples were filtrated, fixed, and dehydrated following the same procedure described for the light microscope at an acceleration voltage of 10 kV and the vacuum of 15 Pa. The dehydrated wheat flour or bran samples were mounted onto SEM stubs with double-sided adhesive tape and were coated with a layer of gold (40–50 nm) prior to observation.

2.8. Statistical Analysis. All experiments were estimated in triplicate, and the data were expressed as mean ± standard deviation (SD). Statistical calculations were carried out using the statistical analysis software OriginPro 9.2 (OriginLab Corporation, Northampton, MA, USA, 2014).

3. Results and Discussion

3.1. Effects of Milling Methods on Flour Yield. There were significant differences in flour yields among the different milling methods (Table 1). 3B and 7B had the highest and lowest flour yields, respectively. GW 3104 showed higher flour yield than YN 19. Flour yield of green wheat (GW 3104) reached 74.04%. However, flour yield of normal wheat (YN 19) only reached 71.65%. Compared with normal wheat, the flour yield of 5B was increased by 27.3%. Interactions between wheat variety and milling method were significant. 3B milling method did not increase significantly flour yield based on GW3104. The most significant reduction was obtained by the 5B and 7B milling methods.

Table 2: Physical and chemical properties of different wheat varieties.

Variety	GW 3104	YN 19
TGW/g	31.32 ± 0.19	36.10 ± 0.13
HI%	60.37 ± 0.22	62.09 ± 0.29
Protein (%)	20.7 ± 0.50	14.59 ± 0.46
Wet gluten (%)	42.3 ± 0.29	29.8 ± 0.17
Lipid (%)	2.13 ± 0.12	1.47 ± 0.15
Ash (%)	2.08 ± 0.07	1.74 ± 0.11

3.2. Kernel Quality Characteristics. Green and normal wheat size fractions were evaluated by kernel quality characteristics. Table 2 shows the kernel characteristics of different varieties of wheat. To different wheat varieties, the changes in physical and chemical properties are shown in Table 2. Thousand grain weight (TGW) is an important factor affecting flour yield as well as grain quality in wheat. TGW is usually controlled by a wide variety of quantitative trait loci (QTLs). TGW of green wheat was lighter than that of normal wheat, but flour yield of green wheat was higher than that of normal wheat, indicating that the bran of the different wheat varieties changed gradually from edge to core during maturation. However, HI of green wheat was softer than that of normal wheat.

The difference between protein content in wheat varieties was due to the protein expression of totally different genotype wheats. Wet gluten content represents the fraction of the total protein in different variety wheats that agglomerates upon hydration leading to dough formation. Wet gluten content was different both in wheats obtained from different milling methods and in kernel size treatments. The basic quality characteristics showed significant differences in protein and wet gluten index, which was higher in green wheat than in normal wheat (Table 2). Lipids, which are entrapped in the gluten network, represent a minor fraction of different wheat varieties. The composition and structure of different wheat varieties strongly influence the end-use quality of the dough. The lipid contents are given in Table 2. Compared with the lipid content of normal wheat, the lipid content of green wheat was increased by 44.9%. The purity of flour milled has been expressed as ash content (Kim and Flores 1999), because the ash content increases from the core to the outer layers of the wheat kernel. Ash contents were significantly ($P < 0.05$) different for green wheat and normal wheat (Table 2); therefore flour refinement was not evaluated by ash content.

3.3. Effects of Milling Methods on Amino Acids. Amino acid compositions can directly address many biological questions by revealing the abundance of specific proteins within organisms. Amino acids have important roles in the quality and nutrient value of dough. Thus, we compared 17 amino acids between green wheat and normal wheat using an automatic amino acid analyzer instrument (Table 3). We found that 17 amino acids were highly accumulated in green wheat with different milling methods. Among these amino acids, the levels of leucine, proline, serine, and glusate were increased the most. The contents of amino acid compositions

Table 3: Amino acid compositions of different wheat milling methods (%).

AA	Type					
	3B		5B		7B	
	GW 3104	YN 19	GW 3104	YN 19	GW 3104	YN 19
Leu	1.63 ± 0.12	1.48 ± 0.14	1.76 ± 0.15	1.60 ± 0.13	1.84 ± 0.15	1.64 ± 0.12
Phe	1.24 ± 0.10	1.16 ± 0.11	1.33 ± 0.11	1.18 ± 0.10	1.38 ± 0.12	1.23 ± 0.11
Val	0.96 ± 0.08	0.92 ± 0.08	1.05 ± 0.10	1.00 ± 0.11	1.12 ± 0.08	1.02 ± 0.10
Ile	0.89 ± 0.06	0.79 ± 0.05	0.96 ± 0.08	0.86 ± 0.04	1.07 ± 0.09	0.87 ± 0.07
Lys	0.56 ± 0.04	0.56 ± 0.03	0.64 ± 0.12	0.63 ± 0.01	0.70 ± 0.04	0.60 ± 0.05
Thr	0.55 ± 0.06	0.53 ± 0.04	0.61 ± 0.04	0.57 ± 0.06	0.63 ± 0.03	0.57 ± 0.03
Met	0.26 ± 0.11	0.23 ± 0.01	0.28 ± 0.01	0.27 ± 0.03	0.28 ± 0.03	0.27 ± 0.01
Glu	7.75 ± 0.54	7.22 ± 0.57	8.28 ± 0.51	7.56 ± 0.48	8.52 ± 0.61	7.95 ± 0.39
Pro	2.83 ± 0.21	2.46 ± 0.21	2.97 ± 0.19	2.55 ± 0.19	3.08 ± 0.23	2.67 ± 0.21
Arg	0.98 ± 0.12	0.93 ± 0.08	1.12 ± 0.11	1.07 ± 0.10	1.22 ± 0.11	1.04 ± 0.11
Asp	0.91 ± 0.07	0.90 ± 0.06	1.04 ± 0.16	1.00 ± 0.07	1.12 ± 0.08	0.99 ± 0.07
Gly	0.84 ± 0.06	0.81 ± 0.07	0.96 ± 0.09	0.90 ± 0.08	1.03 ± 0.07	0.90 ± 0.06
Ala	0.73 ± 0.05	0.68 ± 0.06	0.81 ± 0.06	0.79 ± 0.05	0.87 ± 0.05	0.77 ± 0.04
Ser	0.91 ± 0.02	0.84 ± 0.07	0.98 ± 0.12	0.79 ± 0.06	1.01 ± 0.08	0.90 ± 0.05
Tyr	0.47 ± 0.01	0.44 ± 0.01	0.49 ± 0.05	0.46 ± 0.04	0.49 ± 0.03	0.46 ± 0.03
His	0.56 ± 0.03	0.56 ± 0.03	0.92 ± 0.08	0.60 ± 0.05	0.66 ± 0.05	0.61 ± 0.04
Cys	0.23 ± 0.11	0.20 ± 0.01	0.26 ± 0.03	0.23 ± 0.00	0.26 ± 0.02	0.23 ± 0.01
total	22.32 ± 0.81	20.71 ± 0.76	24.18 ± 0.92	22.16 ± 0.87	25.22 ± 1.12	22.73 ± 0.93

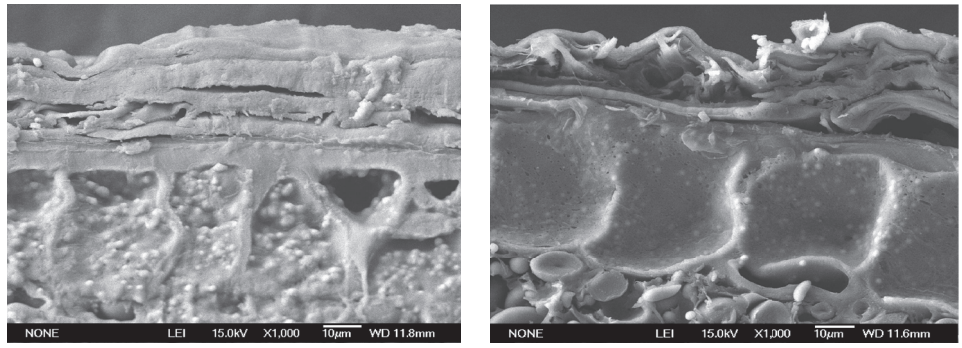
Table 4: Microelement contents of different wheat milling methods (mg/kg).

	3B		5B		7B	
	GW 3104	YN 19	GW 3104	YN 19	GW 3104	YN 19
Ca	505.85 ± 2.9	506.96 ± 3.1	674.47 ± 4.8	830.21 ± 4.2	767.14 ± 3.9	617.17 ± 2.3
Fe	64.05 ± 0.37	91.65 ± 0.49	86.42 ± 0.51	124.12 ± 0.66	148.94 ± 0.72	124.13 ± 0.65
Se	0.0429 ± 0.004	0.0626 ± 0.005	0.0630 ± 0.005	0.0724 ± 0.007	0.0506 ± 0.004	0.0778 ± 0.006

of different milling methods in GW3104 and YN19 are shown in Table 3. The results showed that the percentage of total amino acid values in 3B flour was lower than that in 5B and 7B flour. Compared with total amino acid values of 3B milling method in green wheat, the values of 5B and 7B milling method were increased by 8.3% and 13.0%, respectively.

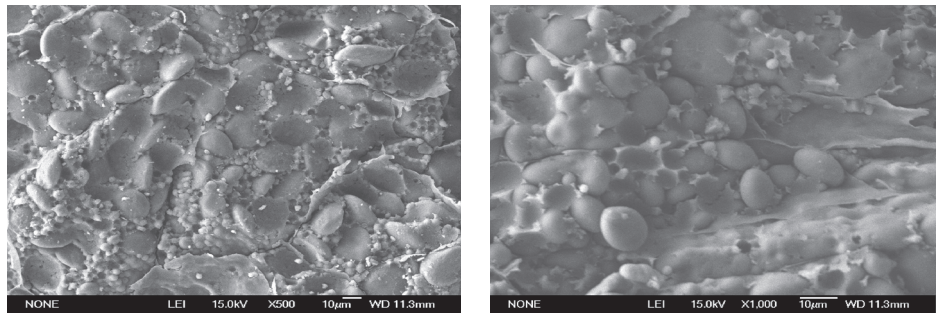
3.4. Effects of Milling Methods on Microelement. Micronutrients are an important factor not only for wheat nutrition, but also for human health. Ca, Fe, and Zn are essential micronutrients for the survival and proliferation of all cells [26]. Calcium ions have been suggested as a trigger for protein aggregation at elevated temperatures, involved in intermolecular protein-Ca^{2+}-protein cross-linking, intramolecular electrostatic screening, or ion-induced protein conformational changes [27, 28]. Iron ions are indispensable for a variety of cellular functions in cells, participating in photosynthesis, respiration, chlorophyll biosynthesis, DNA synthesis, and hormone synthesis. Although the essentiality of selenium to cells has not been established, Se is uptaken by cells from soils mainly as selenate SeO_3^{-2} and selenate SeO_4^{-2}. Selenate is transported by an active process that is mediated by sulfate transporters. Selenite digestion appears to occur passively and phosphate transporters are believed to be involved in this process. A general overview of these microelement interactions in these wheat varieties with different milling methods is summarized in Table 4. The results showed that the percentage of calcium ions values in green wheat was lighter than that of 3B and 5B in normal wheat. Compared with calcium ions value of different milling methods in green wheat, the values of 7B milling method were increased by 24.3%. Compared with iron ions values of normal wheat, iron ions values of green wheat were decreased by 30.1% and 30.4% in 3B and 5B flour, respectively, but iron ions values of green wheat were increased by 20.0% in 7B flour. However, compared with iron ions values of normal wheat, selenium ions values of green wheat were decreased by 31.5%, 13.0%, and 35.0% in 3B, 5B, and 7B flour, respectively. The results showed that the contents of microelement in different layers were of various values. The contents of microelement in 7B were higher than that of 3B and 5B, which indicated the contents of microelement in bran were higher than that of endosperms and aleurone layers.

3.5. SEM Micrographs of Different Milling Methods. Scanning electron microscopy (SEM) was used in order to observe the effect of bran, aleurone layers, and endosperms on the microstructure of the green and normal wheat with



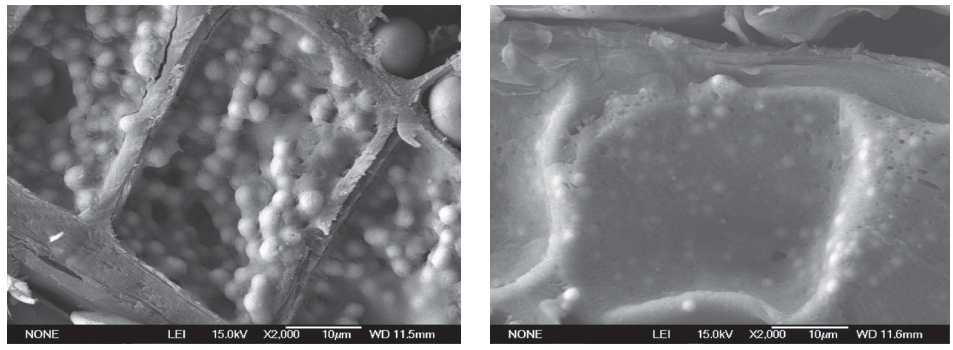
(a) (b)

Figure 2: SEM of green and normal wheat brans. (a) GW3104 and (b) YN19.



(a) (b)

Figure 3: SEM of green and normal wheat endosperms. (a) GW3104 and (b) YN19.



(a) (b)

Figure 4: SEM of green and normal wheat aleurone layers. (a) GW3104 and (b) YN19.

different layering milling methods [15, 29]. Scanning electron microscopy micrographs of different layers from normal and green wheat are shown in Figures 2–4.

Figure 2 shows images of the stratified structure from green and normal wheat brans. Obvious distinctions can be observed in the microstructures of the different wheat bran samples. The normal wheat bran displayed a close-knit structure without larger pores (Figure 2(b)), while green wheat bran displayed a loose structure with larger pores (Figure 2(a)). The micrographs show differences in protein network in green and normal wheat endosperms, which are accordant with the contents of wet gluten (Figure 3). Figure 4 shows images that illustrate the surface graininess of green and normal wheat aleurone layers, and green wheat aleurone layers surface showed more punctate structures. The results showed that the contents of protein in green wheat aleurone layers were higher than that of normal wheat aleurone layers. This observation further verified the results of the effects of milling methods on amino acids.

4. Conclusion

In conclusion, different milling treatment methods are capable of separating the different wheat flour from endosperms,

bran, and aleurone layers. Micro- and physicochemical characterization of different wheat flour and layers by means of microscopy techniques and images analysis provided relevant qualitative and quantitative information, which can be useful for the study of the microstructure of green and normal wheat products and also for its processing and utilization. The images and nutritional ingredient analysis were successful in evaluating the changes on the morphology and surfaces of the different layering milling technology due to mechanical damage induced in the wheat material. This study therefore demonstrated the efficiency of the nutrient distribution and microstructure on the different wheat with layering milling methods. The findings of our study may be applied to the production of highly nutritional wheat flour by the addition of layer flour from different milling methods to meat products.

Conflicts of Interest

The authors declare that they have no conflicts of interest.

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Research Article

Comparison of Sodium Acid Sulfate and UV-C Treatment on Browning and Storage Quality of Fresh-Cut Potatoes

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Fresh-cut vegetables, such as potato chips, get brown quickly and can easily be infected by bacterium during storage. Sodium acid sulfate (SAS) and UV-C treatments are regarded as effective methods for food preservation. In this study, the effects of SAS, UV-C treatment, and their combination on fresh-cut potatoes during storage were evaluated. Compared with the control, all of the treatments were effective in inhibiting the bacterial growth during the whole storage period. Also, both SAS and SAS + UV-C treatments significantly decreased browning and polyphenol oxidase (PPO) activity and increased the firmness and malondialdehyde (MDA) contents, while the UV-C treatment has no good effects on protecting such storage qualities in fresh-cut potatoes. However, when compared with SAS treatment, the combination of SAS and UV-C treatment did not promote the effect in protecting the storage abilities. Thus, it was concluded that SAS is a better treatment in extending shelf life and controlling the quality of fresh-cut potatoes during storage compared to UV-C treatment.

1. Introduction

Fresh-cut vegetables, or minimally processed vegetables, are convenient for consumers while maintaining a high level of nutrients and a good taste [1]. However, these kinds of produces are easily suffering from browning, excessive microbe, and tissue softening due to the exposed surface. With an increasing share of the fresh-cut market during the last two decades, protecting the quality of produce has become particularly important [2, 3].

Discoloration is a consequence of imbalance between phenol and quinone metabolism, which occurs from breakdown of tissue integrity. Numerous technologies have been applied to extend shelf life of fresh-cut produce, particularly the physical methods, such as heat-shock [4], gamma irradiation [5], vacuum packaging [6], modified atmosphere packaging [7], and UV-C [8]. Among them, UV-C is an effective and widely used method in the storage of much fresh-cut produce. Previous reports showed that UV-C can reduce the bacterial population, control browning [9], and inhibit the polyphenol oxidase (PPO) activity [10]. In addition, it maintains nutrients, such as vitamin D_2 [11], and can also lower the risk of disease [12]. Although UV-C has many advantages in protecting quality of fresh-cut produce, no significant effect on browning was observed according to previous reports [13].

Many chemical substances are regarded as effective color-preserving reagents for fresh-cut vegetables, including ascorbic acid [14, 15], citric acid [16], ozone [17], calcium chloride [18, 19], and acidic electrolyzed water [20]. Sodium acid sulfate (SAS), which was certified as generally recognized as safe (GRAS) food additive by FDA in 1998, is effective

in suppressing enzymatic browning and aerobic bacteria in fresh-cut apples [21], but the effects on potato chips are rarely investigated [22].

In the present study, the effects of SAS, UV-C, and their combination on browning, microbial growth, PPO activity, and malondialdehyde (MDA) content of fresh-cut potatoes during storage at 4°C were evaluated. The aim of this study was to provide better alternative method for postharvest storage of fresh-cut produce.

2. Material and Methods

2.1. Sample Preparation and Treatments. Potatoes were purchased from a local supermarket and used for all experiments. Potatoes of uniform size were selected, peeled, and cut into 4-5 mm slices using a slicer. Two pretests were conducted at 4°C to select the suitable treatment time and concentration. In order to select the optimum SAS concentration, the potato slices were dipped into 0%, 1.0%, 1.5%, 2.0%, 2.5%, 3.0%, 4.0%, and 5.0% SAS solutions for 2 min, respectively. For the irradiation time of UV-C treatment, fresh-cut potatoes were irradiated for 2 min, 3 min, 4 min, and 5 min, respectively. After storage for a week, we found that UV-C irradiation for 3 min and 2.5% SAS treatment for 2 min were the most optimum selection.

Prepared potatoes were divided randomly into four groups: (a) control: untreated samples; (b) SAS treatment: dipped in 2.5% SAS solutions for 2 min and then wiped with a clean paper; (c) UV-C treatment: exposed to UV-C irradiation for 3 min; (d) SAS + UV-C treatment: dipped in 2.5% SAS solutions for 2 min and then exposed to UV-C for 3 min. After treatment, the fresh-cut potatoes were packaged in polyethylene bags and stored at 4°C for 25 d. The potato slices were sampled at 0, 5, 10, 13, 16, 19, 22, and 25 d after treatment. Each treatment was repeated for three times and each replication has three slices of potatoes.

2.2. Texture Analysis. Firmness of fresh-cut potatoes was measured using TA-XT2i texture analyzer (Stable Micro Systems Ltd., UK). The samples were indented with a compression probe P50 (Stable Micro Systems Ltd., UK) and firmness was taken as the maximum force (N) required to puncture the slices for 70% of the thickness. Each slice was detected in two symmetrical points of the potato slices. Each treatment was repeated for six times.

2.3. Color Analysis. The color of the treated fresh-cut potatoes was measured using DigiEYE color Measurement and Imagine System (VeriVide Co. Ltd., Leicester, UK). Values of L^*, a^*, and b^* were used for analysis. The results were expressed as mean value from the whole potato slices. Each treatment was replicated for six times.

2.4. PPO Activity Analysis. The PPO enzyme was extracted according to the PPO test kit (Bioengineering Institute, China) protocol. The PPO activity was determined as the amount of enzyme that caused the absorbance decrease of 0.01 at 525 nm for 1 min.

