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Editorial Board

Contents

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Editorial
Bioactive Compounds from Food Byproducts

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Food industry generates large amounts of byproducts that could be considered a source of several bioactive compounds that could be used for technological and nutritional scope. Among others, antioxidants extracted from food byproducts show various technological advantages such as controlling heat-induced contaminant formation, limiting the lipid oxidation, and presenting antimicrobial activity. Moreover, they are used for the formulation of functional foods and nutraceuticals. Thus, the aim of this special issue was to publish research papers as well as review articles addressing recent advances in food science and technology in general and with a deeper insight into food chemistry.

Briefly, several manuscripts have been evaluated in this special issue, and five of them have been published.

G. Gustinelli and coworkers established a new green extraction method based on the use of supercritical fluid extraction (SFE) for the recovery of seed oils enriched with antioxidant compounds. The oils from the seeds of cloudberries, blackcurrants, and bilberries were extracted by SFE at different temperatures and compared with oils that were obtained from the same seeds using conventional solvent extraction with hexane. The obtained oils are a source of PUFAs, and the major fatty acids are linoleic and linolenic acids. They concluded that conventional extraction allows higher yields of oil compared to SFE extraction. Moreover, the oils obtained by SFE reported lower amounts of carotenoids than the other ones obtained by solvent extraction; however, higher values of vitamin E were noticed in the SFE extracts (for blackcurrant and bilberry seed oils). The content of vitamin E was likely correlated with a high antioxidant activity of the obtained oils.

C. Y. Huang et al. carried out studies on the effect of compressional-puffing pretreatment of mango peels on the extraction of bioactive compounds by water (WE) and ethanol (EE); moreover, they compared the results with the same extracts obtained without the compressional-puffed pretreatment. The authors extract the bioactive compounds from six Taiwanese mango peels and demonstrate that the compressional-puffing process increases the extraction yields and polyphenol contents of peel extracts; moreover, ethanol was more effective in the extraction of phenolic compounds than water. The free radical-scavenging, anti-inflammatory, and antibacterial activities of mango peel extracts were also evaluated. The authors reported a positive correlation between polyphenol contents and the free radical-scavenging activities of extracts. Among the analysed samples, the ethanolic extract of TN1 sample exhibited the most antioxidant, anti-inflammatory, and antibacterial properties.

Z. Y. Chen et al. investigated the chemical composition and antioxidant activities of umezu from different factories in South and East China. Umezu is the pickling liquid of *Prunus mume*, and it represents a byproduct that contributes to the environment pollution. However, in order to revalorise this byproduct, the authors studied several chemical constituents, including the content and proportion of organic acids and phenolic acids, and antioxidant activities of different samples collected in two China regions. The results showed that citric acid and malic acid were the main organic acids. About phenolic composition, the authors noticed that neochlorogenic acid, chlorogenic acid, and cryptochlorogenic acid were the predominant phenolic acids. The

phenolic content was correlated with the antioxidant activity of the extracts measured by FRAP and ORAC methods.

In another study, H. Chen and coworkers explored the suitability of extracts of defatted seeds of *Camellia oleifera* as antioxidant ingredients. Briefly, they extracted these byproducts using different solvents demonstrating that isopropanolic extract exhibited the highest yield of total phenolic compounds. Epicatechin, naringenin, and catechin were reported to be the main phenolic compounds. The antioxidant properties of the extracts were tested measuring the antioxidant capacity on the corn oil lipid oxidation. The results underlined that the isopropanolic extract was the best one in terms of peroxide value, anisidine value, conjugated dienes, and thiobarbituric acid reactive substances (TBARS) decrease. These results confirmed that *Camellia* seed byproducts are an useful source of antioxidants for the stabilization of corn oil.

Finally, Jiang et al. investigated the effects of 1-methylcyclopropene (1-MCP) treatment on physicochemical characteristics of yardlong beans during cold storage. They showed the positive effects of 1-MCP treatments on the physicochemical quality of yardlong bean during cold storage. In fact, the application of 1-MCP suppressed the change in skin color and the decrease in firmness,reduced the increase in weight loss, and inhibited the degradation of chlorophyll. In addition, 1-MCP improved activities of antioxidant enzymes, such as SOD and POD, and reduced the accumulation of malondialdehyde (MDA) content.

In conclusion, this special issue offers new information to the readers on the recent advancements in technological approaches for the extraction and preservation of bioactive compounds. The antioxidant, anti-inflammatory, and antimicrobial activity of natural extracts obtained from food byproducts or underutilized crops could be of interest for the development of specific ingredients for food and/or pharmaceutical industry.

Conflicts of Interest

The editors declare that they have no conflicts of interest regarding the publication of this special issue.

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Research Article

Supercritical Fluid Extraction of Berry Seeds: Chemical Composition and Antioxidant Activity

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The influence of supercritical fluid extraction (SFE) and solvent extraction of oils from cloudberry, bilberry, and black currant seeds on the yield, chemical properties, and recovery of antioxidant compounds was investigated. SFE was performed for 1 h at 350 bar and at 50°C and 80°C. Fatty acids, vitamin E, carotenoids, and free radical-scavenging activity (DPPH) were assayed. SFE at 80°C resulted in higher oil yields for cloudberry and black currant seeds. The oils were rich in polyunsaturated fatty acids (PUFAs) (66.8%–75.9% w/w), with high percentages of linoleic and α-linolenic acids. The black currant seed extracts had the highest concentrations of vitamin E (range, 113.0–241.8 mg/100 g oil) and carotenoids (range, 11.5–32.3 mg/100 g oil) and the highest antioxidant activity. The cloudberry seed oils also had high antioxidant content and activity. These findings indicate the potential of SFE for the recovery of PUFA and antioxidant compounds in berry by-products.

1. Introduction

Berries are a common raw material that is processed in the food and juice industry. However, the processing of berries generates large amounts of solid by-products in the forms of peel, pulp, and seeds, which are mostly under utilised [1]. The wastes are usually burnt or deposited in landfills and hence, cause significant impact on the environment [2]. An alternative approach that reduces the amount of waste produced and promotes socioeconomic and environmental benefits is the valorisation of by-products through the extraction of valuable compounds [3].

Berry seeds are rich in oil, and their utilisation offers several advantages as their lipids generally have a high content of polyunsaturated fatty acids (PUFAs) and a favourable $n-6/n-3$ ratio compared to other vegetable oils [4, 5]. In addition, these oils are rich in vitamin E, carotenoids, and bioactive compounds with antioxidant activities [5].