2.5. Aerobic Plate Count (APC) Analysis. APCs were analyzed according to the method described in Chinese National Standards (GB 4789.2-2010) with modifications. All operations were conducted in sterile environment and all the reagents and instruments were sterilized (121°C, 20 min) prior to the analysis. Potato slices were cut into cubes. 10 g aliquots of sample were transferred into a sterile stomacher bag (Beijing Medical, China), which contained 90 mL of sterile saline water, and then pummelled at maximum speed for 2 min by Lab-Blender BILON-08 (Shanghai Bilang Co. Ltd., China). The bacterium solution was diluted using sterile saline water to at least three dilutions based on the count of last period. 0.1 mL of each diluent was plated on plate count agar (Beijing, AoBoXing 02–035A, China). The plates were incubated at 37°C for 48 h and calculated as log CFU·kg^{-1}.

2.6. MDA Content. The MDA content was measured according to the method described by Lin et al. [23]. 1 g of prepared sample was homogenized with 5 mL 10% (w/v) trichloroacetic acid and centrifuged for 10 min at 10,000g. 3 mL 0.6% thiobarbituric acid was added to the supernatant, and it was then held in a boiling water bath for 15 min. Absorbance was measured at 450 nm, 532 nm, and 600 nm. The content of MDA was calculated as follows:

$$\text{MDA}\ \left(\mu\text{mol}\cdot\text{L}^{-1}\right) = 6.45\times\left(A_{532}-A_{600}\right)-0.56\times A_{450}. \quad (1)$$

2.7. Statistical Analysis. All experiments were arranged in a completely randomized design and each treatment contained 3 replicates unless stated otherwise. Standard deviation and figures were drawn using Origin 8.6 (Microbial Software Inc., Northampton, MA, USA). The least significant difference (LSD) test at the 0.05 level was performed by SPSS Statistics 20 Software.

3. Results

3.1. Firmness. The variation in firmness of samples was relatively stable during the whole storage period. SAS and SAS + UV-C-treated samples had significant ($P < 0.05$) higher force values in firmness compared to control and UV-C-treated samples in most storage phases. No significant difference ($P > 0.05$) was found in firmness between UV-C-treated samples and control during the whole storage period. At 5 d of storage, the firmness of potatoes in SAS + UV-C treatment increased by 74.9 N in comparison with that in SAS treatment. In general, the potatoes in SAS and SAS + UV-C treatments were firmer than that in control during the late storage period (Figure 1).

3.2. Color. Control and UV-C-treated potatoes showed an obviously darker appearance compared to SAS and SAS + UV-C-treated samples. The appearances of samples in SAS and SAS + UV-C treatments were similar during the whole storage period and were not affected by the length of the storage period. The UV-C-treated samples had no obvious

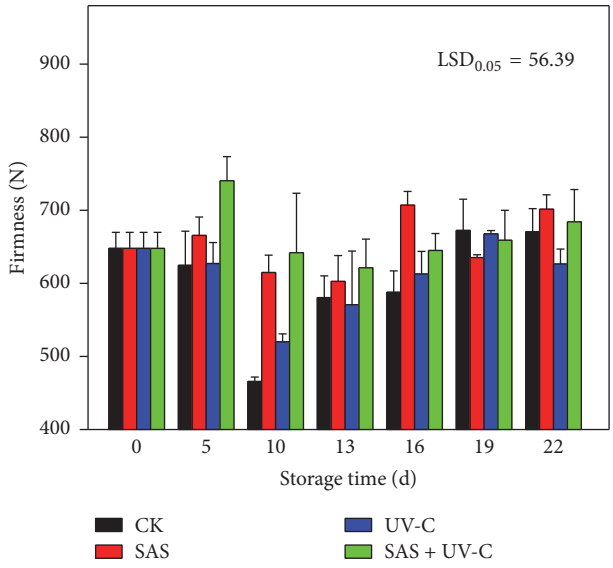

Figure 1: Effects of SAS, UV-C, and SAS + UV-C on firmness of fresh-cut potatoes during storage. The error bars represent the standard deviation. LSDs represent least significant differences at the 0.05 level.

difference with control, which appeared to lose its commodity attribute at 5 d storage (Figure 2).

L^*, a^*, and b^* value of the fresh-cut potatoes are shown in Figure 3. Higher L^* value represents lightness of the potato slices while decreasing a^* indicates decline of redness, and increase in b^* shows increasing yellowness of the potato slices.

L^* values decreased continuously in the control and UV-C-treated potatoes. SAS and SAS + UV-C treatments maintained higher L^* value compared with the control and UV-C-treated samples. However, no significant difference ($P > 0.05$) was observed between SAS and SAS + UV-C. After storage for 25 days, the L^* value was decreased to 67.9 in control but was still maintained at 80.7 and 80.6 in SAS and SAS + UV-C-treated samples, respectively (Figure 3(a)).

The measurement of a^* presented an increasing trend among all treatments. SAS and SAS + UV-C treatments significantly ($P < 0.05$) decreased a^* value during the initial storage periods and the significant difference decreased as the storage period increased. At 10 d storage, a^* value of potato slices in control was 15.7 and 6.70 times higher than that in SAS and SAS + UV-C treatment, respectively (Figure 3(b)).

The b^* value presented a similar variation trend to L^* value. Both SAS and SAS + UV-C treatments showed significantly ($P < 0.05$) higher b^* value throughout whole storage compared to other treatments (Figure 3(c)).

3.3. PPO Activity. PPO activity showed a high value in both the control and UV-C treatment during the storage period, but this phenomenon was significantly ($P < 0.05$) suppressed by SAS and SAS + UV-C treatment. PPO activity had no significant difference ($P > 0.05$) between UV-C treatment and the control during the early storage period, but it was significantly decreased ($P < 0.05$) by UV-C treatment at 13, 16, and 19 d of storage compared to the control. In addition, the PPO activity was significantly ($P < 0.05$) promoted by SAS + UV-C treatment at 10 and 13 d of storage compared to the SAS treatment (Figure 4).

3.4. Microbiological Analysis. The microbe count increased continuously in all the treatments during the whole storage period. SAS and SAS + UV-C treatments had significant effect on preventing bacterial growth during the whole periods while UV-C alone had significant result only in the later storage. SAS combined with UV-C treatment had the lowest bacterial populations among all treatments before storage of 13 d, but the difference was not significant when compared to SAS treatment. At 25 d, the APC in control samples was already too high to measure while it was maintained at 6.00 log CFU·g^{-1} approximately in the other three treatments (Figure 5).

3.5. MDA Content. The content of MDA presented an increasing trend on the whole. UV-C treatment showed no significant difference in MDA content of potatoes compared with the control. But the MDA content was immediately induced and increased by SAS and SAS + UV-C treatment and maintained a high level during the whole storage period. However, the increase of MDA content in potato was significantly suppressed by the combination of SAS and UV-C treatment (Figure 6).

4. Discussion

Fresh-cut products satisfy consumers as they are freshly prepared, convenient, and beneficial for human health. However, fresh-cut produce deteriorates faster than corresponding intact produce, which leads to a reduction in quality and shelf life. The key points in fresh-cut produce deterioration are discoloration and excessive microorganism growth during storage and transportation [24].

SAS has been reported to be an effective treatment for antibrowning of fresh-cut produce compared with other single or combined acidulants. Our results also suggested that 2.5% SAS significantly decreased the extent of browning in potato slices after 25 d storage, which confirmed the results of previous reports [21, 25, 26]. PPO, a key regulation factor in the process of browning, catalyzed the oxidation reaction with oxygen and phenolic compounds and promoted accumulation of quinones, which react with other substances continually leading to the polymerization of quinones [27, 28]. Previous studies produced the low browning potato by silencing the PPO genes, and they confirmed four PPO gene members were closely related to browning of potatoes [29, 30]. The maximum PPO activity in potato occurred at pH 6.8, while it was nearly inactive when pH was below 4.0 [31]. Previous research has demonstrated that SAS is a stronger acidulant (pKa = 2.0) when compared with ascorbic acid (pKa = 4.1) and citric acid (pKa = 3.1) [26]. There is a possibility that SAS treatment can decrease the pH level and

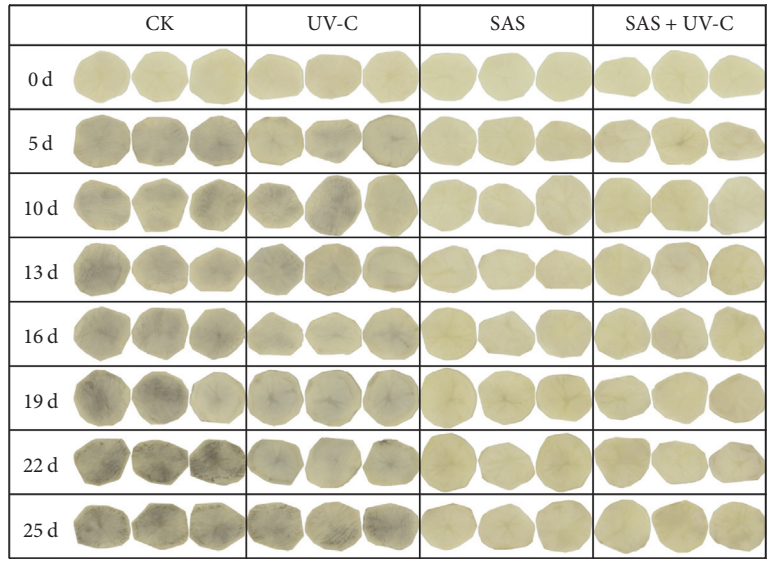

Figure 2: Effects of SAS, UV-C, and SAS + UV-C on appearance of fresh-cut potatoes during storage.

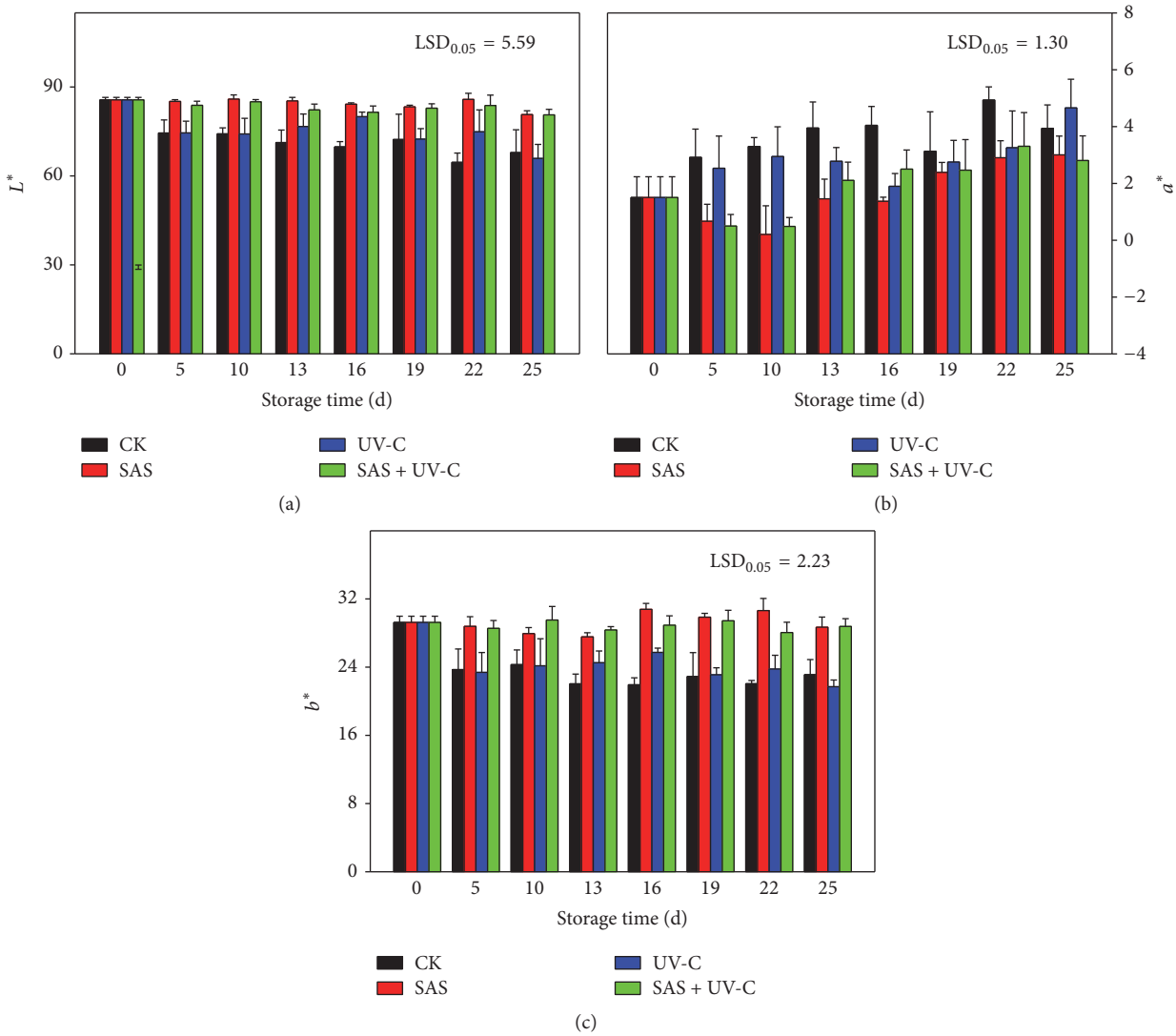

Figure 3: Effects of SAS, UV-C, and SAS + UV-C on color indexes of fresh-cut potatoes during storage. (a) L^* value; (b) a^* value; (c) b^* value. The error bars represent the standard deviation. LSDs represent least significant differences at the 0.05 level.

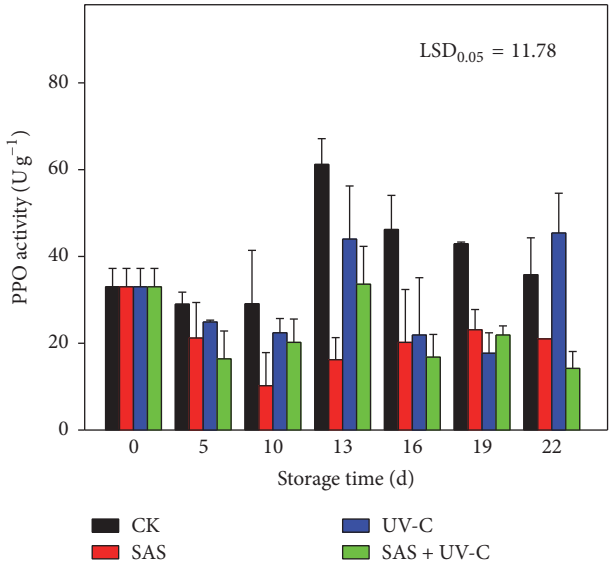

Figure 4: Effects of SAS, UV-C, and SAS + UV-C on PPO activity of fresh-cut potatoes during storage. The error bars represent the standard deviation. LSDs represent least significant differences at the 0.05 level.

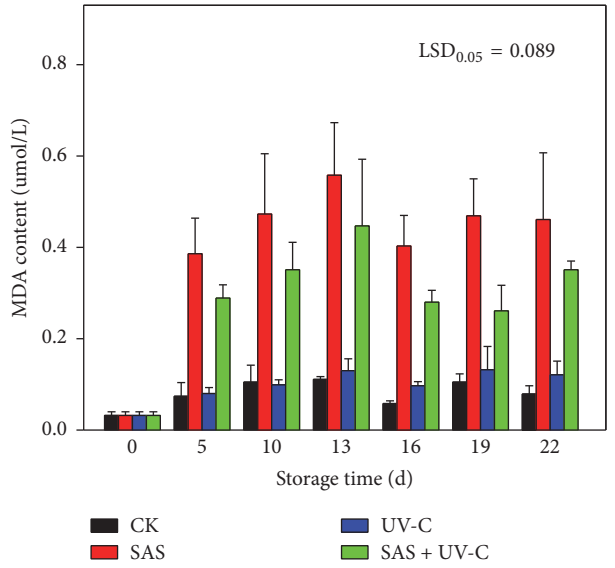

Figure 6: Effects of SAS, UV-C, and SAS + UV-C on MDA content of fresh-cut potatoes during storage. The error bars represent the standard deviation. LSDs represent least significant differences at the 0.05 level.

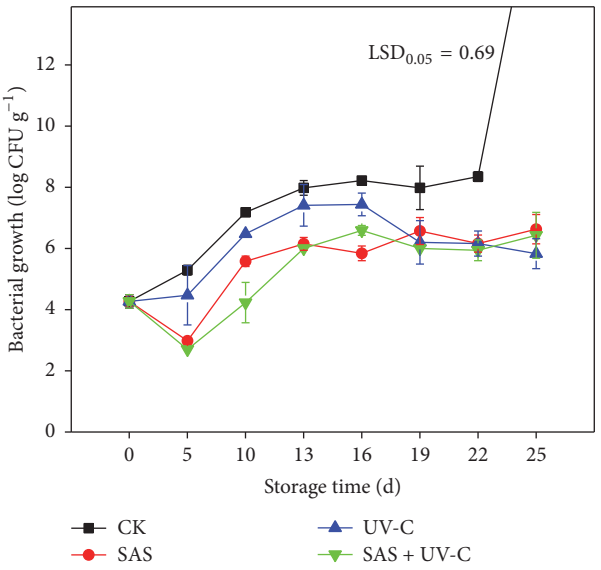

Figure 5: Effects of SAS, UV-C, and SAS + UV-C on APC of fresh-cut potatoes during storage. The error bars represent the standard deviation. LSDs represent least significant differences at the 0.05 level.

PPO activity of the potato tissues, thus, leading to alleviated surface browning.

Excessive microbial population in fresh-cut produce is another problem in fresh-cut industry [32, 33]. UV-C is an environmental-friendly strategy for extending the shelf life of fresh-cut produce and has proved to be effective in reducing bacterial count but not damage other quality attributes [34–36]. Our results showed that UV-C treatment was effective in sterilization of fresh-cut potatoes during storage, and, more interestingly, the SAS treatment had a better effect than UV-C treatment during the early storage period. It was speculated that hydrogen sulfate ion and lower pH might be the main reasons for antimicrobial action in SAS treatment [21], but the regulation mechanism needs further research.

Combined treatments have been widely used in fresh produce during postharvest storage. For example, hot water combined with UV-C treatment has been applied to improve disease resistance and maintain quality of mangos [37]; aqueous chlorine dioxide combined with UV-C treatment was efficient in extending shelf life of blueberries [38]; UV-C combined with heat treatment was efficient in inactivation of the spoilage yeasts in apple juice [39]. According to our results, no significant antibrowning or antimicrobial effect was observed in fresh-cut potato under SAS + UV-C treatment compared with the SAS treatment; thus, it was concluded that SAS combined UV-C treatment did not promote the effect in protection of the storage abilities of fresh-cut potatoes.

In summary, SAS treatment was the most effective method in antibrowning and antimicrobial effects in fresh-cut potatoes during storage, and it was even better than the UV-C treatment. But combination of the selected SAS concentration and UV-C irradiation did not enhance the effects. In addition, the shelf life of fresh-cut potatoes can reach 22 d when SAS combined with UV-C, and it was maintained no more than 5 d when treated with control or UV-C alone. Thus, SAS is effective in controlling quality of fresh-cut produce during storage.

Conflicts of Interest

The authors declare that there are no conflicts of interest regarding the publication of this paper.

Authors' Contributions

Yajing Xie and Qiong Lin contributed equally to this work.

Acknowledgments

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Research Article

The Thawing Characteristic of Frozen Tofu under High-Voltage Alternating Electric Field

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To systematically and comprehensively investigate the high voltage alternating electric field (HVAEF) thawing processing, we investigated the high-voltage electric field thawing characteristic of the frozen tofu at different voltages for alternating current (AC). The thawing time, thawing loss of frozen tofu, and specific energy consumption (SEC) of HVEF system were measured. Seven different mathematical models were then compared to simulate thawing time curves based on root mean square error, reduced mean square of deviation, and modeling efficiency. The results showed that the thawing rate of frozen tofu was notably greater in the high-voltage electric field system when compared to control. Both Linear and Quadratic models were the best mathematical models. Therefore, this work presents a facile and effective strategy for experimentally and theoretically determining the HVAEF thawing properties of frozen tofu.

1. Introduction

Tofu is representative of Chinese traditional food and is a favorite of consumers because of its unique flavor, rich taste, edible convenience, and rich nutritional value. Thawing of frozen materials is an important component of food processing, while freezing is a well-established process for food preservation and is confirmed to increase the storage time significantly. At present, the conventional thawing methods of frozen tofu include cold and warm water thawing, still air thawing, and refrigerator thawing. However, many disadvantages accompany such methods, including higher color deterioration and weight loss, longer thawing time, and decreased nutritional value. Consequently, growing interest has been shown in exploring new thawing methods for frozen tofu.