Supercritical fluid extraction (SFE) is a green method for the extraction of valuable compounds from berry seeds, in contrast to conventional extraction methods that use hazardous organic solvents. The advantages of working with SFE are reduced solvent use, shorter extraction time, and lower energy consumption, as compared with the conventional extraction methods, such as solvent extraction and cold-pressing. SFE also allows the fractionation of compounds by adjusting the pressure and temperature based on the solubilities of the targeted compounds [6]. Moreover, the solvent is easily separated from the extract by depressurisation of the supercritical fluid, thereby saving time, and the fact that organic solvents are not used makes the extract suitable for use in the food industry [7]. The selectivity of SFE for the extraction of target compounds has previously been reported for the extraction of antioxidants in oil from grape seeds [3, 8] and vitamin E and carotenoids from other berries [4, 9]. However, there is little information available

regarding the recovery using SFE of antioxidants from berry seeds.

The plant matrix is an important parameter for extraction [7]. Matrix pretreatments, such as milling, can increase the extraction efficiency by breaking the cells, thereby increasing the surface area for mass transfer [10]. The oil at the surface and in the shallow subsurface is quickly extracted, whereas the oil inside the intact cells, in the particle core, has stronger mass transfer resistance [11]. A better understanding of how the different kinds of berry matrices and cell structure morphologies are affected by milling is essential to improve the extraction efficiency.

The objective of this study was to investigate the yields, chemical compositions, and recovery of antioxidant compounds of SFE-extracted oils from the seeds of cloudberries, black currants, and bilberries. The oils were extracted by SFE at different temperatures (50°C and 80°C) and compared with oils that were obtained from the same seeds using conventional solvent extraction with hexane. In addition, the microstructures of seed particles were visualised using light microscopy to elucidate the influence of cell morphology on the extraction efficacy.

2. Materials and Methods

2.1. Chemicals. Carbon dioxide (>99.99%) was purchased from AGA Gas AB (Växjö, Sweden). The internal standard heptanoic (margaric) acid 17 : 0 (≥99%) and the external standards of FAME mixture GLC 463 and stearidonic acid 18 : 4 n-3 were purchased from Nu-Chek Prep Inc. (Elysian, USA). DL α-tocopherol (≥97%) and 1,1-diphenyl-2-picrylhydrazyl (DPPH) were obtained from Alfa Aesar GmbH (Karlsruhe, Germany). The standards δ-tocopherol (95.5%) and γ-tocopherol (97.3%) were from Supelco (Bellefonte, PA, USA), and α-tocotrienol (97%), (*R*)-γ-tocotrienol (≥97.0%), and acetyl chloride (≥99.0%) were obtained from Fluka, Sigma-Aldrich (Stockholm, Sweden). Methanol (LC-MS grade ≥99.9%), toluene (HPLC grade 99.9%), cyclohexane (HPLC grade ≥99.7%), petroleum ether (ACS reagent, ≥95.0%), *tert*-butyl methyl ether (MTBE) (HPLC grade ≥99.8%), and Tween 80 were all from Sigma-Aldrich (Stockholm, Sweden). 2,2,4-Trimethylpentane (HPLC grade 99.5%) was purchased from Labscan (Dublin, Ireland). Ethanol absolute (AnalaR NORMAPUR) was from VWR International (Spånga, Sweden).

2.2. Berry Seeds. The berries were picked in Norrbotten, northern Sweden. Cloudberries (*Rubus chamaemorus*) and bilberries (*Vaccinium myrtillus* L.) were grown wild, and the black currants (*Ribes nigrum* L.) were cultivated. The seeds were separated from the press cake (obtained after juice removal) in a pureeing machine (Robot Coupe C200) and stored at −40°C. The seeds were thawed at 4°C and dried in a hot-air oven at 40°C (Garomat 142; Electrolux AB, Stockholm, Sweden) until a moisture content of approximately 6.5% was obtained. After drying, the seeds were kept in the freezer at −40°C until use in the extraction experiments. Immediately before extraction, the seeds were ground for 30 s in a coffee mill (model 2393; OBH Nordica, Stockholm, Sweden).

2.3. Solvent Extraction. The extractions were performed with a mixture of 1 g of milled seeds and 10 mL of hexane. The mixtures were mixed for 2 h in an orbital rotary plate (Reax-2; Heidolph GmbH, Schwabach, Germany) at 150 rpm. After shaking, the mixtures were centrifuged in a Heraeus-Kendro Multifuge 1S (Heraeus GmbH, Hanau, Germany) for 10 min (3000 × *g*). The supernatants were collected, and the procedure was repeated. The supernatants were pooled, and the solvent was evaporated by flushing the samples with N_2 at 40°C. The evaporated extracts were stored at −18°C until the analysis. The extractions were performed in 18 replicates for bilberry seeds and 30 replicates for cloudberry and black currant seeds. A high amount of extractions were carried out to ensure that there was sufficient oil for the analysis.

2.4. Supercritical Fluid Extraction (SFE) of Berry Seeds. The supercritical fluid extractions were carried out in triplicate, using a laboratory-scale supercritical fluid system (SFE-500M1-2-C50; Waters Corp., Milford, MA, USA). The unit consisted of a CO_2 pump connected to a cooling bath (F32-HD; Julabo GmbH, Seelbach, Germany), an automated back-pressure regulator, a cylindrical extractor vessel of volume 500 mL equipped with a heating jacket, and one separation vessel (500 mL) kept at 10 bar and 25°C. Ground seeds (50 ± 0.5 g) were loaded into the extraction vessel. For each extraction, 2 g of glass wool was used to protect the vessel filters and to fill up the empty space of the vessel. The extractions were carried out for 60 min at a flow rate of 30 g CO_2/min. The extraction pressure was 350 bar, and the extraction temperature was 50°C or 80°C. The extracts obtained after SFE were used for estimation of fatty acids, tocopherols, tocotrienols, carotenoids, and DPPH. Due to the small volumes obtained from each extraction, the triplicates were pooled; hence, this limited the interpretation of the results. The extracts were stored at −18°C until the analysis.

2.5. Light Microscopy (LM) Analysis. The milled seeds were smeared on the surface of a microscope slide, and a droplet of water was added to disperse the fragments of seeds. The microstructures of the berry seeds were examined under a Microphot FXA microscope (Nikon, Tokyo, Japan). Images were acquired with an Altra 20 camera (Olympus, Tokyo, Japan), and the objective lenses ×10 and ×20 were used.

2.6. Fatty Acid Analysis by Gas Chromatography (GC). The methylation of fatty acids from bilberry, black currant, and cloudberry seed oils was based on the method of Cavonius et al. [12]. Toluene (1 mL) was added to the extracts (0.025 ± 0.005 g) and then mixed with 1 mL of freshly prepared 10% (v/v) acetyl chloride in methanol. The solutions were incubated for 2 h at 70°C. After cooling to room temperature, 1 mL of Milli-Q water was added, followed by

2 mL of petroleum ether. The solutions were vortexed and centrifuged for 5 min at 2500 $\times g$. The supernatant that contained the methyl esters of fatty acids (FAMEs) was collected in a new tube, evaporated under N_2 at 40°C, and redissolved in 1 mL of 2,2,4-trimethylpentane. The samples were analysed by using a GC (7890 A; Agilent Technologies, Santa Clara, CA, USA) with a triple-axis mass spectrometric (MS) detector in the electron impact mode (5975 C; Agilent Technologies) and equipped with a J&W DB-wax column (30 m × 0.250 mm × 0.25 μm). Helium was used as the carrier gas. External standards were used for the identification of the peaks, and an internal standard (17 : 0) was used for the quantification of fatty acids. The limit of detection was 50 μg/mL for 17 : 0. The results were calculated in grams of FA per 100 g of oil.

2.7. Determination of Tocopherols and Tocotrienols by High-Performance Liquid Chromatography (HPLC). Methanol (2 mL) was added to 0.04 ± 0.005 g of extracts and vortexed. The samples were sonicated for 15 min. A 1 mL aliquot of the upper phase was collected and transferred to a vial. The samples were analysed by HPLC coupled with an on-line fluorescence detector (RF-551; Shimadzu, Kyoto, Japan). A reverse phase C18 Kromasil column (pore size, 100 Å; L × ID, 250 mm × 2.1 mm; and particle size, 5 μm) was used to separate the tocopherols and tocotrienols. The mobile phase was composed of a methanol : water mix (95 : 5 v/v). Tocopherols and tocotrienols were detected at 295 nm excitation and 330 nm emission wavelengths. The limits of detection were 2.5 μg/mL for α-tocopherol, 1.25 μg/mL for γ-tocopherol, 0.3135 μg/mL for δ-tocopherol, 0.1 μg/mL for α-tocotrienol, and 0.3125 μg/mL for γ-tocotrienol. The peaks were identified using standards and quantified by calibration curves using their linear regression equations. The correlation coefficients (R^2) of the standard curves were 0.9989, 0.9996, 0.9991, 0.9972, and 0.9992 for α-tocopherol, γ-tocopherol, δ-tocopherol, α-tocotrienol, and γ-tocotrienol, respectively. The concentrations were confirmed spectrophotometrically based on the UV absorption profiles of pure tocols at their maximum wavelengths [13]. The results are expressed in mg of vitamin E/100 g berry seed oil.

2.8. Determination of Carotenoids by HPLC. *Tert*-butyl methyl ether (MTBE; 1 mL) was added to 0.04 ± 0.005 g of extracts and vortexed. The samples were transferred to amber vials and analysed by using HPLC equipped with a UV-visible photodiode array detector (996, Waters; Millipore, MA, USA). The carotenoids were separated using reverse-phase elution on a C30 column (5 μm, 250 × 4.6 mm ID; YMC Europe GmbH, Schermbeck, Germany). The absorption spectrum from 250 nm to 550 nm was measured. The limits of detection were 62.2 μg/mL for β-carotene and 312.5 μg/mL for lutein. The mobile phase consisted of methanol and MTBE. The following gradient mixture was used for black currant and bilberry seed oils: isocratic elution with 85% (v/v) MeOH and 15% (v/v) MTBE for 2 min; the gradient was then built up over 9 min with 75% MeOH and 25% MTBE; for the next 12 min, the gradient attained 10% MeOH and 90% MTBE; in the following 3 min, the gradient reached the initial composition of 85% MeOH and 15% MTBE, followed by isocratic elution for the final 4 min. For cloudberry seed oil, the gradient mixture was obtained as follows: the initial conditions of 85% MeOH and 15% MTBE were established over 6 min as 75% MeOH and 25% MTBE, progressing to 60% MeOH and 40% MTBE over 1 min and 10MeOH and 90% MTBE for 16 min, and the gradient was reset to the initial conditions over 3 min and equilibrated for an additional 6 min. Lutein and β-carotene were identified on the basis of their retention times and the spectral characteristics of pure standards, as described by Svelander et al. [14]. Quantifications were made using the linear regression equations from standard curves. The R^2 was 0.9991 for β-carotene and 0.9999 for lutein, for the analysis of black currant and bilberry seed oils, and 0.9978 for β-carotene and 1 for lutein, for cloudberry seed oils. The concentration of the standard solutions was previously determined spectrophotometrically based on the UV absorption profiles of pure β-carotene and lutein at their maximum wavelengths [15]. The results are expressed in mg of carotenoids/100 g of berry seed oil.

2.9. Free Radical-Scavenging Activity (DPPH Method). The antioxidant activities of the extracts were evaluated using the 1,1-diphenyl-2-picrylhydrazyl (DPPH) method, according to the procedure of Brand-Williams et al. [16]. All the solutions were prepared in ethanol. The samples were diluted to obtain five different concentrations, so as to determine the effective concentration (EC_{50}). An aliquot of 1 mL of each dilution was mixed with 1 mL of freshly prepared DPPH solution (100 μM). Solutions of α-tocopherol were used as the positive control [17]. The mixtures were incubated in the dark at room temperature. After 1 h, the change in the absorbance after the reaction between radical scavengers and DPPH was measured at 517 nm in a spectrophotometer (Ultrospec 1000; Pharmacia Biotech (Biochrom) Ltd., Cambridge, England). The results were expressed in mg oil/mL of ethanol.

2.10. Statistical Analysis. The analyses were carried out in triplicate. Statistical significance was tested by performing analysis of variance (ANOVA), followed by a Tukey post hoc test (level of significance of $p = 0.05$). Correlation among EC_{50} and antioxidants (vitamin E and carotenoids) was calculated using Pearson's correlation coefficient (r) (level of significance of $p = 0.01$). The correlations were analysed for each antioxidant separately and for the sum of the EC_{50} (mg antioxidant/mL ethanol) and concentrations (mg antioxidant/100 g oil) of vitamin E and carotenoids.