High-voltage electric field (HVEF) thawing is a relatively new, nonthermal technique [1], and it has been investigated by many researchers in pork [2, 3], tuna fish [4–6], chicken [7], and apple tissue [8]. The advantages typically include reduced thawing time, food quality preservation, microbial growth inhibition, and reduced energy consumption. When the thawing temperature was set at −3°C, the thawing time of frozen chicken under high-voltage electric field was 2/3 the time taken for thawing meat using a common refrigerator [7]. The HVEF treatment significantly shortened the thawing time of frozen pork tenderloin meat, and thawing time was reduced to 2/3 that of the control [2]. Similarly, in the case of tune fish, HVEF significantly improved the thawing rate [4]. He et al. [9] reported that the thawing time can be maximally reduced by 50% compared to the conventional air thawing treatment. HVEF treatment reduced the total microbial counts in thawed frozen meat by 0.5–1 log CFU/g, without affecting meat quality, and reduced volatile basic nitrogen production during storage [2]. Parameters such as voltage, distance, and electric field strength were studied to determine the relationships and factors that affect HVEF treatment [3, 5]. Mousakhani-Ganjeh et al. reported that the high-voltage electric field could increase susceptibility of tuna fish to lipid oxidation due to ozone generation [6]. The energy consumption of HVEF thawing was comparatively far smaller compared to the other thawing methods [3, 5]. These studies were performed under high-voltage direct current electric field. To the best of our knowledge, few studies have systematically and comprehensively reported on the use of high-voltage alternating electric field (HVAEF) for thawing tofu.

To further investigate the potential of this method for optimizing and improving the thawing efficiency, frozen tofu

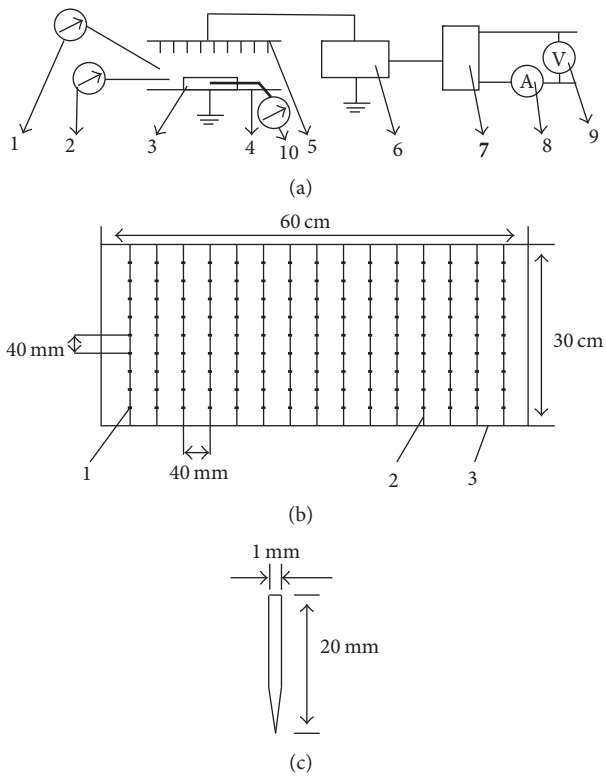

Figure 1: (a) Schematic diagram of HVAEF thawing: 1: thermometer; 2: hygrometer; 3: frozen tofu; 4: grounded plate electrode; 5: needle electrodes; 6: high-voltage power source; 7: voltage regulator; 8: ampere meter; 9: voltmeter; 10: temperature sensor. (b) Arrangement diagram of needle electrodes: 1: needle electrodes; 2: stainless steel wire; 3: stainless steel frame. (c) Schematic diagram of needle electrode.

was thawed in order to study the effect of voltage on thawing rate. The center temperature was measured, in addition to thawing loss and specific energy consumption of frozen tofu to understand the roles of energy consumption and product quality on thawing process.

2. Materials and Methods

2.1. Experimental Equipment. The lab-scale experimental setup for HVAEF thawing is shown schematically in Figure 1(a). This setup is similar to the EHD drying system [10]. It consists of a vertically mounted electrode with multiple sharp pointed needles projected to a fixed horizontal grounded metallic plate on which the frozen tofu samples to be thawed were placed. The electrode gaps between the emitting point and the grounded electrode were 100 mm. The sharp pointed electrodes were connected to a power source that can supply alternating current (AC) high-voltage. In order to set the desired high-voltage parameters for HVAEF thawing, the power was connected to a voltage regulator, with an adjustable voltage ranging within 0–50 kV for alternating current (AC) by a controller. The grounded plate electrode was an 80 cm × 40 cm rectangular stainless steel plate. Temperature and relative humidity were both measured. The voltage and current of HVAEF system were measured by a voltmeter and an ampere meter, respectively. Figures 1(b) and 1(c) show arrangement diagram and schematic diagram of the needle electrodes, respectively. The needles were 20 mm long. The diameter of needles is 1 mm. The distance between two needle electrodes was 40 mm. The needle electrodes were arranged in multiple rows and lined up by stainless steel wire. The distance between two stainless steel wires was 40 mm. All the samples were spread in a single layer on the grounded plate electrode at random. The center temperature of samples was measured by a temperature sensor.

2.2. Experimental Method. The soft tofu was procured from a local market near Inner Mongolia University of Technology, Hohhot, China. The fresh soft tofu was cut into cubes (3.5 cm × 3.5 cm × 3.5 cm) using a knife and immediately frozen at −18°C in a refrigerator. The frozen samples were stored at −18°C until use.

The frozen tofu was thawed at room temperature 20 ± 1°C under an electric field generated by a high-voltage of 4, 8, 12, 16, 20, 24, or 28 kV for alternating current (AC). The control samples were placed on the same kind of stainless steel plate and subjected to HVAEF experimental apparatus in the treatment room (0 kV). The thawing relative humidity was 30 ± 5%, and the ambient wind speed was 0 m/s. A temperature sensor was inserted into the geometric center of the frozen tofu sample and recorded at 5 min intervals during the thawing process. Thawing was continued until the geometric center of the frozen tofu sample temperature reached 10°C. The time required to raise the temperature at the center of the frozen tofu cube from −10°C to 10°C was determined as thawing time. Each experiment was repeated three times and averaged. The electric field strength is calculated from the following equation:

$$E = \frac{V}{G}, \tag{1}$$

where E is electric field strength, V is the thawing voltage, and G is the gap between the emitting point and the grounded electrode. The thawing rate (TR) of frozen tofu samples (g/s) was calculated using the following equation:

$$\mathrm{TR} = \frac{W}{T}, \tag{2}$$

where W is weight of frozen tofu and T is the thawing time of frozen tofu.

2.3. Determination of Evaporation, Thawing, and Drip Losses. Evaporation loss (EL), thawing loss (TL), and drip loss (DL) were determined by weighing the frozen and thawed tofu samples before and after the removal of surface water according to the following equations [4]:

$$\mathrm{EL}\ (\%) = \frac{(M_0 - M_{\mathrm{T}})}{M_0},$$

$$\mathrm{TL}\ (\%) = \frac{(M_0 - M_{\mathrm{TT}})}{M_0}, \tag{3}$$

$$\mathrm{DL} = \mathrm{TL} - \mathrm{EL},$$

Table 1: Mathematical models applied to the thawing time curve.

Model name	Model equation
Power	$T = aE^b$
Exponential	$T = ae^{bE}$
Linear	$T = a + bE$
Logarithmic	$T = a + b\ln(E)$
Quadratic	$T = a + bE + cE^2$
Inverse	$T = a + b/E$
S	$T = e^{(a+b/E)}$

T is thawing time of frozen tofu in min, E is electric field strength in kV/cm, and a, b, and c are constants of mathematical models.

where M_0, M_T, and M_{TT} are the weight of the frozen tofu, the thawed tofu before removing surface water, and the thawed tofu after surface water removal, respectively. Each experiment was repeated three times and averaged.

2.4. Specific Energy Consumption. The specific energy consumption (SEC) for the HVAEF system during thawing of tofu was measured by an ampere meter and a voltmeter, respectively. The specific energy consumption of HVAEF system during thawing of frozen tofu was calculated using the following equation:

$$\text{SEC} = \frac{UIt}{W_d}, \tag{4}$$

where U, I, and t and W_d are voltage of HVAEF system (V), current of HVAEF system (A), thawing time (s), and the weight of frozen tofu (kg), respectively.

2.5. Mathematical Model and Statistical Parameter. The experimental thawing curves were fitted to the seven different empirical models in Table 1. The model best suited for describing the thawing rate curve of frozen tofu was selected based on the values of the statistical parameters at 4, 8, 12, 16, 20, 24, and 28 kV for AC electric field, respectively.

The root mean square error (ERMS), reduced mean square of the deviation (χ^2), and modeling efficiency (EF) were used as the primary criteria to select the equation that best accounts for the variation in the thawing curves of the thawed samples [11–13]. ERMS gives the deviation between the predicted and experimental values. χ^2 was used to determine the goodness of the fit: the lower the values of χ^2, the better the goodness of the fit. EF also gives the model predictive power in relation to the thawing behavior of the product, and its highest value is 1. These statistical values were calculated using the following equation:

$$\begin{aligned}
\text{ERMS} &= \sqrt{\frac{1}{N}\sum_{i=1}^{N}\left(T_{\text{pre},i} - T_{\text{exp},i}\right)^2}, \\
\chi^2 &= \frac{\sum_{i=1}^{N}\left(T_{\text{pre},i} - T_{\text{exp},i}\right)^2}{N-n}, \\
\text{EF} &= \frac{\sum_{i=1}^{N}\left(T_{\text{exp},i} - T_{\text{exp,mean}}\right)^2 - \sum_{i=1}^{N}\left(T_{\text{pre},i} - T_{\text{exp},i}\right)^2}{\sum_{i=1}^{N}\left(T_{\text{exp},i} - T_{\text{exp,mean}}\right)^2},
\end{aligned} \tag{5}$$

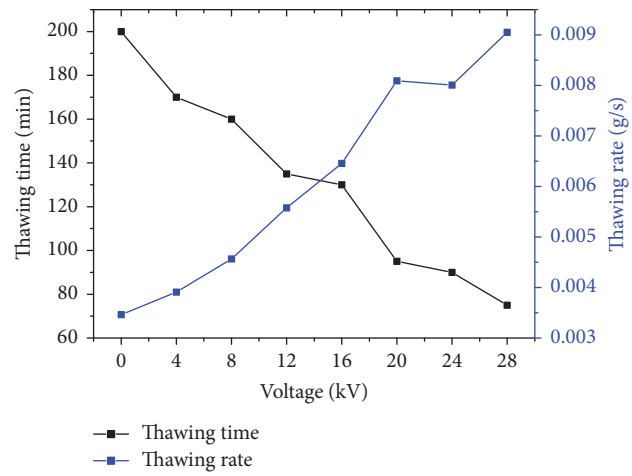

Figure 2: Thawing time and thawing rate of frozen tofu in different voltages.

where $T_{\text{exp},i}$ is the ith experimental thawing time, $T_{\text{pre},i}$ is the ith predicted thawing time, $T_{\text{exp,mean}}$ is the mean value of experimental thawing time, n is the number of constants in the thawing model, and N is the number of observations.

2.6. Statistical Analysis. Single-factor analysis of variance was used to calculate the evaporation loss, thawing loss, and drip loss between the frozen tofu under alternating electric field and without electric field (control). The evaporation loss, thawing loss, and drip loss between different electric field were also calculated using single-factor analysis of variance. The differences in thawing time are considered statistically significant when $p < 0.05$. The results reported in this study are presented as means ± standard deviation (SD).

3. Results and Discussion

3.1. Thawing Time and Thawing Rate of Frozen Tofu by Different HVAEF Treatments. Figure 2 shows the effect of different voltages applied in the HVAEF on the thawing time and thawing rate. The frozen tofu was thawed at 20°C under applied voltages increasing from 4 to 28 kV at increments of 4 kV with a fixed electrode distance of 10 cm. The thawing temperature of all samples was from −10°C to 10°C. The thawing times for voltages of 4, 8, 12, 16, 20, 24, and 28 kV were 170, 160, 135, 130, 95, 90, and 75 min, respectively, which were shortened significantly when compared with 200 min for the control (0 kV). As voltage increased, the thawing time declined. The thawing rate of tofu samples treated with HVAEF increased compared to that of the control, and increasing the voltage had a major effect on the enhancement of the thawing rate, increasing by 0.1277, 0.3180, 0.6099, 0.8638, 1.3349, 1.3116, and 1.6119 times, respectively, at 4, 8, 12, 16, 20, 24, and 28 kV voltages compared to that of the control (0 kV). As can be seen, the results indicate that thawing rate increased with rise in voltage. These results agree with those studies which reported enhancement in thawing rate with increase of applied voltage [3, 4].

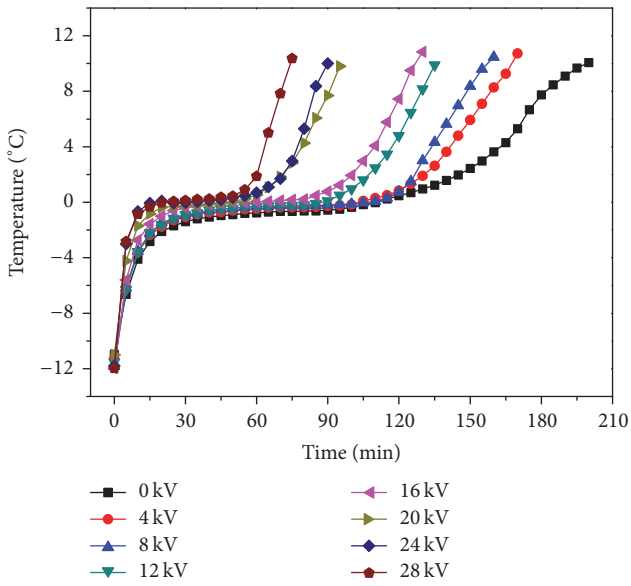

Figure 3: Changes in temperature during thawing of frozen tofu in different voltages.

Higher voltage or electric field strength can induce stronger ionic wind and higher wind velocity [14]. The enhancement in mass transfer rate could be attributed to the corona wind [15]. The corona wind produced impinges on the material and disturbs the liquid part of the thawing tofu, leading to thawing enhancement. The enhancement in thawing rate by the multiple points-to-plate could be attributed to electric wind created by each needle point electrode, resulting in a cumulative effect that could have greatly increased the thawing rate [16]. From Figure 2, it appears that the thawing rate is higher than the control when the voltage is lower than 15 kV. So, apart from corona wind under the AC electric field, another HVAEF thawing mechanism is possible because there is no corona wind. Specifically, as water molecules are highly polar they orient themselves in the direction of the electric field, which in-turn would lead to the conversion of electrical energy into mechanical energy, thereby forcing water molecules out of the material [10]. This effect would be directly proportional to the electric field strength.

3.2. Center Temperatures of Frozen Tofu Thawed with Different HVAEF Treatments. Figure 3 illustrates the center temperatures of frozen tofu exposed to applied voltages 4, 8, 12, 16, 20, 24, 28 kV, and 0 kV (control) at room temperature (20°C). Center temperatures of frozen tofu were measured every 5 min and the initial thaw temperature of all samples was −10°C. The results indicate that the thaw temperatures increased rapidly, reaching about −3°C in the first 10 min. Within the last 20 min, the thaw temperatures also increased rapidly, reaching about 10°C. The thaw temperatures increased slowly between −2 and 0°C. Most of the thawing time is consumed in raising the temperature from −2 to 0°C. In the food freezing industry, −5–−1°C is often considered as the zone of maximum ice crystal formation [2, 5, 7]. Within this temperature range of slow thawing time (−2–0°C), HVAEF treatment exerts its maximum effect. This result coincides with what has been found in other studies [2, 5].

Table 2: The DL, EL, and TL of tofu under different voltages.

Voltage (kV)	DL (%)	EL (%)	TL (%)
0 (control)	25.53 ± 1.71^{a}	2.53 ± 0.43^{a}	27.89 ± 1.73^{a}
4	23.81 ± 0.81^{a}	2.60 ± 0.22^{a}	26.42 ± 1.02^{a}
8	17.72 ± 1.42^{b}	2.63 ± 0.43^{a}	20.35 ± 1.85^{b}
12	11.70 ± 1.19^{c}	2.91 ± 0.29^{a}	14.60 ± 1.18^{c}
16	11.38 ± 1.21^{c}	3.13 ± 0.32^{a}	14.50 ± 2.02^{c}
20	9.49 ± 1.88^{d}	5.25 ± 0.48^{b}	14.74 ± 2.07^{c}
24	5.39 ± 0.34^{e}	5.57 ± 0.11^{b}	10.95 ± 0.44^{d}
28	3.44 ± 0.16^{f}	5.03 ± 0.12^{b}	8.48 ± 0.28^{e}

Data are shown as the mean ± standard deviation (SD). For each treatment, means with different lower case letters are significantly different ($p < 0.05$).

3.3. EL, DL, and TL of Tofu. In this study, EL, DL, and TL were determined for the tofu samples with different voltages. Influence of voltage on the EL, DL, and TL of tofu is given in Table 2. When thawing loss is low, water holding capacity is high [2]. The results showed that evaporation loss increased with increasing applied voltage. The evaporation rate of material samples treated with HVAEF increased compared to that of the control, and increasing the voltage had a major effect on the enhancement of the evaporation rate [17–19]. Drip loss was less in the electric field treatments than in the control and decreased with increasing applied voltage. As can be seen, the results indicate that changes in voltage have significant effects on thawing loss. In other words, water holding capacity of tofu is improved using HVAEF thawing. This result does not coincide with what has been found in other studies [2, 4]. The thawing process can cause the texture and the structural changes of the tofu [20]. Under same thawing method, there could have different results for the different materials. And the thawing loss is related to thawing time. The thawing time was shortened significantly under HVAEF compared to the control.

3.4. Effects of Voltage on Specific Energy Consumption. Figure 4 shows the specific energy consumption (SEC) of HVAEF system during thawing of tofu. The SEC of HVAEF system changes nonmonotonically with voltage, reaching a valley at 4–28 kV. He et al. found that the SEC of HVEF was significantly influenced by voltage and increased with increasing applied voltage [3, 5]. The results showed that the electric field energy was not completely absorbed for thawing frozen tofu and a part of the energy absorbed by the control system. This will affect the energy efficiency of HVEF system.

3.5. Selection of the Best Mathematical Model. To obtain the superior mathematical models of frozen tofu, nonlinear regression analysis was carried on to estimate the constants and parameters of the seven thawing mathematical models given in Table 3. The statistical results of the root mean

Table 3: Statistical results, constants, and coefficients of mathematical models.

Model name	a	b	c	χ^2	ERMS	EF
Power	132.6482	−0.3534		260.3949	24.1728	0.8566
Exponential	202.0317	−0.3364		60.0811	11.6113	0.9658
Linear	188.5714	−41.5179		44.1071	9.9487	0.9722
Logarithmic	137.3362	−50.3776		160.3174	18.9671	0.8991
Quadratic	190.7143	−45.0893	1.1607	54.5239	9.8935	0.9726
Inverse	84.8487	40.2736		470.6708	32.4990	0.6445
S	4.5439	0.2641		571.1514	35.8003	0.5686

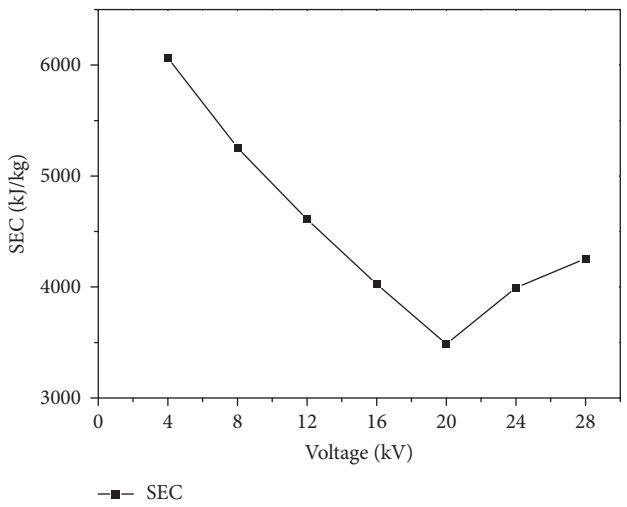

Figure 4: Effects of voltage on special energy consumption of tofu.

square error (ERMS), reduced mean square of the deviation (χ^2), and the modeling efficiency (EF) from mathematical models are given in Table 3. The results indicated that, in Exponential, Linear, and Quadratic models, the values of EF are above 0.96, and χ^2 and RMSE values ranged from 44.1071 to 60.0811 and 9.8935 to 11.6113, respectively, indicating a good fitness. It is clear that all the three mathematical models could satisfactorily describe thawing time curves of frozen tofu treated by HVAEF. Of all the models fitted, the highest values of EF and the lowest values of χ^2 could be obtained by the Quadratic model, but the lowest values of ERMS could be obtained by the Linear model. Thus, both the Linear and Quadratic models were selected as the best models to represent the thawing behavior of frozen tofu by HVAEF thawing technology. He et al. found that all the experimental results under high-voltage electric field fitted well with the Quadratic models for thawing time [3]; these results are in agreement with the present study.