3. Results and Discussion

3.1. Light Microscopy Analysis of Berry Seed Particles. The extraction process involves two different extraction periods; the first period is characterised by the rapid extraction of surface and shallow subsurface oil, while the second period proceeds more slowly and is controlled by diffusion of the

fluid into the matrix [11]. Figures 1(a)–1(c) show the LM images of the dry and ground seeds of cloudberry (a), black currant (b), and bilberry (c) before extraction. The pretreatments disrupted the seeds, revealing the interior part divided by the lamellar structure. The milling step broke several of the cells, releasing immediately some of the oil from the cells, which could be easily extracted. The oil droplets spread around the seed particles were dispersed from the particle surface by the addition of a water droplet to the objective glass. In Figure 1(a), the presence of a big oil droplet is shown (indicated by a white arrow). The milling step disrupted each berry seed in a different way, depending on the size of the seed and the cell shape. The seeds of cloudberry are larger and have higher density than the bilberry and black currant seeds [18]. Differences in the weights and sizes of seeds can produce heterogeneous particles after milling, with different shapes and sizes. The disrupted cells are indicated by arrows (black) in Figures 1(a)–(c). Cloudberry seeds have the largest cells of the three berry types, while bilberry seeds have the longest and narrowest cells (Figures 1(a) and 1(c)), which may have facilitated the extraction from the inner part of the broken bilberry seed cells. Black currant seeds (Figure 1(b)) have the smallest cells and particles. Longer milling times might break the cloudberry and bilberry seeds into smaller particles with larger surface areas, resulting in more efficient extractions. Further investigations into the effects of pretreatments of the investigated berries are necessary to optimise the extraction.

The small cells and seed particles present a larger membrane surface area, facilitating the extraction of nonpolar compounds from the membranes, for example, of the vitamin E located in the cell membranes [19]. Milling of the seeds broke the cell membranes and released to the surface the oil than contains vitamin E. This may explain why a major fraction of the vitamin E was extracted during the first part of the extraction, which was followed by a decrease in the extraction rate due to mass transfer resistance from the cell membrane. As shown in Figures 1(a)–1(c), the particle cores have many intact cells, which may have decreased the extraction rates of other compounds present in the oil.

3.2. Extraction Yields. The yields and chemical compositions of the cloudberry, black currant, and bilberry seed oils were assessed after SFE at 50°C and 80°C or after hexane extraction (which was performed at room temperature and evaporated at 40°C). Figure 2 presents the yield% (w/w) values of the cloudberry, black currant, and bilberry seed oils. Significantly higher yields were obtained with hexane extraction compared to SFE. Studies comparing SFE and conventional extraction using hexane as solvent often report higher extraction yields with the conventional extraction method [20, 21]. However, since hexane is hazardous to workers and the environment, more gentle extraction procedures are needed [22]. In addition, a higher yield may not reflect the quality of the extracts. Johansson et al. [18] assessed the oil contents of berry seeds with moisture contents of 6%, similar to the current work, using the Folch procedure, which is another conventional extraction method. They reported for cloudberry, black currant, and bilberry seeds oil content of 11.9%, 15.9%, and 30.5%, respectively. In the current work, lower oil levels were obtained with hexane extraction, which might be due to the differences in the type of cultivar, geographical origin, and the genotype [23]. The highest yields were obtained for bilberry seeds. Gustinelli et al. [24] have reported a higher extraction yield (21.78%) for bilberry seed oil extracted at 350 bar and 50°C. Since extraction time and flow rate are important parameters for SFE, the higher value seen in the present study might be explained by the longer extraction time (80 min) and higher flow rate (40 g CO_2/min). The measured yields were 2.0% and 18.8% for black currant seeds extracted by SFE at 50°C and for bilberry seeds extracted by hexane, respectively. For SFE, the yields of cloudberry and black currant seed oils were higher at 80°C than at 50°C. The temperature is considered to influence the yield of oil based on two counteracting forces [25]. At constant pressure, an increase in temperature decreases the solvent density, leading to a decrease in solubility. However, an increase in temperature increases the vapour pressure of the solutes, thereby improving the solubility of the oil during exposure to SC-CO_2. The effect of the increase in temperature depends on which one of the two forces is predominant. In the present study, the vapour pressure predominated during SFE of the black currant and cloudberry seeds at 350 bar. In mixtures of similar compounds, the intermolecular interactions are similar, and the vapour pressure depends on the molecular weights of the compounds [26]. However, in complex mixtures such as berry seed oils, complex intermolecular interactions result in significant differences compared with solubilities of the pure lipids (fatty acids and mono-, di-, and triacylglycerols) [27]. Therefore, the vapour pressure and its effects on the extraction yields of different berry seeds extracted under the same conditions are more difficult to predict.

3.3. Fatty Acid Composition. Table 1 shows the fatty acid compositions of cloudberry, black currant, and bilberry seed oils extracted by SFE at different temperatures and by hexane extraction. The major fatty acids were linoleic (18 : 2 $n-6$) and α-linolenic acid (18 : 3 $n-3$). Only the bilberry seed oil had $n-6/n-3$ ratios ≤1. The sum of the PUFAs ranged from 66.8% to 75.9% of the total fatty acids for bilberry seeds extracted by hexane and for black currant seeds extracted by SFE at 50°C, respectively. There were small but significant differences in the fatty acid compositions of the oils obtained by either SFE or hexane extraction. The largest relative compositional difference measured was the concentration α-linolenic acid (18 : 3 $n-3$), which was significantly lower in the black currant seed oils extracted by hexane. The α-linolenic acid (18 : 3 $n-3$) is mostly in the triacylglycerol fraction and therefore, its concentration relies on the solubility of the triacylglycerols, which depends on their molecular weights [28]. Johansson et al. [28] have reported 26 different triacylglycerol species for black currant seed oil.

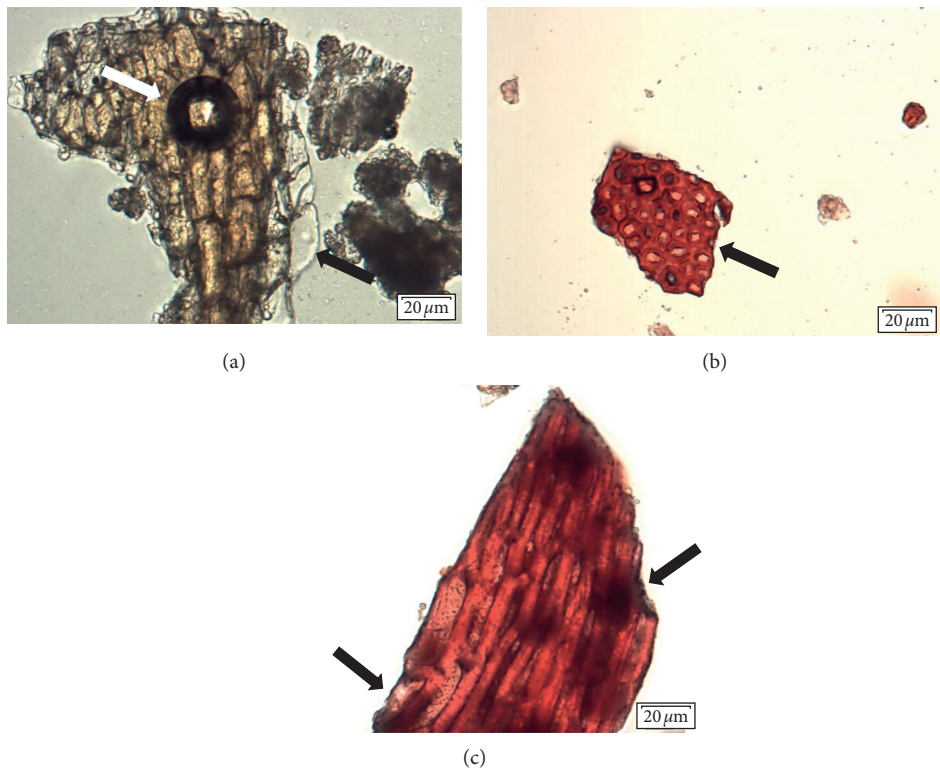

Figure 1: Seeds of cloudberry (a), black currant (b), and bilberry (c) dried and milled for 30 s. White arrow indicates oil droplet released after milling. Black arrows indicate disrupted cells.

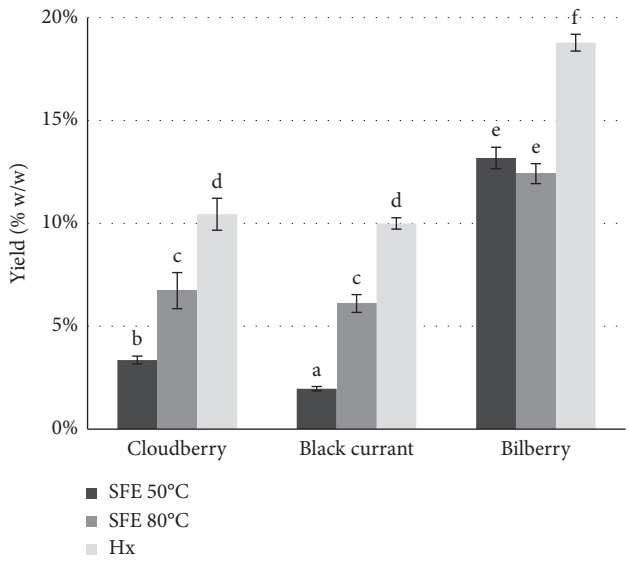

Figure 2: Extraction yield (% w/w) of cloudberry, black currant, and bilberry seed oils extracted by SFE at different temperatures and by hexane extraction. Results are mean values ± standard deviation of triplicate extractions. Different letters above error bars denote significant difference ($p < 0.05$) according to ANOVA followed by Tukey's post hoc test.

All the oils analysed had high percentages of PUFA and the major fatty acids were linoleic and α-linolenic acid. Furthermore, the black currant seed oil had a high level of γ-linolenic acid (18 : 3 $n-6$) and was the only berry oil in which stearidonic acid (18 : 4 $n-3$) was detected. Both γ-linolenic and stearidonic acids are associated with reduced inflammation [29, 30]. Similar results for the fatty acid profile have been reported in previous studies of SFE of cloudberry, black currant, and bilberry seed oils [4, 24, 31, 32].

3.4. Recovery of Antioxidants and Antioxidant Activity. Table 2 lists the contents of tocopherols and tocotrienols in the berry seed oils. The major vitamin E isomers detected in the seed oils from cloudberry, black currant, and bilberry were γ-tocopherol, α-tocopherol, and γ-tocotrienol, respectively. The highest concentration of vitamin E was obtained in black currant seed oil extracted by SFE at 50°C, while bilberry seed oil extracted by hexane extraction had the lowest vitamin E content. For cloudberry seeds, hexane extraction resulted in the highest recovery of vitamin E. Increasing the temperature of SFE enhanced the extraction of vitamin E for cloudberry and bilberry seeds; although for black currant seeds, increasing the temperature was found to decrease the extraction yield. In general, the oils with lower yields contained higher concentrations of vitamin E. A strong negative correlation between the oil yield and the concentration of vitamin E was found ($r = -0.846$, $p = 0.01$), which suggests that most of the vitamin E could be extracted. Vitamin E is soluble in triacylglycerols and can be easily extracted during SFE of seeds [25]. In plant seeds, the vitamin E is mostly located in the membranes [33]. The

Table 1: Fatty acid composition of cloudberry, black currant, and bilberry seed oils extracted by SFE at different temperatures and by hexane extraction.

Oils	Fatty acids% (w/w)										$n-6/n-3$ ratio
	16 : 0	18 : 0	18 : 1 $n-9$	18 : 1 $n-7$	18 : 2 $n-6$	18 : 3 $n-3$	18 : 3 $n-6$	18 : 4 $n-3$	20 : 0	20 : 1 $n-9$	
Cloudberry 50°C	3.7 ± 0.06^{a}	1.8 ± 0.04^{ab}	16.0 ± 0.15^{b}	1.0 ± 0.05^{a}	41.6 ± 0.21^{bc}	32.4 ± 0.08^{d}	1.5 ± 0.02^{a}	n.d.	1.1 ± 0.04^{a}	0.9 ± 0.03^{c}	1.3 ± 0.00^{b}
Cloudberry 80°C	3.4 ± 0.04^{a}	1.8 ± 0.03^{ab}	16.6 ± 0.04^{b}	1.0 ± 0.01^{a}	41.3 ± 0.12^{bc}	33.8 ± 0.04^{ef}	n.d.	n.d.	1.2 ± 0.03^{b}	0.9 ± 0.03^{c}	1.2 ± 0.00^{b}
Cloudberry Hx	3.4 ± 0.01^{a}	1.9 ± 0.01^{abc}	16.6 ± 0.04^{b}	1.0 ± 0.01^{a}	40.8 ± 0.04^{b}	34.1 ± 0.12^{ef}	n.d.	n.d.	1.2 ± 0.01^{b}	1.0 ± 0.01^{d}	1.2 ± 0.01^{b}
Black currant 50°C	7.9 ± 0.04^{c}	2.1 ± 0.02^{bc}	12.3 ± 0.04^{a}	1.0 ± 0.03^{a}	42.5 ± 0.04^{cd}	16.4 ± 0.01^{b}	13.4 ± 0.02^{c}	3.5 ± 0.07^{a}	n.d.	0.8 ± 0.01^{b}	2.8 ± 0.01^{c}
Black currant 80°C	7.7 ± 0.08^{c}	2.1 ± 0.06^{bc}	13.0 ± 0.08^{a}	1.1 ± 0.02^{a}	42.1 ± 0.42^{bc}	17.5 ± 0.11^{c}	12.7 ± 0.04^{b}	3.2 ± 0.22^{a}	n.d.	0.7 ± 0.03^{a}	2.7 ± 0.06^{c}
Black currant Hx	7.7 ± 0.64^{c}	2.4 ± 0.05^{c}	12.9 ± 0.12^{a}	1.1 ± 0.05^{a}	44.1 ± 0.09^{d}	14.5 ± 0.60^{a}	12.8 ± 0.26^{b}	3.6 ± 0.39^{a}	n.d.	0.8 ± 0.02^{b}	3.2 ± 0.18^{d}
Bilberry 50°C	5.9 ± 0.14^{b}	1.6 ± 0.03^{a}	24.2 ± 0.85^{c}	1.0 ± 0.12^{a}	32.6 ± 15.6^{a}	34.7 ± 0.59^{f}	n.d.	n.d.	n.d.	n.d.	0.9 ± 0.06^{a}
Bilberry 80°C	6.1 ± 0.50^{b}	1.8 ± 0.49^{ab}	23.9 ± 0.05^{c}	1.1 ± 0.06^{a}	33.4 ± 0.46^{a}	33.7 ± 0.43^{e}	n.d.	n.d.	n.d.	n.d.	1.0 ± 0.00^{a}
Bilberry Hx	6.1 ± 0.16^{b}	1.7 ± 0.10^{ab}	24.3 ± 0.13^{c}	1.1 ± 0.04^{a}	32.9 ± 0.63^{a}	34.0 ± 0.22^{ef}	n.d.	n.d.	n.d.	n.d.	1.0 ± 0.02^{a}