4. Conclusion

The HVAEF technique can strengthen the thawing rate of frozen tofu. The thawing rate increases with strengthening applied voltage. By the root mean square error (ERMS), reduced mean square of the deviation (χ^2), and modeling efficiency (EF), both Linear and Quadratic models were found to be suitable for describing the thawing characteristics of frozen tofu under different voltages. Voltage has a major effect on thawing loss. Overall, HVAEF appears to be a feasible methodology for thawing frozen tofu. However, more studies are needed, especially for its comparison with other existing techniques for its full-scale utility.

Conflicts of Interest

All authors declare that they have no conflicts of interest regarding the publication of this paper.

Acknowledgments

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Research Article

Effect of Low-Frequency Ultrasonic-Assisted Enzymolysis on the Physicochemical and Antioxidant Properties of Corn Protein Hydrolysates

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The aim of this study was to investigate the effect of low-frequency ultrasound on the enzymolysis of corn protein. A L_9 (3^4) orthogonal design was used to optimize ultrasound pretreatment conditions. Degree hydrolysis (DH), conversion rate of protein (CR), and DPPH IC_{50} were selected as analytical indicators. Under the optimal ultrasound conditions (5 W/L power, 2 s/2 s on/off time, 50°C temperature, and 25 min time), the DH, CR, and radical (DPPH$^{\bullet}$, $^{\bullet}$OH) scavenging capacities were significantly increased. Molecular weight distribution and amino acid profile analysis showed that ultrasound pretreatment enhanced the formation of short-chain peptides with molecular weight of 200–3000 Da, especially the peptides containing hydrophobic amino acids. Moreover, 40 potential antioxidant peptides were purified by C18 semipreparative column and identified by UPLC-ESI-MS. The results suggest that the optimal ultrasonic-assisted enzymolysis technology could be useful for preparation of antioxidant peptides from corn.

1. Introduction

Corn, the third most widely cultivated cereal in the world, supplies approximately 42 million tons of protein per annum. China is the second largest country for production and consumption of corn [1]. Corn gluten meal (CGM), a main byproduct of the corn wet-milling process, contains 60–71% (w/w) protein [2]. Currently, CGM is mainly used as feedstuff or disposed due to low water solubility and severely imbalanced amino acid profile [3]. But on the other hand, CGM contains a high proportion of hydrophobic amino acids (leucine, alanine, phenylalanine, etc.) [4], which makes it become a potential source for peptides with biological activities such as antioxidant activity [5], antihypertensive effect [6], facilitating alcohol effect [7], and antitumor efficacy [8], while the poor solubility of corn protein and the special structure have created barrier for enzymes to cleave the protein, which seriously limited the functional peptides' release and reduced the corn protein's bioavailability. Therefore, developing a more efficient technique to overcome these problems is of great interest to food scientists all over the world.

Several kinds of new technologies have used extensively in food industry and its related fields, such as high pressure treatment, microwave assist, ultrasonication, heating at ambient and moderate pressures, and super-high frequency electromagnetic field, which could increase the extraction yield, achieve higher quality, protect environment, and so forth; our team has been devoted to the research of application of the ultrasound technology in food physical processing for many years. Its mechanism is attributed to the thermal, cavitation, and mechanical efficacies and they can enhance mass

transfer and increase the contact frequency between substrate and enzyme or change the substrate configuration [9, 10]. Ultrasound pretreated-assisted enzymolysis could induce protein unfolding and enhance proteolysis through increased exposure of susceptible peptide bonds [11] that enhance the efficiency of enzymatic protein hydrolysis for bioactive peptides production. Recent studies have shown that ultrasound pretreatment could facilitate release of angiotensin converting enzyme-inhibitory peptides from corn protein [2, 12] and wheat gluten [13], as well as antioxidant peptides from peanut protein [14] and wheat gluten [15]. In consideration of the ultrasonic mechanism and the characteristic of corn protein, more scientific studies are required to investigate the effect of ultrasound pretreatment on the physicochemical and antioxidant properties of corn protein hydrolysates.

Oxygen free radicals are very reactive molecules that can react with every cellular component and cause functional and morphologic disturbances in cells [16]. The bioactive peptides derived from food can play a significant role in an oxidative systems, protecting the human body from free radicals and retarding the progress of many chronic diseases, such as brain disorders, cancer, obesity, and cardiovascular diseases [17, 18]. Several studies have reported the antioxidant activity of corn protein hydrolysates [1, 3, 19], and only a few antioxidant peptides have been identified [5]. Thus, there are much more antioxidant peptides that need to be purified and determined.

In the present study, the optimum ultrasound pretreatment factors were developed by using orthogonal L_9 $(3)^4$ tests. The effects of ultrasound pretreatment on DH, protein conversion rate of corn protein and hydrolysates' antioxidant activities, molecular weights, and amino acid composition were evaluated. Furthermore, the antioxidant peptides from the corn protein hydrolysates with highest antioxidant activity were identified by mass spectrometry.

2. Materials and Methods

2.1. Materials and Chemicals. CGM, with 65.2% protein, was purchased from Yishui Earth Corn Development Co., Ltd. (Shandong, China). The concentrated corn protein with 92.5% protein purity was prepared according to a previous study [20]. Alcalase 2.4 L with an activity of 23,400 U/ml was purchased from Novozymes Co., Ltd. (Tianjin, China). All other chemicals used were of analytical grade.

2.2. Ultrasound Pretreatment of Corn Protein. Prior to the enzymolysis reaction, the corn protein was pretreated by the multifrequency energy-gathered ultrasound equipment. This apparatus was developed by our research team and manufactured by Meibo Biotechnology Co., Ltd (Zhenjiang, Jiangsu, China). Before ultrasound treatment, the corn protein dispersions (2.0%, w/v) were adjusted to pH 9.0 with 1.0 M NaOH. Then the protein dispersions were processed at a constant frequency of 28 kHz. The probe (2.5 cm in diameter) was dipped into the reaction solution to a depth of 0.5 cm and sonication was done at different sonication times ranging from 15 to 25 min and powers ranging between 45 and 65 W/L. Three kinds of ultrasound pulse modes of on time/off time (2 s/2 s, 3 s/2 s, and 4 s/2 s) were used.

2.3. Enzymatic Hydrolysis of Corn Protein. The enzymatic hydrolysis was carried out immediately after low-frequency energy-gathered ultrasound (LFEU) pretreatment. After 10 min preheating at 50°C, Alcalase (E/S = 2500 U/g) was added to initial the reaction and the pH was maintained at 9.0 by continuously adding 0.5 M NaOH during the enzymolysis process. The enzymolysis time was 60 min and the reaction was terminated by boiling the mixtures for 10 min. Then it was centrifuged at 5030g for 15 min to get the supernatant. The traditional enzymolysis (control) was conducted with a magnetic stirring apparatus under the same conditions, instead of ultrasound.

2.4. Determination of Degree of Hydrolysis (DH) and Protein Conversion Rate (CR). The DH of corn protein was determined using the pH-state method [4], which was calculated as follows:

$$\mathrm{DH}\,(\%) = \frac{h}{h_{\mathrm{tot}}} = \frac{N_b \times B \times 100}{\alpha \times M_p \times h_{\mathrm{tot}}}, \quad (1)$$

where B is the NaOH volume consumed (mL), N_b is the normality of the NaOH (mol/L), M_p is the protein weight (g), α is the average dissociation degree of α-NH_2 in substrate (0.99 for corn protein), and h_{tot} is the total number of peptide bonds in the protein substrate (9.2 mmol/g for corn protein).

The conversion rate of protein (CR) was calculated based on the following:

$$\mathrm{CR}\,(\%) = \frac{C \times V}{10M} \times 100\%, \quad (2)$$

where C is the concentration of corn protein hydrolysates (mg/mL), which was determined by Lowry method, using bovine serum albumin (BSA) as standard; V is the volume of the hydrolysates (mL); M is the mass of corn protein (mg).

2.5. Molecular Weight Distribution and Amino Acid Profile Analysis. The molecular weight distribution of the hydrolysate was analyzed by size exclusion chromatography with a TSK gel-G2000 SWXL column (7.8 mm × 30 cm, Tosoh). The elution was done using 45% acetonitrile plus 1% trifluoroacetic acid (TFA) with the flow rate of 0.5 mL/min for 65 min. The absorbance was monitored at 220 nm. Molecular weight of the peptide fractions was calculated and compared with the standards [21, 22].

The total amino acid profile of the sample was analyzed using an amino acid analyzer (RP-HPLC, Agilent 1100, US), after hydrolyzing under vacuum with 6 M HCl at 110°C for 24 h and 1% phenol (v/v), as described by Wang et al. [23] The free amino acid profile was measured by the analyzer after precipitation with 10% cold trichloroacetic acid for 2 h.

2.6. Radical Scavenging Activity. The DPPH radical scavenging activity was determined by the method described by Hu et al. [24] with slight modification. Two mL of sample was added to 2 mL of 0.2 mM ethanol solution of DPPH. After reacting for 30 min in the dark at 37°C, the absorbance of the solution

was monitored immediately at 517 nm. The scavenging rate was calculated as follows:

$$\text{Scavenging rate}\,(\%) = \left[1 - \frac{A_1}{A_0}\right] \times 100, \quad (3)$$

where A_0 was the absorbance of the control and A_1 was the absorbance of the sample.

The hydroxyl radical ($^{\bullet}$OH) scavenging activity was determined by the method reported previously, with slight modification [25]. Briefly, 0.5 ml of 9 mmol/L $FeSO_4$, 1 mL of 9 mmol/L salicylic acid in ethanol, 1 mL of 4.4 mmol/L H_2O_2, and 2 ml distilled water were sequentially added to 1 mL of sample. Absorbance of the mixture was measured at 510 nm after reacting for 30 min at 37°C. The $^{\bullet}$OH scavenging rate was calculated as follows:

$$^{\bullet}\text{OH scavenging rate}\,(\%) = \left[1 - \frac{(A_1 - A_2)}{A_0}\right] \times 100, \quad (4)$$

where A_0 is the absorbance of the blank, A_1 is the absorbance of the sample, and A_2 is the absorbance of the sample without H_2O_2.

2.7. Isolation of Antioxidant Peptides. The lyophilized corn peptides were dissolved in water contained 0.1% trifluoroacetic acid and subjected to an Agilent ZORBAX Eclipse XDB C18 semipreparative column (9.4 × 250 mm, 5 μm) and separated by a gradient of acetonitrile (2–15% in 0–20 min, 15–30% in 20–35 min, and 30–80% in 35–40 min) at 2.5 mL/min. Online UV absorbance was monitored at 220 nm. The elution fractions collected every minute were concentrated and freeze-dried. The antioxidant activity of each fraction was expressed as the DPPH radical scavenging rate per unit weight peptide (mg).

2.8. Identification of Antioxidant Peptides by UPLC-ESI-MS. The purified peptides were dissolved in 0.1% aqueous formic acid and identified using Waters ACQUITY UPLC coupled to SYNAPT Q-TOF MS with electrospray ionization. 10 μL sample was injected into a BEH130 C18 column (2.1 × 150 mm, 1.7 μm) and eluted with a linear gradient of acetonitrile (0–30% in 8 min and 30–80% in 8–15 min) at 0.2 mL/min. The amino acid sequence was analyzed using BioLynx software and confirmed by performing searches against manually restricted NCBI database with entries only from G. max (11, 1525 sequences, 03-2016) taxonomy [26]. To analyze the antioxidant activity, all identified peptides were searched against online available databases BioPep [27].

2.9. Statistical Analysis. All the tests were conducted in triplicate and data were presented as mean and standard deviations. The results obtained were subjected to one-way analysis of variance (ANOVA). Differences were considered significant at $p < 0.05$. All computations were done with SPSS 15.0.

3. Results and Discussion

3.1. Analysis of Orthogonal Design Experiment of LFEU Pretreatment. Over the past decades, ultrasound has been increasingly investigated for application of controlled release of peptides. High power ultrasound treatment for longer treatment time can generate high temperature and pressure conditions which can alter the native state of food protein or peptide [11]. It is necessary to optimize the treatment conditions for specific ultrasound applications to achieve optimum results. In the present study, L_9 (3^4) orthogonal design was used to optimize processing conditions to obtain peptides, which might possess potent high antioxidant activity. The full experimental design, with respect to the evaluation indicators (DH, DPPH IC_{50}, and CR) were presented in Table 1.

The results of the orthogonal test are shown in Tables 2 and 3. Fisher's F test, however, provided a decision at some confidence level so as to make sure whether these factors have significant effect on experimental indicators. Table 2 indicates that the F-test on all four factors was significant ($p < 0.05$) for DPPH IC_{50}. For the other two indicators, only factors A and C were significant. Results of the range analysis (Table 3) showed that the influential orders of the four factors to DPPH IC_{50}, DH, and CR were $A > D > C > B$, $A > C > D > B$, $A > C > B > D$, respectively. Based on the results of range analysis and ANOVA, the optimum pretreatment condition for DPPH IC_{50}, DH, and CR were $A_3D_2C_2B_1$, $A_3C_3D_2B_1$, and $A_3C_3B_3D_1$, respectively. Based on the frequent best levels obtained for the reference indicators of DPPH IC_{50}, DH, and CR (Table 1), the best pretreatment condition was $A_3B_1C_3D_2$, namely, 65 W/L ultrasound powers, 2 s/2 s ultrasound on/off time, 50°C ultrasound temperature, and 25 min ultrasound time. These corresponded to number 7 in Table 1, which achieved the highest DPPH IC_{50}, DH, and CR.

3.2. DH and CR of Corn Protein after Ultrasonic Pretreatment. Table 1 shows the change of DH and CR during enzymolysis process of corn protein for the different pretreatments. For the traditional hydrolysis, the DH was 19.28% and 51.58% for CR. Obviously, compared to traditional hydrolysis, ultrasonic pretreatment caused increase of 5.42% and 11.27% in DH and CR. The current study results are in agreement with the that of ultrasound-assisted enzymolysis of solid leather waste [28], zein [12], and soy protein isolates [29]. This enhancement in DH and CR could be attributed to the particle size reduction and change in molecular conformation of protein [2, 30].

3.3. Molecular Weight Distribution and Amino Acids Profile after Ultrasonic Pretreatment. The molecular weight distribution of the corn peptides obtained from traditional and LFEU assisted enzymolysis is presented in Figure 1. The ultrasound pretreatment (the best conditions) caused an increase of 11.84% for 200–1000 Da peptides fraction and 21.29% for the fraction of 1000–3000 Da peptides. Meanwhile, the fraction of <200 Da and >3000 Da peptides decreased by 15.61% and 46.5%, respectively. This increase in the amounts of small peptides fraction (200–3000 Da) after ultrasound pretreatment might be due to exposure of more hydrophobic cores buried inside the protein. Moreover, Alcalase is a typical endoprotease with a preference for sites containing hydrophobic residues [31]. You et al. [32] also reported that the peptides were absorbed by the body mainly in the form of tetrapeptide, tripeptide, and dipeptide, rather than mainly

Table 1: Factors and levels of the orthogonal design L_9 (3^4) of ultrasound pretreatment process and experimental results for corn protein hydrolysis (n = 3, mean ± SD); number 10 represents traditional hydrolysis.

Test number	Factors (levels)				Experimental results		
	(*A*) Ultrasound powers (W)	(*B*) Ultrasound on/off time	(*C*) Ultrasound temperature (°C)	(*D*) Ultrasound time (min)	DPPH IC_{50} (mg/ml)	Degree hydrolysis (%)	Conversion rate of protein (%)
1	45 (1)	2/2 (1)	30 (1)	15 (1)	3.125 ± 0.078	19.528 ± 0.402	51.451 ± 3.065
2	45 (1)	3/2 (2)	40 (2)	20 (2)	2.495 ± 0.092	20.720 ± 1.065	54.288 ± 2.539
3	45 (1)	4/2 (3)	50 (3)	25 (3)	2.643 ± 0.118	21.497 ± 2.102	56.615 ± 2.457
4	52 (2)	2/2 (1)	40 (2)	25 (3)	2.322 ± 0.045	22.266 ± 1.402	56.304 ± 4.258
5	52 (2)	3/2 (2)	50 (3)	15 (1)	2.647 ± 0.035	23.713 ± 0.645	58.067 ± 4.831
6	52 (2)	4/2 (3)	30 (1)	20 (2)	2.581 ± 0.184	20.755 ± 0.056	56.985 ± 3.112
7	65 (3)	2/2 (1)	50 (3)	20 (2)	2.035 ± 0.113	24.704 ± 0.495	62.85 ± 0.491
8	65 (3)	3/2 (2)	30 (1)	25 (3)	2.289 ± 0.045	21.640 ± 0.112	56.768 ± 0.328
9	65 (3)	4/2 (3)	40 (2)	15 (1)	2.354 ± 0.168	23.502 ± 0.682	60.288 ± 2.539
10	—	—	—	—	2.402 ± 0.098	19.275 ± 0.608	51.581 ± 2.654

Table 2: Analysis of variance (ANOVA) on indicator parameters obtained from the L_9 (3^4) orthogonal experiment (n = 3, Mean ± SD). *A*, ultrasound powers; *B*, ultrasound on/off time; *C*, ultrasound temperature; *D*, ultrasound time.

Indicators	Source	Sum of squares	df	Mean square	*F*	*p*	Sig.
DPPH IC_{50}	Model	170.874	9	18.986	3.073*E*3	<0.05	significant
	A	1.260	2	0.630	102.012	<0.05	significant
	B	0.511	2	0.245	39.902	<0.05	significant
	C	0.384	2	0.192	31.070	<0.05	significant
	D	0.604	2	0.302	48.863	<0.05	significant
	Error	159.653	18	8.870			
	Total	50178.870	27				
DH	Model	50019.217	9	5557.691	626.600	<0.05	significant
	A	154.079	2	77.040	8.686	<0.05	significant
	B	11.891	2	5.945	.670	0.524	
	C	76.142	2	38.071	4.292	<0.05	significant
	D	12.778	2	6.389	.720	0.500	
	Error	13.700	18	.761			
	Total	13191.364	27				
CR	Model	13177.664	9	1464.185	1.924*E*3	<0.05	significant
	A	33.400	2	16.700	21.942	<0.05	significant
	B	0.279	2	.139	.183	0.834	
	C	32.144	2	16.072	21.116	<0.05	significant
	D	0.905	2	.453	.595	0.562	
	Error	13.700	18	.761			
	Total	13191.364	27				

Note. Differences were considered significant at $p < 0.05$.

free amino acids. Therefore, the result indicated that the LFEU pretreatment helped peptides absorb rapidly and had a high bioactivity.

Table 4 shows the amino acid composition of corn protein hydrolysates obtained by control and LFEU assisted enzymolysis. The total AAs significantly increased after ultrasound pretreatment, which is in accordance with the influence of ultrasound on corn protein's DH and CR. The corn protein hydrolysates contained considerable amounts of hydrophobic AAs (429.95 μg/ml), which increased by 13.41% after being pretreated by ultrasound. However, the percentage of free hydrophobic AAs was barely changed. By comparison, we

Table 3: Range analysis (R) on indicator parameters obtained from the L_9 (3^4) orthogonal experiment (n = 3, mean ± SD).