Results are mean values ± standard deviation of triplicates, and different letters within the same column indicate significant difference ($p < 0.05$) according to ANOVA followed by Tukey's post hoc test. n.d. = not detected.

Table 2: Content of tocopherols (T) and tocotrienols (Tr) in cloudberry, black currant, and bilberry seed oils extracted by SFE at different temperatures and by hexane extraction.

Oils	mg/100 g oil						mg/100 g dry seeds
	α-T	γ-T	δ-T	α-Tr	γ-Tr	Total	Total
Cloudberry 50°C	38.2 ± 1.44^{d}	67.1 ± 2.95^{b}	4.9 ± 0.01^{b}	n.d.	0.8 ± 0.03^{a}	111.0 ± 5.38^{c}	3.7 ± 0.18^{a}
Cloudberry 80°C	40.2 ± 1.34^{d}	74.9 ± 1.91^{bc}	4.9 ± 0.03^{b}	0.2 ± 0.00^{a}	1.1 ± 0.01^{a}	121.3 ± 4.67^{cd}	8.2 ± 0.31^{c}
Cloudberry Hx	40.3 ± 0.13^{d}	84.6 ± 1.53^{c}	5.5 ± 0.05^{b}	n.d.	1.0 ± 0.06^{a}	131.5 ± 2.37^{d}	13.7 ± 0.25^{e}
Black currant 50°C	122.1 ± 1.24^{f}	102.7 ± 6.10^{d}	16.0 ± 0.67^{d}	0.3 ± 0.02^{b}	0.7 ± 0.02^{a}	241.8 ± 9.65^{f}	4.7 ± 0.19^{b}
Black currant 80°C	93.2 ± 1.21^{e}	87.4 ± 0.02^{c}	13.7 ± 0.06^{c}	0.3 ± 0.01^{b}	2.4 ± 0.01^{a}	197.0 ± 1.58^{e}	12.0 ± 0.09^{d}
Black currant Hx	30.7 ± 1.11^{c}	69.7 ± 1.78^{b}	12.5 ± 0.17^{c}	n.d.	0.1 ± 0.02^{a}	113.0 ± 4.34^{cd}	11.3 ± 0.43^{d}
Bilberry 50°C	18.1 ± 0.12^{b}	3.6 ± 0.17^{a}	0.3 ± 0.01^{a}	0.6 ± 0.00^{c}	36.4 ± 2.15^{c}	59.1 ± 3.14^{b}	7.8 ± 0.41^{c}
Bilberry 80°C	25.8 ± 0.32^{c}	4.8 ± 0.00^{a}	0.5 ± 0.01^{a}	0.7 ± 0.01^{d}	37.4 ± 1.84^{c}	69.3 ± 3.06^{b}	8.6 ± 0.38^{c}
Bilberry Hx	4.7 ± 0.19^{a}	1.6 ± 0.03^{a}	0.2 ± 0.01^{a}	n.d.	10.7 ± 0.39^{b}	17.2 ± 0.85^{a}	3.2 ± 0.16^{a}

Results are mean values ± standard deviation of triplicates, and different letters within the same column indicate significant difference ($p < 0.05$) according to ANOVA followed by Tukey's post hoc test; n.d. = not detected.

milling damages the cells in the membranes (Figure 3), which could facilitate the extraction of the vitamin E in the initial phase of extraction. Shen et al. have reported a higher extraction rate at the beginning of extraction of α-tocopherol at similar pressure (310 bar) and low temperature (40°C) [34]. In the present work, oil samples with high yields had lower vitamin E concentrations because of a dilution effect from the other nonpolar compounds, such as mono-, di-, and tri-acylglycerols, which continued being recovered during the final part of the extraction.

Previous studies have reported the vitamin E concentrations for cloudberry, black currant, and bilberry seed oils extracted at 350 bar and 50°C [4, 24]. Yang et al. [4] reported a higher concentration of vitamin E for cloudberry seed oil (260 mg/100 g oil) and lower concentrations for black currant and bilberry seed oils (110 and 40 mg/100 g oil, resp.). Gustinelli et al. [24] have reported a higher concentration of vitamin E for bilberry seed oil (62.5 mg/100 g oil). The longer extraction time used in both studies (120 min and 80 min) may have influenced the recovery rates of antioxidants. Moreover, variations in the chemical composition of the initial material could also have resulted in differences in the eventual extracts.

The use of SFE at 350 bar and 50°C for the extraction of black currant seed oil was advantageous for the recovery of α-tocopherol (122 mg/100 g oil), whereas for bilberry seeds, extraction at 80°C was more efficient. Temperature is an important parameter for extraction, as it influences the solubility as well as the cellular matrix that contains the targeted compound. In plant matrices, the vitamin E is located in the cell membranes and forms complexes with lysophospholipids and free fatty acids [19]. As vitamin E is fat-soluble, it is concentrated in domains that are rich in lipids, thereby protecting the lipids from oxidation [35]. In plant seeds, the lipids (predominantly triacylglycerols) are also stored in organelles, called "oil bodies" [36]. When the seeds are dried and ground, the cell membranes and walls are broken and the oil bodies are easily accessed by the SC-CO_2 or the hexane, increasing their extractability.

Analyses of the levels of vitamin E in bilberry seed oil extracted by SFE (59 to 69 mg/100 g oil) and black currant seed oil extracted by SFE (242 to 113 mg/100 g oil) showed that the concentrations were higher than those reported in the literature for oils extracted with SFE (40 and 110 mg/100 g oil, resp.) [4]. In contrast, the concentration of vitamin E in cloudberry seed oil extracted by SFE (111 to 132 mg/100 g oil) was considerably lower than that described in the literature for cloudberry seed oils extracted by SFE (260 to 270 mg/100 g oil) [4, 9]. The differences in the concentrations of vitamin E compared to other studies may reflect differences in extraction efficiency between the different extraction methods, differences in drying and milling of the seeds before extraction and environmental and physical factors, such as weather conditions, geographical region, ripeness, and berry variety, all of which are known to affect the chemical composition of the raw material [37, 38].

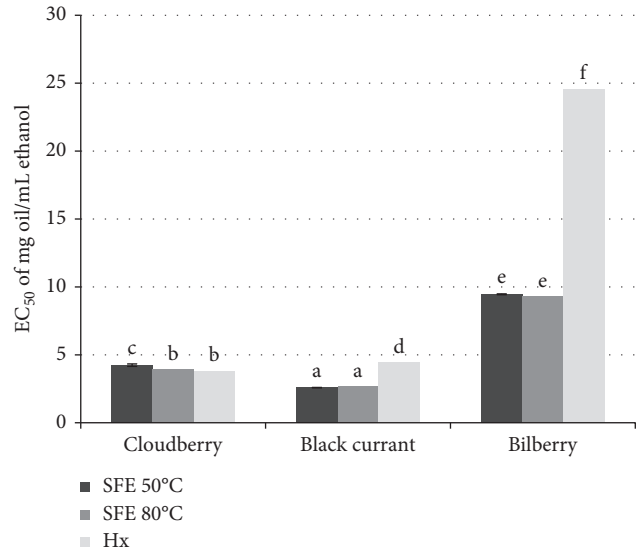

Figure 3: Values of EC_{50} of cloudberry, black currant, and bilberry seed oils extracted by SFE at different temperatures and by hexane extraction. Results are mean values ± standard deviation of triplicates. Different letters above error bars denote significant difference ($p < 0.05$) according to ANOVA followed by Tukey's post hoc test.

Table 3: Content of carotenoids in cloudberry, black currant, and bilberry seed oils extracted by SFE at different temperatures and by hexane extraction.

Oils	mg/100 g oil				mg/100 g seeds
	Lutein	β-carotene	Carotene equivalents	Total	Total
Cloudberry 50°C	0.9 ± 0.00^{a}	4.3 ± 0.10^{g}	31.3 ± 0.09^{f}	36.5 ± 0.18^{f}	1.2 ± 0.01^{d}
Cloudberry 80°C	0.2 ± 0.00^{a}	2.6 ± 0.03^{e}	34.9 ± 0.14^{g}	37.7 ± 0.16^{g}	2.5 ± 0.01^{e}
Cloudberry Hx	n.d.	3.4 ± 0.04^{f}	53.5 ± 0.09^{h}	57.0 ± 0.06^{h}	5.9 ± 0.01^{h}
Black currant 50°C	12.1 ± 0.02^{e}	n.d.	4.8 ± 0.02^{b}	16.9 ± 0.02^{e}	0.3 ± 0.00^{a}
Black currant 80°C	10.1 ± 0.07^{d}	1.4 ± 0.0^{d}	1.7 ± 0.01^{d}	13.2 ± 0.09^{c}	0.8 ± 0.01^{c}
Black currant Hx	30.8 ± 0.12^{f}	1.5 ± 0.02^{d}	5.6 ± 0.13^{e}	38.0 ± 0.23^{g}	3.8 ± 0.03^{g}
Bilberry 50°C	2.4 ± 0.01^{b}	0.1 ± 0.00^{a}	0.4 ± 0.00^{a}	2.8 ± 0.02^{a}	0.4 ± 0.00^{ab}
Bilberry 80°C	3.2 ± 0.03^{c}	0.3 ± 0.01^{b}	0.6 ± 0.01^{a}	4.1 ± 0.05^{b}	0.5 ± 0.01^{b}
Bilberry Hx	12.1 ± 0.56^{e}	0.9 ± 0.03^{c}	2.6 ± 0.02^{c}	15.6 ± 0.57^{d}	2.9 ± 0.13^{f}

Results are mean values ± standard deviation of triplicates, and different letters within the same column indicate significant difference ($p < 0.05$) according to ANOVA followed by Tukey's post hoc test; n.d. = not detected.

Cloudberry seed oil extracted by hexane gave the highest recovery of vitamin E, when analysing the normalised data, expressed as mg of vitamin E/100 g of dry seeds (Table 2). The normalised data were derived by the vitamin E concentration (mg vitamin E/100 g oil) in relation to the extraction yield and represent the total amount of vitamin E extracted, independently of its final oil concentration. Black currant seed oil extracted by SFE at 50°C, which had the highest recovery of vitamin E per oil, had a comparatively low normalised concentration. This indicates that at 80°C a higher level of vitamin E can be extracted from black currant seeds, although the high extraction yield under this condition exerts a dilution effect. The pure lipids belonging to the major lipid classes act as cosolvents during SFE and might influence the extraction of minor compounds, such as vitamin E and carotenoids [26].

Table 3 gives the concentrations of carotenoids in the berry seed oils. Lutein and β-carotene were identified in all the berry seed oils. Significantly higher recoveries (16 to 57 mg/100 g oil) of carotenoids were obtained by hexane extraction than by SFE for all three berry seeds. This was also the case when analysing the normalised data, expressed as mg of carotenoids/100 g of dry seeds. Lutein was the major carotenoid in the black currant and bilberry seed oils. The cloudberry seed oils had the highest concentrations of β-carotene and carotene equivalents, which contributed to the intense orange colour of the extracted oils. The concentrations of carotenoids were slightly lower than the value reported by Manninen et al. for the sum of α-carotene and β-carotene in cloudberry seed oil extracted at 300 bar and 40°C [9]. Black currant seed oil extracted by hexane showed a high recovery of carotenoids, similar to that of the cloudberry seed oils extracted by SFE.