Indicators	(A) Ultrasound powers (W)	(B) Ultrasound on/off time	(C) Ultrasound temperature (°C)	(D) Ultrasound time (min)
DPPH IC_{50} (mg/ml)				
K_1	2.754 ± 0.096	2.477 ± 0.236	2.665 ± 0.307	2.709 ± 0.281
K_2	2.517 ± 0.983	2.494 ± 0.172	2.39 ± 0.305	2.37 ± 0.389
K_3	2.226 ± 0.109	2.526 ± 0.47	2.442 ± 0.208	2.418 ± 0.208
R	0.528	0.049	0.275	0.339
Best level	A_3	B_2	C_2	D_2
Degree hydrolysis (%)				
K_1	20.615 ± 0.656	22.199 ± 3.299	20.674 ± 1.858	22.106 ± 1.729
K_2	22.245 ± 0.834	22.024 ± 1.822	22.163 ± 1.633	22. 281 ± 3.016
K_3	23.282 ± 0.763	21.918 ± 1.640	23.305 ± 2.642	21.801 ± 2.016
R	2.667	0.281	2.631	0.48
Best level	A_3	B_1	C_3	D_2
Conversion rate of protein (%)				
K_1	54.118 ± 2.687	56.868 ± 2.605	55.068 ± 2.168	58.041 ± 3.478
K_2	57.119 ± 4.067	56.374 ± 2.566	56.960 ± 3.112	56.602 ± 2.047
K_3	59.969 ± 1.119	57.963 ± 2.702	59.177 ± 2.593	56.562 ± 2.347
R	5.851	1.589	4.109	1.479
Best level	A_3	B_3	C_3	D_1

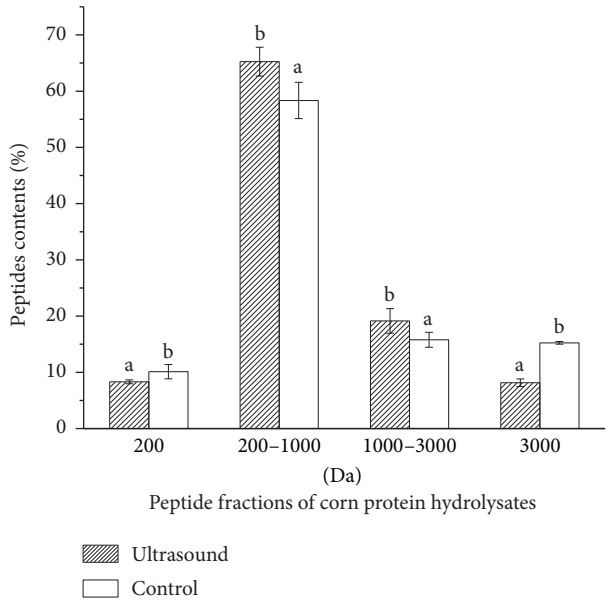

Figure 1: Molecular weights distribution of corn protein hydrolysates. [a,b]Values which are significantly different at $P < 0.05$.

found a large amount of increased hydrophobic AAs in form of peptides. This result also indicated that ultrasonic treatment to corn protein tended to produce more peptides with hydrophobic amino acid residues than traditional hydrolysis, which is in line with the finding reported by Jia et al. [13].

3.4. Antioxidant Activity of Corn Protein Hydrolysates after Ultrasonic Pretreatment. Antioxidant activity depends on many different factors, such as DH, CR, molecular weight distribution, and AAs composition. The results above showed that ultrasound pretreatments improved the DH and CR obviously and significantly ($p < 0.05$) changed the molecular weight distribution and amino acids composition of the corn peptides. Therefore, the radical (DPPH, $^{\bullet}OH$) scavenging capacities were analyzed to evaluate the antioxidant activity of corn peptides after ultrasonic pretreatment.

Table 4: Total and free amino acids composition of corn protein hydrolysates.

	Total amino acids				Free amino acids			
	Control		Ultrasound		Control		Ultrasound	
	mg/ml	%	mg/ml	%	mg/ml	%	mg/ml	%
Asp	44.83	6.01	37.21	4.50	0.14	0.26	0.18	0.36
Glu	143.46	19.22	195.12	23.60	5.60	10.12	5.50	11.08
Ser	34.77	4.66	31.42	3.80	0.88	1.59	0.67	1.35
His	24.96	3.34	21.50	2.60	0.36	0.66	0.31	0.62
Gly	23.25	3.12	19.84	2.40	3.99	7.23	3.37	6.78
Thr	28.67	3.84	23.98	2.90	0.36	0.69	0.36	0.72
Arg	30.53	4.09	28.94	3.50	0.28	0.52	0.31	0.62
Ala	59.67	8.00	81.02	9.80	14.13	25.60	11.48	23.15
Tyr	30.80	4.13	34.72	4.20	0.46	0.84	0.41	0.83
Cys	15.16	2.03	9.92	1.20	0.33	0.54	0.33	0.67
Val	35.46	4.75	28.94	3.50	1.46	2.66	2.03	4.09
Met	15.37	2.06	14.06	1.70	10.46	18.93	9.66	19.47
Phe	42.66	5.72	62.09	7.51	8.08	14.58	6.01	12.12
Ile	29.94	4.01	28.11	3.40	5.78	10.44	5.99	12.07
Leu	106.75	14.30	125.92	15.23	0.78	1.38	0.98	1.97
Lys	19.87	2.66	18.19	2.20	0.91	1.62	0.54	1.09
Pro	60.60	8.12	65.83	7.96	1.29	2.34	1.49	3.00
The total of hydrophobic amino acids (HAAs)	379.12	50.80	429.95	52.00	42.34	76.62	38.00	76.59
The total of amino acids (AAs)	746.76	100.0	826.79	100.00	55.24	100.00	49.61	100.00

Hydrophobic AAs: Ala, Val, Leu, Ile, Phe, Pro, Thr, and Met.

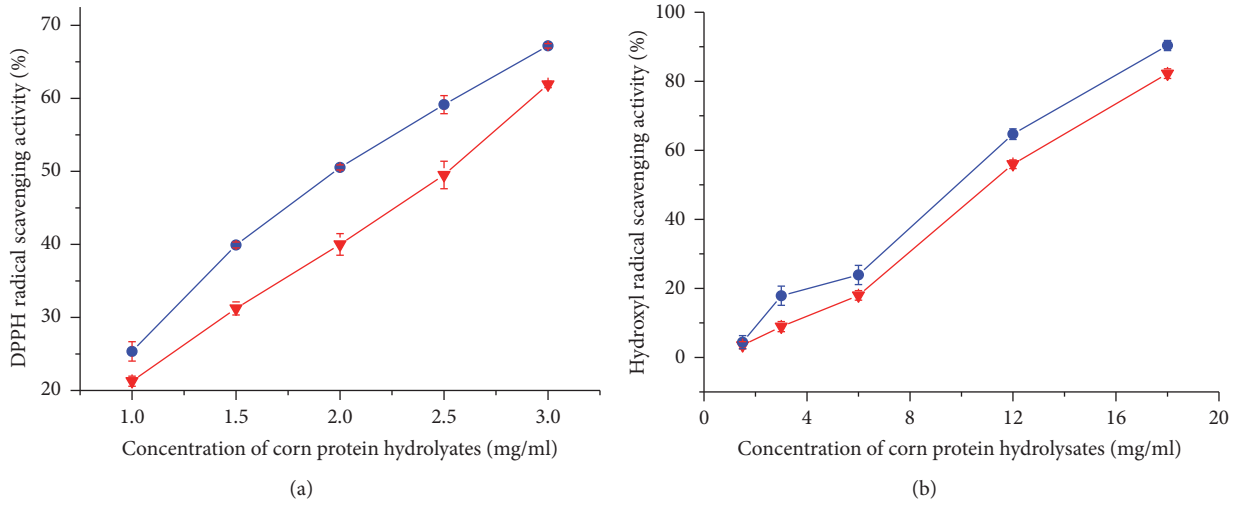

Figure 2: Antioxidant capacities of corn protein hydrolysates on DPPH radical scavenging activity (a) and hydroxyl radical scavenging activity (b). Ultrasonic (●); control (▼).

Generally, hydrolysates contain peptides or AAs, which were hydrogen donors that could react with radicals to convert them to more stable products, thereby terminating the radical chain reaction [33]. Figure 2 shows the changes of the antioxidant activity at various concentrations in traditional and LFEU assisted enzymolysis. As depicted, the corn protein hydrolysates showed dose-dependent antioxidant activity to varying extents. At any tested concentration, the DPPH radical and $^{\bullet}$OH scavenging activity of corn peptides prepared by ultrasound pretreatment were significantly higher than that of control. With respect to IC_{50}, the lower the value means, the higher the antioxidant activity and vice versa. The IC_{50} values of corn peptides were 2.41 and 9.98 mg/ml (control) 1.95 and 7.61 mg/ml (ultrasound pretreatment), respectively, for DPPH and $^{\bullet}$OH radical scavenging activities. The result suggested that LFEU assisted enzymolysis could significantly increase the antioxidant activity of corn protein hydrolysates. Additionally, comparing DPPH IC_{50} values to that of other

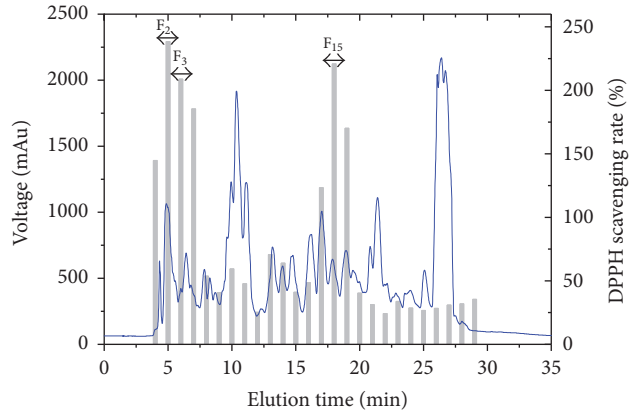

Figure 3: Chromatographic and antioxidant profiles of corn protein hydrolysates using Eclipse XDB C18 semipreparative column. Histogram, DPPH radical scavenging rate per unit weight peptide (mg).

protein hydrolysates, such as loach peptides (17.0 mg/mL) [34], albumen peptides (5.767 mg/mL), and soybean peptides (6.268 mg/mL) [35], corn peptides exhibited remarkable DPPH radical scavenging activity. It indicated that LFEU pretreatment might help in releasing the higher antioxidant peptides from corn protein hydrolysates, which could be used as source of antioxidant peptides for the further purification and identification.

3.5. Purification and Identification of Antioxidant Peptides. The hydrolysates from Alcalase-hydrolyzed corn protein after ultrasonic pretreatment were chromatographically fractionated by the C18 semipreparative column (Figure 3). As shown in Figure 3, three fractions displayed the strong DPPH radical scavenging activity, namely, F_2, F_3, and F_{15}. The fractions displaying the strong activity were collected and further purified and identified using UPLC-ESI-MS spectrometry. Identification of a large amount of peptide sequences in complex food protein hydrolysates is challenging. Therefore, all of the identified peptides were further searched against the sequences of corn (NCBI database) and 40 peptides were obtained with a probability of certainty of 100 (Table 5).

It has been reported that the majority of antioxidant peptides derived from food protein consist of 2 to 20 amino acids and the lower the molecular weight, the higher their chance to cross the intestinal barrier and exert biological effects [36]. Dávalos et al. [37] suggested that hydrolysates with higher proportion of low molecular weight peptides could access more easily the oxidant system and lead to high values of TEAC and DPPH radical scavengers. The number of amino acids ranged from 2 to 5 and the MW range of the identified antioxidant peptides was 200–500 Da, which corresponded to the above research findings.

Additionally, the antioxidant activity of peptides was highly dependent on their sequences and amino acid compositions. Statistical analyses found that the most popular AAs in our current study appeared to be Gly, Ala, Ser, Leu, Phe, Val, Pro, and His. The result is in agreement with the finding of Mendis et al. [38] who purified the peptides from jumbo squid skin gelatin and reported that the peptides containing Pro, Gly, Ala, Val, and Leu in the peptides sequence had strong antioxidant activity. Another frequent amino acid residue of the identified peptides was His, an important amino acid residue responsible for the radical scavenging activity of peptides due to their special structure characteristics (the imidazole group in His has the proton-donation ability) [39]. Furthermore, it has been reported that hydrophobic amino acids (Ala, Leu, Phe, Val, Met, and Pro in the identified peptides) have a significant effect on radical scavenging, which can enhance the presence of peptides at the water lipid interaction and facilitate the permeability to the lipid phase to scavenge the generated free radicals [40].

Sequenced peptides were checked through BIOPEP bioactive peptide databases. We found 7 peptides with significant antioxidant activity that have been reported in previous studies. Especially, LPH and LLPH were also found in corn peptides hydrolyzed by Alcalase [36]. Furthermore, there were 33 peptides, which still need to be identified and their antioxidant activity and physiological effects need to be validated after synthesizing.

4. Conclusion

In this study, an orthogonal design L_9 (3^4) was applied to ultrasonic-assisted enzymolysis in the preparation of corn antioxidant hydrolysates. Under the optimal ultrasound pretreatment, the radical (DPPH, $^{\bullet}OH$) scavenging capacities were significantly increased. The increase in DH, CR, short-chain peptides with molecular weight 200–3000 Da, and the peptides containing hydrophobic AAs coincided with the improvement in the antioxidant activity of corn protein hydrolysates. Furthermore, the potential antioxidant peptides were purified by C18 semipreparative column and identified by UPLC-ESI-MS. For the identified 40 peptides, except the 7 peptides that have been reported before, further studies are

Table 5: Amino acid sequence of purified peptide (F_2, F_3, and F_{15}) identified using UPLC-ESI-MS.

Fraction number	Peptide sequence	Calculated mass	Probability of certainty	Activity
F_2	SGV	261.28	100	Antioxidant
	FNV	378.44	100	
	AL	202.25	100	
	MT	250.32	100	
	SPL	315.37	100	
	LAH	339.40	100	
	PEA	315.33	100	
	YPQ	406.45	100	
	LDV	345.40	100	
	ENN	375.34	100	
	EDL	375.38	100	
	EPDE	488.46	100	
	LPF	375.47	100	Antioxidant
F_3	LLPH	478.60	100	Antioxidant
	LLPF	478.60	100	Antioxidant
	FLPF	512.62	100	Antioxidant
	VGA	245.29	100	
	PAAQ	385.43	100	
	AAV	259.30	100	
	LGA	259.30	100	
	AH	226.24	100	
	AHL	339.39	100	Antioxidant
	LAH	339.40	100	
	RLQ	415.50	100	
F_{15}	LGV	287.36	100	Antioxidant
	SHL	355.39	100	
	SPGA	330.34	100	
	NGGGA	374.35	100	
	PSAQ	401.43	100	
	VGSP	358.40	100	
	TNLA	417.47	100	
	ANLT	417.47	100	
	SPSP	385.43	100	
	ALSP	386.45	100	
	MM	280.41	100	
	SF	252.27	100	
	SH	242.24	100	
	SHQ	370.37	100	
	HSQ	370.38	100	
	SPL	315.37	100	

needed to validate their antioxidant activity and physiological effects after synthesizing.

Additional Points

Practical Application. The ultrasound technology has been widely applied to solve traditional enzymatic hydrolysis problems. The results of this study suggest that the low-frequency ultrasonic pretreatment could significantly increase DH, CR, and radical ($DPPH^{\bullet}$, $^{\bullet}OH$) scavenging capacity of corn derived protein hydrolysates. The optimal ultrasonic pretreatment parameters were attained by orthogonal design. This study could be useful for producing antioxidant peptides for foods and pharmaceuticals industries.

Disclosure

This article does not contain any studies involving human or animal subjects.

Conflicts of Interest

There are no conflicts of interest to declare.

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Research Article

Effects of Combined Heat and Preservative Treatment on Storability of Ponkan Fruit (*Citrus reticulata* Blanco cv. Ponkan) during Postharvest Storage

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Heat treatment and preservative application have been widely used during postharvest storage of many fresh products, but the effect of their combination on citrus storage has rarely been investigated. In this study, the optimal heat treatment (HT) conditions and HT combined with preservative treatment were investigated for Ponkan fruit (*Citrus reticulata* Blanco cv. Ponkan) storage. Results indicated that HT at 55°C for 20 s can significantly reduce the decay rate of Ponkan fruit, and a combination of HT and 25% of the preservative dosage used in production of iminoctadine tris (albesilate), 2,4-dichlorophenoxyacetic acid, and imazalil significantly reduced the decay rate without affecting fruit quality. In addition, the increased fiber contents in fruit receiving the HT combined with preservative treatments may be a response preventing fungus infection and enhancing fruit storability and resistance. The above results suggested that the combination of HT and 25% of the preservative production dosage was optimal for controlling Ponkan fruit decay during storage.

1. Introduction

Ponkan (*Citrus reticulata* Blanco cv. Ponkan) is one of the most widely grown and economically important citrus fruit species in China. However, the fruit is often subjected to huge losses during postharvest storage and transportation, which presents a big challenge to the citrus industry. Biological diseases and physical damage are reported to be the most important reasons causing significant economic losses during storage [1]. Many postharvest treatments have been used in industry to reduce the decay rate during storage and transport, including physical measures [2, 3] and preservative applications [4, 5].

Application of preservative is an effective way to reduce the decay rate, which can control the growth and spread of microorganisms. The most commonly used preservative mixture in the Ponkan industry contained 200 mg L^{-1} iminoctadine tris (albesilate), 100 mg L^{-1} 2,4-dichlorophenoxyacetic acid, and 200 mg L^{-1} imazalil [6–8]. However, the long-term application of preservative can generate pathogen resistance against many site-specific fungicides [9], and some preservatives even have bad effects on human health and the environment. Nowadays, people pay more attention to food safety; thus, to reduce or abandon the use of preservative becomes a new challenge for postharvest storage of fruit and vegetables and for preservation of other fresh foods.

Heat treatment (HT) is one of the most commonly used physical measures to reduce fruit decay, which has been reported to be effective in decreasing fly infestation, inducing resistance to chilling injury and enhancing the

biocontrol effectiveness of antagonistic yeasts [10–12]. Previous reports also showed that heat treatment enhanced the efficacy of preservative and decreased the amount of chemicals used for decay control in fruits [13, 14]. However, the effects of HT combined with iminoctadine tris (albesilate), 2,4-dichlorophenoxyacetic acid, and imazalil mixture on storability and quality in Ponkan fruit have rarely been studied.

In this study, the effect of HT combined with application of preservative mixture, including iminoctadine tris (albesilate), 2,4-dichlorophenoxyacetic acid, and imazalil, on storability and quality of Ponkan fruit was evaluated by analyzing the decay rate, weight loss, color, superficial microbial population, ethanol content, acetaldehyde content, pectin content, and fiber content during postharvest storage. The future applications of this method are discussed.

2. Materials and Methods

2.1. Fruit Materials and Treatments. Fruits of uniform size and color were picked at economic harvest time from an orchard located in Quzhou, China, in 2012–2014.

Fruits used for heat treatments were picked in 2012. The control fruits were submerged in water at room temperature (RT), and the heat treatment groups were submerged in hot water at 55°C for 20 s, 30 s, and 40 s, respectively. After the treatments, fruits were stored in a ventilated warehouse at 10°C and were sampled at 0, 30, 60, 90, and 120 d storage.

Fruits used for combined HT and preservative treatments were picked in 2013 and 2014. The preservative mix includes iminoctadine tris (albesilate), 2,4-dichlorophenoxyacetic acid, and imazalil. 0%, 25%, 50%, and 100% (w/v) of the preservative dosage used in production, which contained 200 mg L^{-1} iminoctadine tris (albesilate), 100 mg L^{-1} 2,4-dichlorophenoxyacetic acid, and 200 mg L^{-1} imazalil, were dissolved in both RT and 55°C water and were used for the HT combined preservative treatments. The CK, HT, 25% C ("C" represents the concentration of preservative used in production), HT + 25% C, 50% C, HT + 50% C, 100% C, and HT + 100% C fruits were submerged in corresponding preservative solutions for 20 s. After the treatments, fruits were stored for 0, 30, 60, 90, 120, and 150 d in a ventilated warehouse at 10°C and the fruits with 150 d storage were transferred to shelf storage for analysis at 7 and 15 d. These experiments were repeated twice over two years.

2.2. Decay Rate Analysis. Each treatment contained 300 fruits, which were divided equally into three groups and were considered as three biological replicates. Fruits with obvious appearance of decay were recorded on each sampling day. Decay rate were calculated according to the following formula: decay rate (%) = $n/100 \times 100\%$, where "n" represents the total number of decayed fruits in each replicate.

2.3. Weight Loss Analysis. Thirty fruits were randomly selected from each treatment and marked. The weight of each fruit was regularly recorded on each sampling day. The weight loss rate was calculated according to the following formula: weight loss rate (%) = $(m_0 - m)/m_0 \times 100\%$, where "$m_0$" represents fruit weight on 0 d storage and "m" represents fruit weight on each sampling day.

2.4. Color Analysis. Color measurement was carried out with a Hunter Lab Mini Scan XE Plus colorimeter (Hunter Associates Laboratory, Inc., Reston, VA) at four evenly distributed equatorial sites of each fruit. The CIE 1976 L^* a^* b^* color scale was adopted. Citrus color index (CCI) was calculated according to the following formula: CCI = $1000 \times a^*/(L^* \times b^*)$.

2.5. Ethanol and Acetaldehyde Contents Analysis. Ethanol and acetaldehyde production was determined with a gas chromatograph instrument (Agilent 6890N, Folsom, CA, USA) with an FID column (HP-INNOWAX, 0.25 mm, 30 m, 0.25 μm, Agilent J&W, Folsom, CA, USA) according to the method described by Min et al. [15] with modifications. The injector, detector, and oven temperatures were 150°C, 160°C, and 100°C, respectively. Sec-butyl alcohol was added to each vial as an internal control. The results were calculated using standard curves for acetaldehyde and ethanol, respectively.

2.6. Microbial Population Analysis. Microbial medium was obtained from peel of Ponkan fruit and cultured on potato dextrose agar (Difco, Detroit, MI). Twenty grams fruit peel was put into a blender and homogenized with 100 mL sterile water. 1 mL mixtures for each sample were taken out and homogenized with 9 mL sterile water. A volume of 100 μL mix was cultured on PDA medium. Colony-forming units (CFU) were counted after incubation for 72 h at 28°C.

2.7. Pectin Content Analysis. One gram fruit tissue samples were homogenized with 25 mL 95% ethanol, followed by 30 min boiling water bath. Then the homogenate was centrifuged at 8000 rpm for 30 min to remove the supernatant. Repeat the extraction step for three times. The resulting pellet was incubated with 20 mL distilled water at 50°C water bath for 30 min to dissolve the pectin. The homogenates were then centrifuged at 8000 rpm for 15 min. Transfer the supernatant into a 100 mL volumetric flask and wash the precipitate with distilled water. Move the supernatant into the volumetric flask and dilute with distilled water to volume. This is the soluble pectin. Add 25 mL 0.5 mol L^{-1} sulfuric acid into the precipitate and incubate it in boiling water for 1 h to dissolve the pectin. The homogenates were centrifuged at 8000 rpm for 15 min; then transfer the supernatant into a 100 mL volumetric flask and dilute it with distilled water to volume. This is the protopectin. Incubate 1 mL of the extracted solution with 6 mL concentrated sulfuric acid for 20 min in boiling water. Add 0.2 mL 1.5 g L^{-1} carbazole-ethanol into the mixture, and keep it in dark for 30 min. The absorbance of the reaction mixture was measured at 530 nm to calculate the content of galacturonic acid by contrast with the standard curve. The total pectin content includes both the solution pectin and protopectin.

2.8. Fiber Content Analysis. Add 5 mL mixture of acetic acid and nitric acid into 100 mg drying samples, incubated with boiling water bath for 60 min; the homogenate was

Table 1: Effects of different heat treatments on decay rate of Ponkan fruit during storage.

Treatments	Decay rate (%)				
	0 d	30 d	60 d	90 d	120 d
CK	nd	nd	3.33 ± 0.58	13.3 ± 2.31[a]	13.3 ± 1.53[a]
55°C 20 s	nd	nd	nd	6.10 ± 2.07[b]	8.54 ± 1.15[c]
55°C 30 s	nd	nd	nd	6.67 ± 1.15[b]	10.0 ± 2.31[b]
55°C 40 s	nd	nd	nd	6.67 ± 1.53[b]	10.0 ± 3.51[b]

"nd" indicates no decayed fruit observed.

centrifuged at 5000 rpm for 10 min to remove the supernatant. The precipitate was washed with 8 mL water and 8 mL acetone, respectively, and centrifuged at 5000 rpm for 10 min to remove the supernatant. The precipitate was dried at 30°C for 30 min, and then 9 mL 67% suspension samples were stewing for 1 h. Repeat the step for three times. Dilute the supernatant for 50 times. The reaction mixture consisted of 1 mL supernatant, 1 mL phenol, and 5 mL concentrated sulfuric acid (ice cold), incubated at RT for 30 min. The absorbance of fiber was measured at 490 nm.

2.9. Statistical Analysis. Standard errors and figures were drawn using Origin 8.0 (Microcal Software Inc., Northampton, MA, USA). The test of statistical significance was based on the total error criteria with a confidence level of 95.0%, calculated by SPSS Statistics 20.0 Software.

3. Results and Discussion

3.1. Effects of Heat Treatments on Decay Rate of Ponkan Fruit during Storage. The decay rate of Ponkan fruit increased continuously in all the treatments during storage. No decayed fruit were found in heat treatments until 90 d storage, while the control fruit reached a decay rate of 3.33% at 90 d storage. Compared with the control, the decay rate was reduced by 36%, 25%, and 25% in heat treatments at 55°C for 20 s, 30 s, and 40 s, respectively (Table 1). Thus, heat treatment was effective in reducing decay during storage, which was similar to results from previous reports [16–18]. In addition, heat treatment at 55°C for 20 s was the optimal condition to extend storage time of Ponkan fruit and was used for further research.

3.2. Effects of Combined Heat and Preservative Treatments on Decay Rate, Shelf Life, and Quality of Ponkan Fruit during Storage. The decay rate of Ponkan fruit increased continuously in all the treatments during the whole storage period. Compared with the control, the decay rates in all the treated samples were significantly decreased during 90 to 150 d storage. At 150 d storage, the decay rate in the control reached a level of 30%, while all the treated samples exhibited decay rates lower than 18%, with a ranking order of HT, 25% C, 50% C, HT 50% C, HT + 25% C, HT + 100% C, and 100% C from high to low (Figure 1(a)). When transferred to shelf storage, the three combined treatments showed lower decay rates compared with the corresponding preservative treatments alone. Compared with 25% C treatment, the HT + 25% C treatment decreased the decay rate by about 75% and 50% after transferring to shelf storage for 7 and 15 d, respectively (Figure 1(b)). Weight loss and CCI in all the treatments increased continuously during the whole storage period, but no significant difference was found between the control and treated samples in either weight loss or CCI (Figures 1(c) and 1(d)).

From the above results, the combined heat plus 25% preservative treatment significantly decreased the decay rate of Ponkan fruit during storage, showing a similar effect to that with 100% preservative treatment. This may be because the heat treatment was effective in enhancing the efficacy of preservative [14] and was an effective measure to decrease the preservative content while still controlling fruit decay, which was similar with the effect of heat treatment combined with preservative on other fresh produce [19–21]. Also, the heat treatment may promote resistance to microorganisms in Ponkan fruit during storage, which has been reported in other fruits [22–24]. In addition, no significant difference in weight loss and CCI between the treatments was observed, indicating that the HT combined with preservative treatments had no adverse effects on fruit quality during storage. Thus, the optimal combination for long-term storage of Ponkan fruit is heat treatment at 55°C for 20 s and preservative with a concentration of 25% of the dosage used in production, and this method can be further applied during fruit storage in the citrus industry.

3.3. Effects of Combined Heat and Preservative Treatment on the Epiphytic Microbial Population of Ponkan Fruit during Storage. The epiphytic microbial population increased in all the treatments during the whole storage period. No significant difference was investigated between the treated and untreated samples during the early storage period. Compared with the control, the 100% C and HT + 100% C treatments significantly decreased the epiphytic microbial population during the later storage period. At 120 d storage, the microbial population was 363, 290, 162, and 60 CFU L^{-1} in CK, HT + 25% C, HT + 50% C, and HT + 100% C treatment, respectively, showing a decreasing trend with increasing preservative concentration. At 150 d storage, the microbial population was decreased to 65% and 60% by 100% C and HT + 100% C treatments, respectively, while no significant difference was observed between the other treatments and the control (Figure 2). The observation that the HT + 25% C treatment showed a similar effect to the 100% preservative treatment in decreasing fruit decay rate suggests that the epiphytic microbial population is not the only determinative factor for fruit decay during storage.

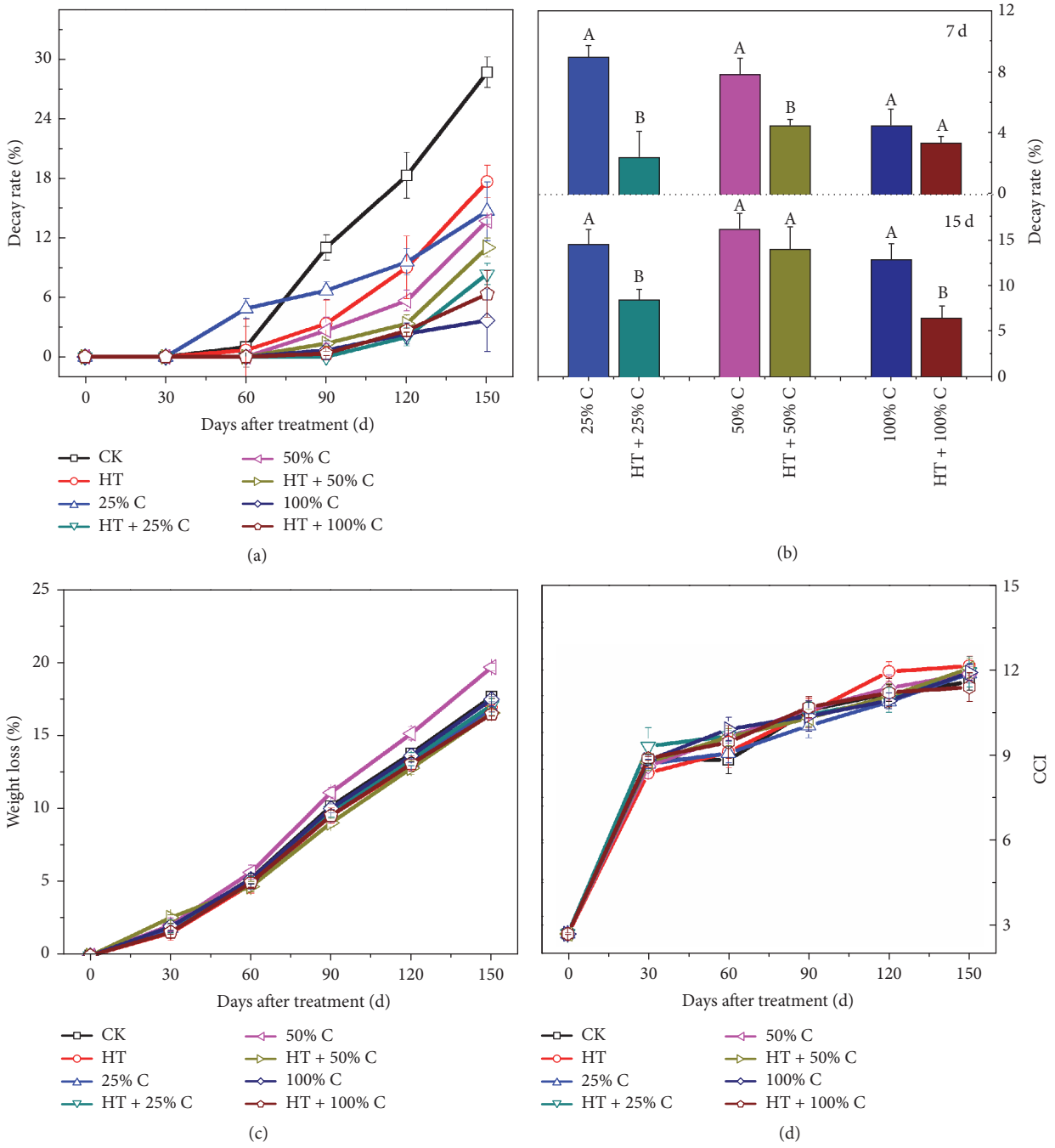

Figure 1: Effects of different combined heat and preservative treatments on the decay rate (a), shelf life (b), weight loss (c), and CCI (d) of Ponkan fruit during storage. "C" represents the concentration of preservative used in production. The error bars represent the standard errors. Letters on columns represent significant differences at the 0.05 level.

3.4. Effects of Combined Heat and Preservative Treatments on Ethanol and Acetaldehyde Contents in the Fruit Pulp during Storage. The content of ethanol in fruit pulp showed an increasing trend during storage in general, and it occurred more rapidly during the later storage period. A sharp increase was observed in HT treatments at 90 and 120 d storage compared with the control and other treatments. During the late storage period, the ethanol content of fruits in the HT + 25% C treatment was significantly lower than with the 25% C treatment alone. At 150 d storage, the ethanol contents in fruits of all the combined treatments were lower than those where preservative was used alone (Figure 3(a)). The variation of acetaldehyde content was more stable compared to ethanol. At 60 and 90 d storage, acetaldehyde content in fruits of HT + 50% C treatment was significantly lower than when preservative was used alone. No consistent variation was found between different treatments during storage (Figure 3(b)).

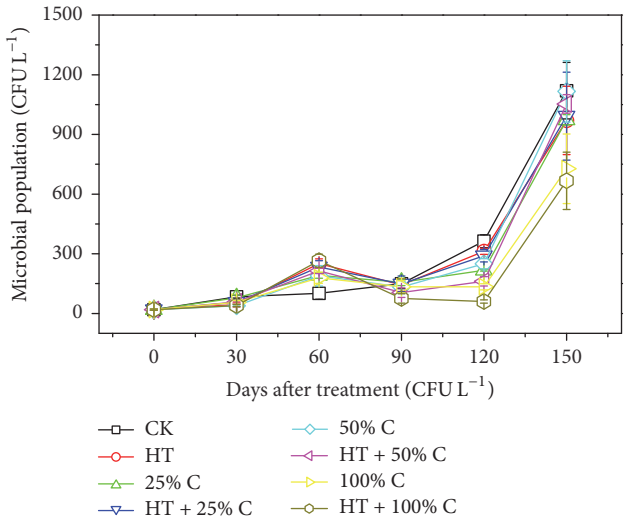

FIGURE 2: Effects of combined heat and preservative treatments on the epiphytic microbial population of Ponkan fruit during storage. "C" represents the concentration of preservative used in production. The error bars represent the standard errors. Three repetitions were used in this analysis.

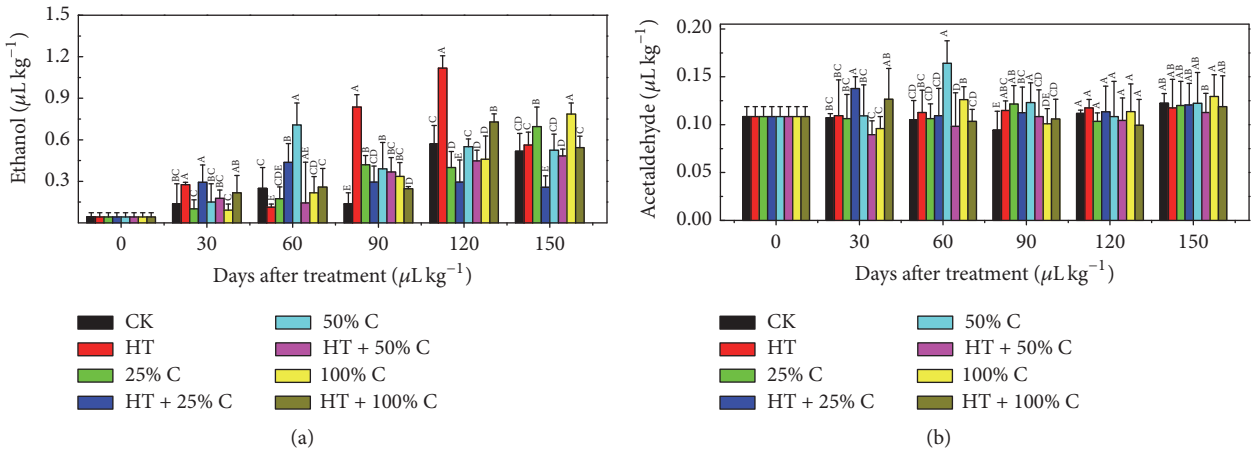

FIGURE 3: Effects of combined heat and preservative treatments on ethanol (a) and acetaldehyde (b) contents in the fruit pulp during storage. "C" represents the concentration of preservative used in production. The error bars represent the standard errors. Different superscripts between columns represent significant differences between samples at the 0.05 level.

Acetaldehyde and ethanol accumulated under anaerobic conditions during fruit development or storage, for example, through coating with films or in modified atmospheres or packages [25]. Previous reports showed that fruits tend to accumulate more ethanol and acetaldehyde during the later storage stage, which contributed to dramatic change in the fruit flavor and appearance of browning [26, 27]. In addition, these molecules can reflect the fruit status or predict the quality characteristics [28]. From our results, the ethanol content of fruits in HT combined with 25% preservative treatment was significantly lower than that of the 25% preservative treatment during the late storage period, which may indicate better internal quality and long-term storage ability of fruits in HT combined with 25% preservative treatment.

3.5. Effects of Combined Heat and Preservative Treatments on Pectin and Fiber Contents in the Fruit Peel during Storage. The variation of fiber contents was relatively stable in all the treatments during storage. Compared with the control, the fiber content was lower in HT treatments but showed no regular trend during the whole period. No significant difference was observed between samples in 50% C and HT + 50% C treatments or between the 100% C and HT + 100% C treatments. However, the HT + 25% C treatment significantly increased the pectin content between 60 to 150 d storage when compared with the 25% C treatment alone (Figure 4).

The fiber contents showed irregular variation in all the treatments during storage. No significant difference was observed in fiber contents among different treatments at 30 and 60 d storage. Generally, the combined treatments

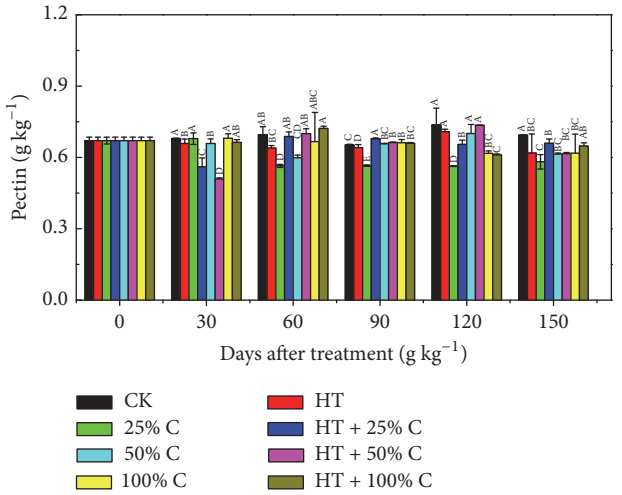

Figure 4: Effects of combined heat and preservative treatments on pectin content in the fruit peel during storage. The error bars represent the standard errors. "C" represents the concentration of preservative used in production. Different superscripts between columns represent significant differences between samples at the 0.05 level.

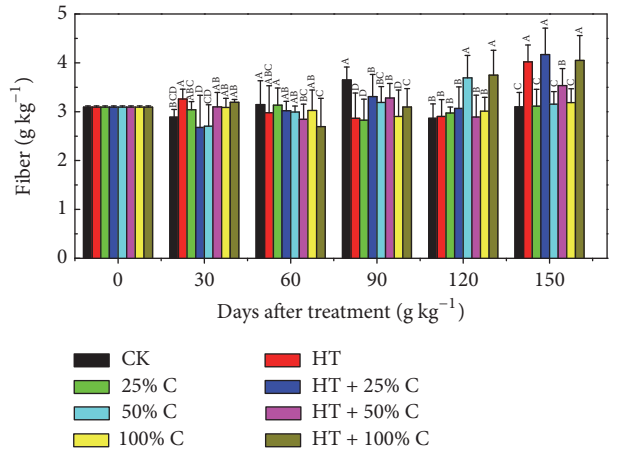

Figure 5: Effects of combined heat and preservative treatments on total fiber content in the fruit peel during storage. "C" represents the concentration of preservative used in production. The error bars represent the standard errors. Different superscripts between columns represent significant differences between samples at the 0.05 level.

increased the fiber content compared with the single preservative treatments from 90 to 150 d storage. Significant differences were investigated in all the combined treatments when compared with the corresponding single preservative treatments at 150 d storage, indicating that the HT treatment was effective in increasing the fiber content in fruit peel during long time storage (Figure 5).

Pectin and fiber are important constitutes of the main structural support in the cell wall, playing important roles in material transportation and protection against adversity. Previous reports showed that the cell wall participated in perception of external signals by changing the components in response to various environmental stimuli or stresses [29]. It has also been reported that heat shock treatment can inhibit the degradation of pectin and cellulose and lead to an increase in water soluble pectin, delaying the process of persimmon softening, alleviating chilling injury and extending the shelf life of fruits [30, 31]. The fiber contents were significantly increased by the combined HT and preservative treatments, suggesting that the structure and intensity of fruit cell wall were affected so as to prevent fungus and enhance fruit storage and resistance.

4. Conclusions

Heat treatment at 55°C for 20 s was effective in extending the storage life of Ponkan fruit. Furthermore, heat combined with 25% preservative treatment significantly decreased the decay rate of Ponkan fruit during storage, showing a similar effect to that observed with 100% preservative treatment. This may be because the heat treatment was effective in enhancing the efficacy of the preservative, permitting a decrease in the amount of chemicals used for decay control in Ponkan fruit. In addition, the increased pectin and fiber contents in fruit treated with HT combined with preservative suggested that the structure and intensity of cell wall were affected so as to prevent fungus infection and enhance fruit resistance during postharvest storage.

Conflicts of Interest

The authors declare that they have no conflicts of interest.

Authors' Contributions

Dandan Tang and Qiong Lin contributed equally to this work.

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Research Article

Effects of Different Processing Methods on the Antioxidant Activity of 6 Cultivars of Foxtail Millet

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The total phenolic content (TPC) of millet was whole > dehulled > cooked > steamed and the bound phenolic content (BPC) was the main form. Compared with dehulled millet, the TPC, TFC, and phenolic acid contents were decreased significantly ($P < 0.05$). The retention rate of TPC of steamed millet ranged from 47% to 55% and cooked millet ranged from 55% to 79%. Additionally, the mean cinnamic acid content of cooked millet was 1.29 times as much as steamed millet. The antioxidant activity of millet was whole > dehulled > cooked > steamed. Therefore, cooked millet was a good choice for human.

1. Introduction

Foxtail millet (*Setaria itatica*), a member of the Poaceae grass family, is one of the world's oldest crops and is a valuable source of human food in Africa and Asia. Foxtail millet originates in the Yellow River Basin country, the main producing areas in China, accounting for 80% of world production [1]. India is the second largest producing areas of foxtail millet, accounting for 10% of world production. The foxtail millet mainly in arid and semiarid regions in the north is one of the major food crops in northern China [2].

Phenolic compounds of millet, presented in free and bound forms, are ubiquitous which possess health benefits such as antioxidant and antimicrobial properties [3, 4]. At present, most papers have reported the nutritional composition of millet without processing [5, 6] and some papers focused on the effects of dehulling or milling on the nutrients or antioxidant activity of millet [7–12]. Singh and Srivastava [5] reported that the nutritional composition including minerals and antinutritional factors of finger millet (Africa) and the variety "VL-204" was found to be exceptionally rich in iron and very low in antinutritional factor phytate content. Bachar et al. [6] also reported calcium and magnesium were the most concentrated nutrients in all finger millet, followed by potassium, sodium, and phosphorus. Chandrasekara and Shahidi [7] also showed Kodo millet (Sri Lanka) had the highest total phenolic content, up to 114.03 ± 1.08 μmol of ferulic acid equiv/g of defatted meal, and all millet varieties showed high antioxidant activities. Choi et al. [4] reported that the polyphenolics content of the methanolic extracts obtained from whole millet (Morejo) was 47 ± 1.4 mg GAE/100 g of grain (wet weight basis). Zhang and Liu [12] reported the phenolic compounds and antioxidant activity of two cultivars (China) of dehulled foxtail millet. However, there were few researches on the effect of steaming and cooking on phenolic compounds and antioxidant activity of millet, which were the main homely cuisine ways in China or other countries. Thus, the primary objectives of this study were to entirely discuss the effect of different processing methods of dehulling, cooking, and steaming on the nutraceutical of FPC, BPC, phenolic acid, the free flavonoid contents (FFC), bound flavonoid contents (BFC), and phytic acid contents

of 6 cultivars of foxtail millet planted in China, further investigating the changes of their antioxidant activity and their correlation with the nutraceutical.

2. Materials and Methods

2.1. Raw Material. The 6 cultivars of foxtail millet, which were Yugu01 (Henan province), Jingfen02 (Shanxi province), Jinggu21 (Shanxi province), Fenghonggu (Inner Mongolia province), Jigu31 (Hebei province), and Longgu12 (Gansu province), were selected for analysis in this experiment. The samples in the experiment are well representative, no sample wormhole, mildew, groats, in which there is no pollution and no hybridization of pure millet.

2.2. Sample Preparation. Foxtail millet was dehulled three times by the huller under the power of 2 kw. After the dehulling, the ratio of hull removal was 90%. After washing and draining, the millet was cooked at 100°C for 30 min and the ratio of seed : water was 1 : 20 (w/w) [13]. The steaming millet was processed at 100°C for 10 min and seed : water ratio 1 : 5 (w/w) [14, 15]. After cuisine, the cooked and steamed millet were prefrozen in a cold room (−80°C, 4 h) and then lyophilized to dryness and milled with a coffee mill. The flour was passed through an 80-mesh sieve and stored in refrigerator (−20°C) until analyzed.

2.3. Extraction of Free Phenolic Compounds. Free phenolic compounds were extracted using the method as described by Taylor and Duodu [11] and Xu and Chang [16] with modifications. Free phenolic compounds in grains were extracted by blending 15 g of whole grain flour with 180 mL of solvent (the ratio of absolute ethanol : water : acetic acid was 70 : 29.5 : 0.5, v/v/v) for 4 h at 45°C. After centrifugation at 5000 r/min for 10 min, the supernatant was pooled, evaporated at 50°C to 10 mL, reconstituted with methanol to a final volume of 25 mL, and stored at 4°C until analysis. The residues were lyophilized.

2.4. Extraction of Bound Phenolic Compounds. Bound phenolic compounds were extracted using the method as described by Adom and Liu [17] with modifications. Briefly, 1.0000 g of the residues after extracted free phenolic compounds was then digested with 18 mL of 1.5 M sodium hydroxide at room temperature for 1 h with shaking under nitrogen gas. The mixture was neutralized with 6 M hydrochloric acid until the pH value up to 2 and extracted with hexane (4 × 10 mL, 30 min) to remove lipids. The final solution was extracted with ethyl acetate (4 × 10 mL, 30 min). The ethyl acetate fraction was evaporated to dryness. Phenolic compounds were reconstituted in 5 mL of methanol.

2.5. Determination of Total Phenolic Content. Total phenolic contents of the extracts were determined using Folin-Ciocalteu colorimetric method as described by Singleton et al. [18] with modifications. Briefly, 1 mL of foxtail millet extract was oxidized with 1 mL of Folin-Ciocalteu reagent, and the reaction was neutralized with sodium carbonate. The resulting blue color was reconstituted with methanol to a final volume of 10 mL and the absorbance was measured at 760 nm after 2 h. Total phenolic content was expressed as mg gallic acid equivalents/100 g dry weight (mg GAE/100 g DW). Data are reported as a mean value ± standard deviation (SD) for three replications.

2.6. Determination of Phenolic Acid. Phenolic acid contents were determined by HPLC equipped with an autosampler and a diode-array detector. The analytical column was Agilent ZORBAX SB-C18. The mobile phase consisted of 0.05% TFA in water (v/v) (solvent A) and 0.05% TFA in acetonitrile (solvent B). The flow rate was kept at 0.8 mL/min. The injection volume was 20 μL and peaks were monitored simultaneously at 260 nm. All samples were filtered through a 0.45 μm filter before injection. Values were expressed as μg/g DW.

2.7. Determination of Total Flavonoid Content. Total flavonoid contents of the extracts were determined using the method as described by Singleton et al. [18] with modifications. Briefly, appropriate dilutions of sample extracts were reacted with 3 mL of aqueous solution of 1 M potassium acetate, followed by a flavonoid-aluminum complex formation using 2 mL of aqueous solution of 0.1 M aluminum chloride. The sample was reconstituted with 30% ethanol to a final volume of 10 mL. The absorbance at 420 nm was measured after 0.5 h and the result was expressed as mg rutin equivalents/100 g dry weight (mg RE/100 g DW). Data are reported as a mean value ± standard deviation (SD) for three replications.

2.8. Determination of Phytic Acid Content. The phytic acid contents of the 6 cultivars of foxtail millet were determined using the method as described by Badau et al. [19] and Wheeler and Ferrel [20].

The absorbance at 480 nm was measured and the result was expressed as mg/g dry weight (mg/g DW). Data are reported as a mean value ± standard deviation (SD) for three replications.

2.9. Determination of Antioxidant Activity. For ABTS assay, the procedure followed the method of Arnao et al. [21] and Thaipong et al. [22]. The FRAP assay was carried out according to Thaipong et al. [22].

The DPPH assay was carried out according to Brand-Williams et al. [23] with some modifications. The stock solution was prepared by dissolving 19.716 mg DPPH with 50 mL anhydrous methanol and then stored at −20°C until needed. The working solution was obtained by mixing 1 mL stock solution with 9 mL anhydrous methanol to obtain an absorbance of 1.1 ± 0.02 units at 515 nm using the spectrophotometer. Foxtail millet extracts (200 μL) were all owed to react with 3.8 mL of 0.1 M DPPH solution for 0.5 h in the dark. The absorbance at 765 nm was measured. The standard curve was linear between 50 and 500 μM Trolox and the results were expressed in μmol Trolox equivalents/g dry weight (μmol TE/g DW). Data are reported as a mean value ± standard deviation (SD) for three replications.

The ORAC procedure, measuring a compounds ability to scavenge a free radical from ABAP compared to that Trolox,

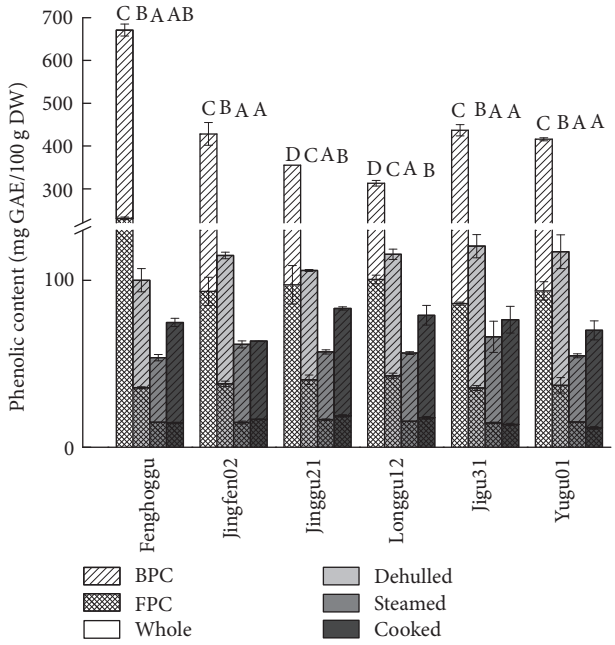

Figure 1: The FPC, BPC, and TPC of 6 cultivars of foxtail millet with different treatment. Error bars represent the standard deviation (±SD). The *different uppercase letters (A–D)* indicate significant differences of TPC within different processing methods of the same cultivar ($P < 0.05$).

used an automated plate reader with 96-well plates [24]. Briefly, 40 μL phosphate buffer was pipetted to the F well and 20 μL sample, blank (phosphate buffer), or Trolox standard was, respectively, pipetted to the appropriate well except the F and incubated 10 min at 37°C. Then add 200 μL fluorescein working solution to each well. Add 20 μL AAPH working solution to each well after 20 min and read plate immediately. Fluorescence conditions were as follows: excitation at 485 nm and emission at 538 nm. The standard curve was linear between 0 and 50 μM Trolox and the results were expressed in μmol Trolox equivalents/g dry weight (μmol TE/g DW). Data are reported as a mean value ± standard deviation (SD) for three replications.

2.10. Statistical Analysis. The analysis of variance (ANOVA) technique was used to analyze the experimental data and Duncan's multiple range tests were used to determine the significance of differences among treatments at 95% confidence level. Correlations among data were calculated using Pearson's correlation coefficient (r).

3. Results and Discussion

3.1. Effects of Processing Methods on the Phenolic Content of Foxtail Millet. The free phenolic contents (FPC), bound phenolic contents (BPC), and total phenolic contents (TPC) of the 6 cultivars of foxtail millet with different treatment were shown in Figure 1, which have significant difference ($P < 0.05$). For the different processing methods of foxtail millet, the TPC were whole > dehulled > cooked > steamed. In whole foxtail millet, the FPC and BPC of Fenghonggu were higher than the other millets, up to 230.95 ± 3.26 and 440.14 ± 13.86 mg GAE/100 g DW, respectively. At the same time, Longgu12 showed the lowest content. The different cultivars may be responsible for these results.

The FPC accounted for 26.34% (ranging from 19.72% to 34.41%), 34.18% (ranging from 29.45% to 38.18%), 26.41% (ranging from 22.09% to 28.88%), and 20.90% (ranging from 16.59% to 26.33%) of the TPC in whole, dehulled, steamed, and cooked foxtail millet. Thus the phenolic compounds existed mainly in the bound form, which was consistent with Zhang and Liu [12] who showed that FPC constituted around 32% and 38% of the TPC in Jingu28 and Jingu34, respectively. In addition, Adom and Liu [17] showed the FPC contributed 15% of the total in corn, 24% in wheat, 25% in oats, and 38% in rice. This may be due to the different grain.

When the foxtail millet was dehulled, the phenolic contents had a significant reduction ($P < 0.05$). The TPC of Jigu31 (120.53 ± 8.45 mg GAE/100 g DW) and Yugu01 (117.22 ± 19.73 mg GAE/100 g DW) were higher than other dehulled foxtail millets. In dehulled foxtail millet, the mean value of TPC was 112.42 mg GAE/100 g DW which was slightly higher than the results of Zhang and Liu [12] who reported that the mean TPC of Jingu28 and Jingu34 were 96.51 mg GAE/100 g DW. The TPC of dehulled foxtail millet constituted around 15% and 37% of the TPC of whole foxtail millet in Fenghonggu and Longgu12, respectively. Kim et al. [25] and Vaher et al. [26] reported that the phenolic compounds were mainly found in the bran. Ivanišová et al. [9] reported that 73% of grain phenolic contents were found in the bran. Thus the results of this study gave further support to the notion that phenolic compounds of grains were concentrated mainly in the bran.

The TPC were ranged from 53.70 ± 1.97 (Fenghonggu) to 66.23 ± 9.60 (Jigu31) mg GAE/100 g DW in steamed foxtail millet with a mean value of 58.38 mg GAE/100 g DW. Compared with dehulled millet, the retention rate of TPC of steamed millet ranged from 47% (Yugu01) to 55% (Jigu31). In cooked foxtail millet, the TPC were ranged from 63.61 ± 0.25 to 83.19 ± 1.78 mg GAE/100 g DW with a mean value of 74.54 mg GAE/100 g DW. Compared with dehulled millet, the retention rate of TPC of cooked millet ranged from 55% (Jiingfen02) to 79% (Jinggu21). The different TPC may be due to the different ratio of seed : water involved in steamed and cooked foxtail millet. Additionally, the FPC and BPC of millets decreased significantly after cooking and steaming ($P < 0.05$). The heating process involved in steamed and cooked foxtail millet might be responsible for the reduced level of the FPC and BPC. Compared with cooked millet, the BPC of steamed millet decreased significantly ($P < 0.05$), which may be because the moisture activity and oxygen concentration were different. Meanwhile, a part of bound phenolic degraded into free phenolic and the free phenolic could be oxidized easier in the high concentration of the moisture and oxygen; thus the FPC of steamed and cooked millet had no significant difference ($P < 0.05$).

3.2. Effects of Processing Methods on the Phenolic Acid Composition. The phenolic acids compositions of 6 cultivars of

Table 1: Phenolic acid composition of 6 cultivars of whole foxtail millet.

Phenolic acid	Coumaric acid	*P*-Hydroxy benzoic acid	Vanillic acid	Caffeic acid	Cinnamic acid	Ferulic acid
Longg12	75.15 ± 0.11	108.89 ± 1.26	63.31 ± 0.98	5.67 ± 0.01	2075.03 ± 25.78	0.89 ± 0.00
Jigu31	105.18 ± 1.23	22.50 ± 0.02	271.31 ± 8.23	3.70 ± 0.01	3128.72 ± 19.08	6.17 ± 0.01
Yugu01	61.91 ± 1.01	12.87 ± 0.01	74.56 ± 0.63	1.07 ± 0.01	1837.38 ± 10.01	1.67 ± 0.00
Jinggu21	180.39 ± 3.27	19.53 ± 0.25	109.85 ± 0.16	6.18 ± 0.02	1995.29 ± 9.87	2.49 ± 0.01
Jingfen02	334.60 ± 9.01	15.02 ± 0.01	122.25 ± 1.21	3.08 ± 0.01	1828.43 ± 21.01	2.33 ± 0.01
Fenghonggu	116.36 ± 1.06	30.90 ± 0.78	167.72 ± 1.85	4.96 ± 0.05	2091.76 ± 13.47	13.33 ± 0.01

Values expressed as μg/g DW. Values are means ± SD of three determinations.

whole millet which were coumaric acid, *P*- hydroxy benzoic acid, vanillic acid, caffeic acid, cinnamic acid, and ferulic acid were presented in Table 1. In the 6 kinds of phenolic acids, cinnamic acid was higher than any others in 6 cultivars of whole millet, ranged from 1828.43 (Jingfen02) to 3128.72 (Jigu31) μg/g DW, and accounted for 87% of the 6 kinds of phenolic acids. The result was higher than the report of Mcdonough et al. [27] who showed that the contents of cinnamic acid of foxtail millet, pearl millet, and finger millet were 781.7, 345.3, and 35.1 μg/g, respectively. In addition, the values of vanillic acid and coumaric acid were relatively higher. The vanillic acid of whole foxtail millet ranged from 63.31 to 271.31 μg/g DW, accounting for 5% of the 6 kinds of phenolic acids. The coumaric acid of whole foxtail millet ranged from 61.91 to 334.60 μg/g DW, accounting for 6% of the 6 kinds of phenolic acids, which were different with Chandrasekara and Shahidi [7] who showed that *p*-coumaric acids of Kodo, finger (Ravi), finger (local), foxtail, proso, little, and pearl millets were 698.67, 37.56, 39.81, 915.90, 1155.40, 1027.26, and 41.45 μg /g of defatted meal, respectively. This may be due to the different cultivars and different planting region.

When the foxtail millets were dehulled, steamed, and cooked, coumaric acid, *P*-hydroxy benzoic acid, vanillic acid, and caffeic acid were hard to detect in 6 cultivars of foxtail millet. Figure 2 showed the contents of cinnamic acid and ferulic acid of 6 cultivars of foxtail millet with different treatment and that they had significant differences ($P < 0.05$). The heat treatment in wetted preparations may cause phenolic acid to decrease. The cinnamic acids of cooked millet were 46.27% lower than dehulled foxtail millet, ranging from 26.47% (Longgu12) to 69.27% (Jinfen02). For the steamed millet, the range (46.09% to 63.12%) was different from cooked millet. However, the mean cinnamic acid content of cooked millet was 1.29 times as much as steamed millet. As Figure 2(b) has shown, the ferulic acids of cooked and steamed millet were 83.40% and 42.07% lower than dehulled millet, respectively. However, the mean ferulic acid content of cooked millet was 3.42 times as much as steamed millet. This may be due to the different ratio of seed : water and processing time.

3.3. Effects of Processing Methods on the Flavonoid Content. Flavonoid presented in grains had potent antioxidant properties associated with the health benefits of grains and grain products [17]. The flavonoid contents of the 6 varieties of foxtail millet, expressed as mg rutin equivalents/100 g of dried foxtail millet weight, were shown in Figure 3. Significant differences were found in the free flavonoid contents (FFC), bound flavonoid contents (BFC), and total flavonoid contents (TFC) between whole and dehulled foxtail millet ($P < 0.05$). In whole foxtail millet, the FFC of Jigu31 was lower than any other cultivars and the BFC of Fenghonggu was lower than others, but the TFC of Jigu31 was lowest. The different cultivars may be responsible for these results.

In dehulled foxtail millet, the TFC were ranged from 485.25 ± 26.01 (Yugu01) to 620.77 ± 18.43 (Fenghonggu) mg RE/100 g DW with a mean value of 577.52 mg RE/100 g DW, which were 4 to 6 times that of whole millet. The TFC of dehulled foxtail millet were significantly ($P < 0.05$) higher than whole foxtail millet, because of the conversion of the major flavones into the C-glycosylflavanols form in the seeds [28].

Compared with dehulled foxtail millet, cooking and steaming were significantly ($P < 0.05$) increased in the BFC for the 6 cultivars of foxtail millet. The BFC of steamed foxtail millet were 31% higher than dehulled foxtail millet, ranging from 0.3% (Jinfen02) to 96% (Jigu31). The BFC of cooked foxtail millet were 23% higher than dehulled foxtail millet, ranging from 2% (Jingfen02) to 74% (Jigu31). In contrast, the FFC of steamed and cooked foxtail millet were lower than dehulled foxtail millet. The FFC of dehulled foxtail millet were ranged from 189.86 ± 11.77 (Yugu01) to 379.83 ± 1.39 (Fenghonggu) mg RE/100 g DW with a mean value of 301.26 mg RE/100 g DW. However, the FFC of cooked foxtail millet were ranged from 23.66 ± 2.81 (Longgu12) to 120.41 ± 0.42 (Jingfen02) mg RE/100 g DW with a mean value of 86.93 mg RE/100 g DW. The FFC of steamed foxtail millet were ranged from 42.95 ± 0.54 (Longgu12) to 149.83 ± 7.82 (Fenghonggu) mg RE/100 g DW with a mean value of 86.61 mg RE/100 g DW. Cooking and steaming involving heat treatment seemed to be extremely important for reducing the FFC of the 6 cultivars of foxtail millet [29]. Additionally, the heat treatment in wetted preparations could favour the hydrolysis of C-glycosylflavones and the release of corresponding aglycones, which led to an increase in the BFC of cooked and steamed foxtail millet [30, 31].

For the cooked and steamed millet, the TFC of Jigu31, Yugu01, and Jinggu21 had no significant difference ($P < 0.05$). For Jingfen02, the TFC of cooked millet was 1.06 times as

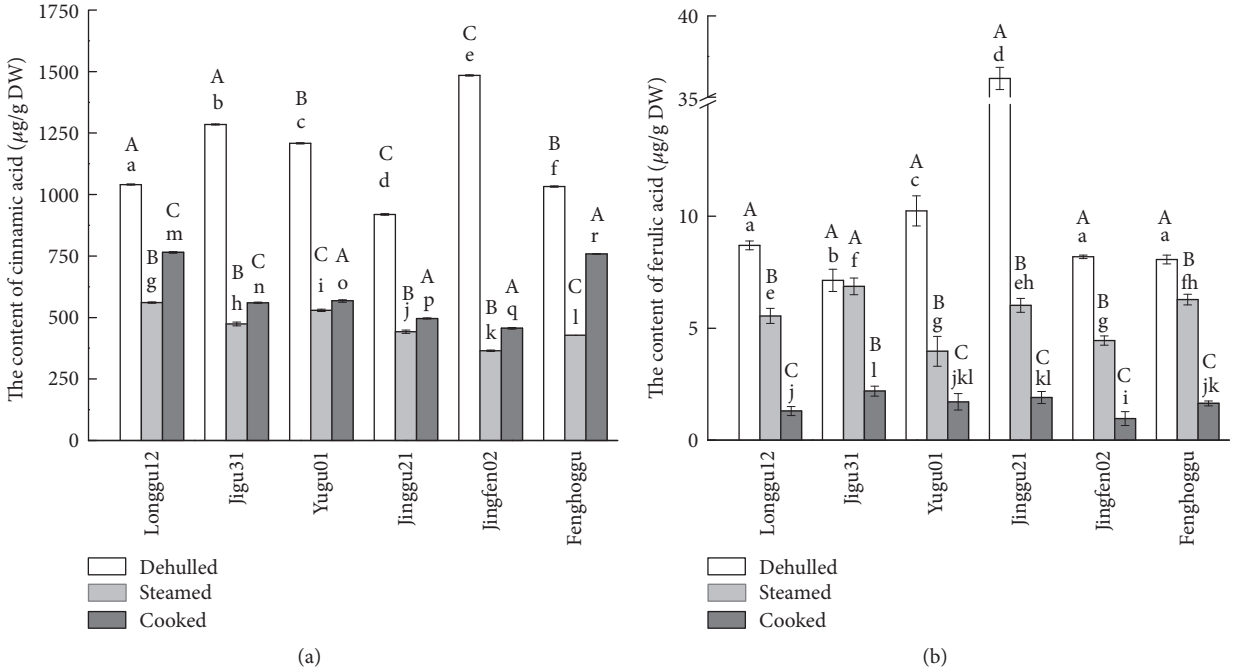

Figure 2: (a) The cinnamic acid of 6 cultivars of foxtail millet with different treatment. Error bars represent the standard deviation (±SD). The *different uppercase letters (A–C)* indicate significant differences within different processing methods of the same cultivar ($P < 0.05$). The *different lowercase letters (a–r)* indicate significant differences within different cultivars with the same treatment ($P < 0.05$). (b) The ferulic acid of 6 cultivars of foxtail millet with different treatment. Error bars represent the standard deviation (±SD). The *different uppercase letters (A–C)* indicate significant differences within different processing methods of the same cultivar ($P < 0.05$). The *different lowercase letters (a–l)* indicate significant differences within different cultivars with the same treatment ($P < 0.05$).

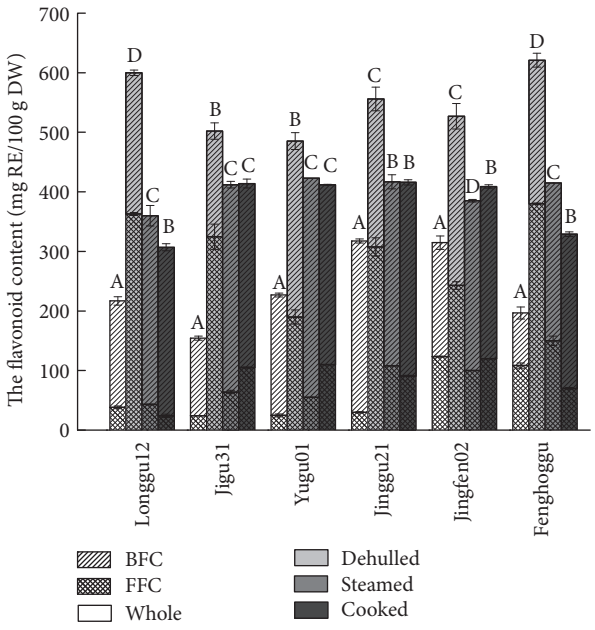

Figure 3: The FFC, BFC, and TFC of 6 cultivars of foxtail millet with different treatment. Error bars represent the standard deviation (±SD). The *different uppercase letters (A–D)* indicate significant differences of TFC within different processing methods of the same cultivar ($P < 0.05$).

much as steamed millet. For Longgu12 and Fenghonggu, the TFC of cooked millet accounted for 85% and 79% of the TFC of steamed millet, which may be the result of the different processing time.

3.4. Effects of Processing Methods on the Phytic Acid Content. The phytic acid contents of whole, dehulled, steamed, and cooked foxtail millet were presented in Figure 4. Significant differences in the phytic acid contents among Longgu12, Jigu31, Yugu01, Jinggu21, and Fenghonggu were observed ($P < 0.05$). The phytic acid contents of whole foxtail millet ranged from 27.96 ± 0.01 (Yugu01) to 28.95 ± 0.04 (Jinfen02) mg/g DW. Thus the results of this study were not consistent with El Hag et al. [32] who reported that the phytic acid contents of two cultivars of whole pearl millet were 9.43 ± 0.04 and 10.76 ± 0.16 mg/g, respectively. As a result of the analysis, the phytic acid contents of 6 cultivars of dehulled foxtail millet were 2.80%, which were consistent with Badau et al. [19] who reported that the phytic acid content of ten pearl millet cultivars ranged from 2.91% to 3.30%. This might be due to the fact that phytic acid content varied depending upon the cultivar, irrigation conditions, and type of soil in which they were grown [33].

Phytic acid had the ability to form insoluble chelates with various metal ions such as iron, zinc, and calcium [34]. When cooking and steaming, the phytic contents of 6 cultivars of

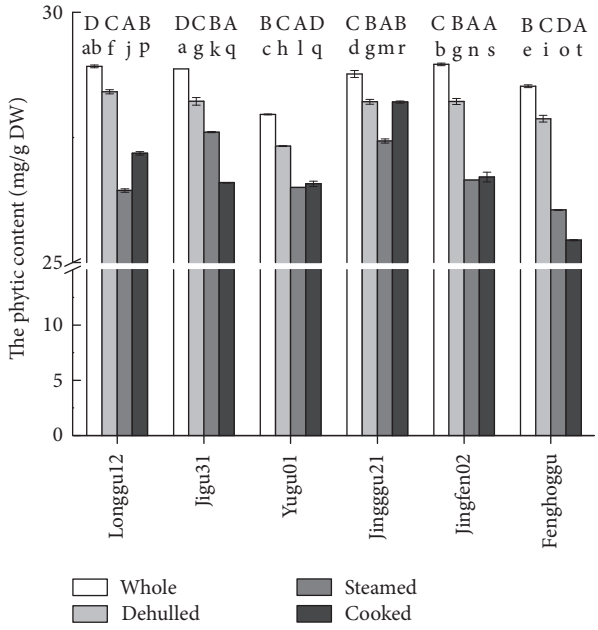

Figure 4: Phytic acid contents of 6 cultivars of foxtail millet with different treatment. Error bars represent the standard deviation (±SD). The *different uppercase letters (A–D)* indicate significant differences within different processing methods of the same cultivar ($P < 0.05$). The *different lowercase letters (a–t)* indicate significant differences within different cultivars with the same treatment ($P < 0.05$).

foxtail millet reduced. The phytic acid contents of steamed foxtail millet ranged from 26.05 ± 0.01 to 27.61 ± 0.01 mg/g DW. The phytic acid contents of cooked foxtail millet ranged from 25.45 ± 0.01 to 28.21 ± 0.03 mg/g DW. The phytic acid contents of cooked and steamed foxtail millet were 4.49% and 4.51% lower than dehulled millet. This might be due to the fact that any formation of calcium/magnesium salts of phytic acid, compounds with limited solubility, occurs as a result of heat treatment [35, 36].

3.5. Effects of Processing Methods on the Antioxidant Activity. The antioxidant potential of 6 varieties of foxtail millet was determined on the basis of the scavenging activity of the stable free radicals DPPH, FRAP, ABTS, and ORAC (Tables 2(a) and 2(b)). Significant differences within different processing methods of the same cultivar in the antioxidant activity measured by DPPH, FRAP, and ABTS assays were observed ($P < 0.05$). The value of DPPH-measured antioxidant activity was ranged from 0.93 ± 0.03 (steamed foxtail millet) to 4.74 ± 0.06 (whole foxtail millet) μmol TE/g DW.

For the different processing methods of foxtail millet, the antioxidant activity, measured by DPPH and FRAP, was whole > dehulled > cooked > steamed. Jigu31, whole foxtail millet, showed the highest antioxidant activity compared to the other cultivars when measured by FRAP (26.94 ± 0.61 μmol TE/g DW), DPPH (4.74 ± 0.06 μmol TE/g DW), ABTS (59.92 ± 0.04 μmol TE/g DW), and ORAC (624.1 ± 2.79 μmol TE/g DW) assays. By contrast, Yugu01, whole foxtail millet, showed the lowest antioxidant activity when measured by FRAP (16.60 ± 0.62 μmol TE/g DW), DPPH (4.38 ± 0.02 μmol TE/g DW), and ABTS (41.76 ± 3.14 μmol TE/g DW) assays. However, for dehulled foxtail millet, Yugu01 showed the highest antioxidant activity compared to the other cultivars when measured by FRAP (7.75 ± 0.17 μmol TE/g DW), DPPH (4.02 ± 0.11 μmol TE/g DW), ABTS (15.24 ± 0.36 μmol TE/g DW), and ORAC (182.2 ± 40.21 μmol TE/g DW) assays. The antioxidants of cooked and steamed millet, measured by DPPH, FRAP, ABTS, and ORAC assays, were not consistent with each other, which may be because ABTS, FRAP, and DPPH were measured single electron transfer and ORAC represented hydrogen atom transfer [37]. Therefore, antioxidant potency composite index (APC) was used to measure the antioxidant activity of 6 cultivars of foxtail millet [38].

Table 2(c) showed that the antioxidant activity of millet was whole > dehulled > cooked > steamed and the mean APC of whole, dehulled, steamed, and cooked millet were 0.83, 0.33, 0.20, and 0.21, respectively. Additionally, the rank order of antioxidant activity of dehulled millet was Yugu01 > Jigu31 > Jingfen02 > Jinggu21 > Longgu12 > Fenghonggu. However, the antioxidant activity of steamed foxtail millet was consistent with cooked foxtail millet. The rank order of antioxidant activity was Fenghonggu > Longgu12 > Jinggu21 > Yugu01 > Jigu31 > Jingfen02. The reason might be the fact that cooking and steaming involving heat treatment led to reducing the polyphenols of the 6 cultivars of foxtail millet in various degrees.

3.6. Correlations. Total phenolics of the foxtail millet (Folin-Ciocalteu method) correlated strongly with their antioxidant activity measured by all four methods ($P < 0.01$). The correlation coefficients were as follows: TPC versus DPPH, $r = 0.83$; TPC versus ABTS, $r = 0.91$; TPC versus FRAP, $r = 0.91$; TPC versus ORAC, $r = 0.87$ (Table 3), which was consistent with previous findings that there was significant correlation between TPC and antioxidant [20]. Awika et al. [39] reported that phenol contents of the sorghums correlated strongly with their antioxidant activity measured by DPPH, ABTS, and ORAC assays and the correlation coefficients were as follows: phenol versus ORAC, $r = 0.96$; phenol versus ABTS, $r = 0.97$; phenol versus DPPH, $r = 0.96$. In addition, correlations among the antioxidant activities measured by ABTS, DPPH, FRAP, and ORAC assays and CA were positively high and ranged between 0.75 and 0.89. FRAP, ABTS, and ORAC values were significantly correlated ($P < 0.01$). Moreno-Montoro et al. [40] also reported significant correlation between FRAP and ABTS values.

4. Conclusion

From the data in this study, it can be concluded that the different processing methods of foxtail millet made an effect on the TPC, TFC, and the 6 kinds of phenolic acids. Compared with whole millet, the TPC of dehulled millet decreased and TFC of dehulled millet increased. Compared with dehulled millet, the TPC and TFC of cooked and steamed millet decreased. However, the total phenolic content and cinnamic acid content were rich in cooked millet. In addition, cooked

Table 2: (a) The antioxidant activity of 6 cultivars of foxtail millet with different treatment measured by the FRAP and DPPH methods. (b) The antioxidant activity of 6 cultivars of foxtail millet with different treatment measured by the ABTS and ORAC methods. (c) Antioxidant potency composite index of 6 cultivars of foxtail millet with different treatment.

(a)

Foxtail millet	FRAP				DPPH			
	Whole	Dehulled	Steamed	Cooked	Whole	Dehulled	Steamed	Cooked
Longg12	20.06 ± 0.03^{dC}	6.59 ± 0.09^{cC}	3.92 ± 0.23^{aB}	5.11 ± 0.13^{bC}	4.63 ± 0.06^{dC}	2.34 ± 0.11^{cB}	1.50 ± 0.02^{b}	1.77 ± 0.03^{aA}
Jigu31	26.94 ± 0.61^{dD}	7.16 ± 0.14^{cD}	4.11 ± 0.19^{aB}	4.89 ± 0.21^{bC}	4.74 ± 0.06^{dC}	3.14 ± 0.15^{cC}	1.37 ± 0.02^{bB}	1.70 ± 0.05^{aB}
Yugu01	16.60 ± 0.62^{dA}	7.75 ± 0.17^{cE}	4.09 ± 0.09^{bC}	4.57 ± 0.04^{aA}	4.38 ± 0.02^{dA}	4.02 ± 0.11^{cE}	0.93 ± 0.03^{bD}	2.03 ± 0.01^{aC}
Jinggu21	20.48 ± 1.61^{cC}	5.79 ± 0.08^{bB}	3.80 ± 0.06^{aB}	4.19 ± 0.23^{aB}	4.60 ± 0.10^{dBC}	3.34 ± 0.08^{cD}	1.75 ± 0.05^{bD}	2.07 ± 0.04^{aD}
Jingfen02	18.38 ± 1.23^{dB}	6.43 ± 0.18^{cC}	3.02 ± 0.20^{aA}	3.92 ± 0.25^{bB}	4.45 ± 0.08^{dAB}	3.32 ± 0.01^{cD}	1.15 ± 0.04^{bA}	1.41 ± 0.04^{aE}
Fenghonggu	19.87 ± 0.32^{cC}	5.13 ± 0.18^{bA}	3.98 ± 0.33^{aB}	4.87 ± 0.29^{bC}	4.58 ± 0.25^{dBC}	2.74 ± 0.02^{aA}	2.04 ± 0.05^{cCE}	2.39 ± 0.03^{bF}

The results were expressed as μmol Trolox equivalents/g dry weight (μmol TE/g DW). Values are means ± SD of three determinations.
The *different lowercase letters* indicate significant differences within different processing methods of the same cultivar ($P < 0.05$). The *different uppercase letters* indicate significant differences within different cultivars with the same treatment ($P < 0.05$).

(b)

Foxtail millet	ABTS				ORAC			
	Whole	Dehulled	Steamed	Cooked	Whole	Dehulled	Steamed	Cooked
Longg12	47.79 ± 3.46^{cC}	11.34 ± 0.28^{bA}	4.34 ± 0.28^{aA}	6.56 ± 0.19^{aC}	465.1 ± 39.88^{cB}	136.8 ± 3.26^{aD}	193.5 ± 17.47^{bC}	144.4 ± 0.47^{aE}
Jigu31	59.92 ± 0.04^{dD}	14.41 ± 0.31^{cB}	4.93 ± 0.22^{aB}	6.67 ± 0.12^{bC}	624.1 ± 2.79^{dD}	123.0 ± 0.77^{bCD}	133.7 ± 0.72^{cB}	102.5 ± 6.62^{aC}
Yugu01	41.76 ± 3.14^{cA}	15.24 ± 0.36^{bC}	5.45 ± 0.04^{aC}	3.69 ± 0.29^{aA}	537.5 ± 34.89^{cC}	182.2 ± 40.21^{bE}	202.1 ± 2.35^{bC}	101.0 ± 4.26^{aC}
Jinggu21	48.12 ± 2.41^{cC}	9.53 ± 0.06^{bD}	6.33 ± 0.38^{aD}	6.37 ± 0.24^{aC}	486.0 ± 33.98^{cB}	90.68 ± 2.73^{aAB}	128.6 ± 10.76^{bB}	113.1 ± 2.84^{abD}
Jingfen02	45.04 ± 2.81^{cBC}	11.88 ± 0.79^{bE}	5.01 ± 0.06^{aB}	4.29 ± 0.21^{aB}	423.0 ± 30.67^{bA}	111.6 ± 6.54^{aBC}	111.4 ± 0.58^{aA}	91.0 ± 6.55^{aB}
Fenghonggu	44.43 ± 1.11^{dAB}	10.30 ± 0.07^{cF}	7.10 ± 0.42^{aE}	8.61 ± 0.55^{bD}	414.6 ± 20.59^{cA}	71.6 ± 1.77^{aA}	103.0 ± 2.21^{bA}	82.7 ± 0.55^{aA}

The results were expressed as μmol Trolox equivalents/g dry weight (μmol TE/g DW). Values are means ± SD of three determinations.
The *different lowercase letters* indicate significant differences within different processing methods of the same cultivar ($P < 0.05$). The *different uppercase letters* indicate significant differences within different cultivars with the same treatment ($P < 0.05$).

(c)

Millet	APC			
	Whole	Dehulled	Steamed	Cooked
Longg12	0.82	0.29	0.21	0.23
Jigu31	1.00	0.34	0.19	0.20
Yugu01	0.78	0.42	0.19	0.21
Jinggu21	0.83	0.31	0.21	0.22
Jingfen02	0.76	0.33	0.15	0.17
Fenghonggu	0.78	0.28	0.22	0.24

Table 3: Pearson's correlation coefficients of antioxidant activities, phytic acid, total phenolics, total flavonoid content, and cinnamic acid.

	ORAC	FRAP	ABTS	DPPH
ORAC	1.00			
FRAP	0.95^{**}	1.00		
ABTS	0.96^{**}	0.99^{**}	1.00	
DPPH	0.79^{**}	0.88^{**}	0.89^{**}	1.00
CA	0.75^{**}	0.83^{**}	0.86^{**}	0.89^{**}
PA	0.61^{**}	0.70^{**}	0.72^{**}	0.72^{**}
TPH	0.87^{**}	0.91^{**}	0.91^{**}	0.83^{**}

TPC = total phenolics, PA = phytic acid, CA = cinnamic acid, and $**$ = significant at $P < 0.01$.

millet demonstrated remarkable radical scavenging capacity, which was associated with its high contents of natural antioxidants found in the samples, such as phenolic compounds, cinnamic acid, and phytic acid. Correlations between the antioxidant activity and CA ranged from 0.75 to 0.89, while the antioxidant activity and total phenolic content ranged from 0.83 to 0.91. Therefore, cooked millet was a good choice for human.

Additional Points

Practical Applications. The foxtail millet mainly in arid and semiarid regions is one of the major food crops in northern China. Steaming and cooking are two main homely cuisine

ways in China or other countries. Moreover, phenolic compounds of millet, presented in free and bound forms, are ubiquitous and beneficial to human health. Therefore, we research the effect of steaming and cooking on the phenolic content and antioxidant activity, in order to determine a good processing way for millet. Then, for the steamed and cooked millet, we can establish optimal conditions to improve the retention rate of TPC in the further research. Lastly, for the dehulled millet, we can develop an effective method of millet dehulling to improve the retention rate of TPC in the further research.

Conflicts of Interest

All authors declare that they have no conflicts of interest regarding the publication of this paper.

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