The EC_{50} value is the concentration at which an extract reduces the DPPH absorbance by 50%. The lower the value is for an extract, the stronger is its antioxidant activity. As is evident from Figure 3, bilberry seed oils have the highest EC_{50} values, and thus, the lowest antioxidant activity compared with the oils extracted from cloudberry and black currant seeds. This is probably because of its low vitamin E and carotenoid contents. A significant negative correlation was found for the EC_{50} values and vitamin E content ($r = -0.763$, $p = 0.01$). The lowest EC_{50} values were obtained for black currant seed oils extracted by SFE (at 50°C and 80°C), which also had the highest content of vitamin E. The correlation of the EC_{50} values and carotenoid contents was also negative, albeit not statistically significant ($r = -0.377$), indicating only a trend. This is probably explained by the fact that the antioxidant activities of carotenoids are not only restricted to scavenging activities, but these compounds also quench singlet oxygen [39]. However, when the values for vitamin E and carotenoids were combined, a higher negative correlation was found ($r = -0.802$), indicating a synergistic effect between vitamin E and carotenoids. Carotenoids can transfer electrons to the α-tocopheroxyl radical to regenerate tocopherol, which can then continue acting as an antioxidant [40]. A previous study has also reported a synergistic contribution of vitamin E and carotenoids to the antioxidant activities of lentils [23].

The reason for the variability of the extraction yields and recovery rates of antioxidants obtained for hexane extraction and for SFE at the two different temperatures may be related to differences in the structures and compositions of the three berry seeds. Therefore, the SFE conditions need to be optimised for each individual raw material. In addition, pretreatments (e.g., drying and milling) of the seeds should be taken into account. Milling seeds of different sizes and with different structural features lead to different particle sizes and surface areas for mass transfer, both of which influence the yield and composition of the oil.

4. Conclusions

This study investigated the yields, chemical compositions, and recovery rates of antioxidant compounds of seed oils extracted from cloudberries, bilberries, and black currants by solvent extraction and by SFE at 350 bar and at 50°C and 80°C. Higher extraction yields were obtained with solvent extraction than SFE. The oils extracted from cloudberry, bilberry, and black currant seeds are excellent sources of PUFAs. Oils extracted by solvent extraction had better recovery of carotenoids than SFE while SFE showed higher vitamin E content and better antioxidant activity for black currant and bilberry seed oils. The lower EC_{50} of black currant seed oils is likely correlated to its high content of antioxidants. Black currant seed oils had the highest

antioxidant activities, as seen by the low EC_{50} values and also their high concentrations of vitamin E and carotenoids. The results suggest that SFE extraction can be used as an efficient alternative to conventional extraction for concentration of tocopherols and carotenoids but due to the limited sample size, further studies are needed to support the findings. The seed cells differed in size and shape, and the milling step led to differences in the numbers of broken cells and particle sizes. These aspects warrant further investigation for optimisation of the pretreatments, so as to increase the total surface area of the cell wall particles and the number of broken cells.

Data Availability

The data (carotenoids, vitamin E, DPPH, and fatty acids) used to support the findings of this study are currently under embargo, while the research findings are commercialized. Requests for data (6 months), after publication of this article, will be considered by the corresponding author.

Disclosure

The results of this manuscript were partially presented at the 15th Euro Fed Lipid Congress at the European Federation of Food Science and Technology (EFFoST).

Conflicts of Interest

The authors declare no conflicts of interest.

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Research Article

Effects of 1-Methylcyclopropene Treatment on Physicochemical Attributes of "Hai Jiang" Yardlong Bean during Cold Storage

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The yardlong bean belongs to nonclimacteric fruit. The objective of this study was to investigate the effects of 1-methylcyclopropene (1-MCP) treatment on physicochemical characteristics of yardlong beans during cold storage. Freshly harvested yardlong beans were treated with different concentrations of 1-MCP (0, 0.75, 1.0, 1.25, and 1.5 $\mu L \cdot L^{-1}$) and stored at 8°C for 21 days. The results showed that, compared with the control, the decrease in firmness and good fruit rate and the degradation of chlorophyll and vitamin C (Vc) content could be inhibited, change in skin color could be delayed, activities of superoxide dismutase (SOD) and peroxidase (POD) could be improved, and the increasing of malondialdehyde (MDA) content and weight loss could be inhibited significantly by 1-MCP treatments. Of the different concentrations of 1-MCP, 1.0 $\mu L \cdot L^{-1}$ proved to have the best preservative effects, extending storage time and delaying ripening and senescence of yardlong beans. These results indicated that 1-MCP treatment provided an effective method for delaying the postharvest senescence of fresh yardlong beans.

1. Introduction

The yardlong bean (*Vigna unguiculata* (Linn.) subsp. (sesquipedalis)) is a very popular and healthy horticultural product with a high nutritional value. It is commercially cultivated, and its green pods are eaten throughout the tropical and subtropical areas, covering Asia, Africa, South America, and Southern Europe [1–3]. The yardlong bean, which is a crisp and tender legume, is a good source of proteins, dietary fibers, vitamins, anticarcinogenic compounds, etc. [4, 5]. However, yardlong bean is easy to deteriorate and lose commodity, mainly due to its browning skin color, dehydration, and softening characteristics after harvest, ultimately leading to quality decrease during the transportation and selling [6].

Ethylene is one of the important factors that influence vegetable preservation, which promote the process of vegetable senescence and accelerate its quality deterioration. 1-Methylcyclopropene (1-MCP) is an ethylene action inhibitor that blocked the ethylene signaling transduction by interacting with ethylene receptors in climacteric fruits and vegetables [7]. Suppression of ethylene activity neutralizes many adverse effects on postharvest fruits and vegetables such as increased respiration rate and ethylene production, accelerated softening, senescence, color change, starch breakdown, and other physiological disorders [8–10]. Commercial application of 1-MCP in edible crops was introduced by Rohm and Haas Company [11]. Due to its nontoxic mode of action, low product application rate, and nonexistent residues in postharvested fruits and vegetables, 1-MCP has been widely used in postharvest fruits and vegetables preservation [12].

Previous studies reported that 1-MCP has positive effects on delaying ripening and senescence of climacteric fruits and vegetables, such as guava [13], pear [9], plum [10], and tomato [14]. However, it has been found that 1-MCP may also show significant preservation effects on inhibition of senescence, physiological disorders development, degreening, and color change in some nonclimacteric fruit and vegetables [15], e.g., eggplant [16], jujube [17], broccoli [18], and pitaya [15].

Yardlong bean belongs to the nonclimacteric group [19]. The preservation methods of yardlong bean mainly focused on modified storage atmosphere, coating, hot water treatment, chemical treatment, etc. [6, 20]. Influence of 1-MCP on physicochemical attributes is rarely studied. Therefore, the aim of the present study is to investigate the effects of

different concentrations of 1-MCP on physicochemical characteristics, including skin color, firmness, weight loss, and content of Vc and MDA, and the effects on activities of SOD and POD were investigated too.

2. Materials and Methods

2.1. Yardlong Bean Fruit and 1-MCP Treatment. Fresh yardlong beans (cv. "Hai Jiang" with dark green color, crisp, and tight flesh) were harvested from a commercial field in Yongxin town, Hainan province, China. Beans were packed into cardboard boxes and transported to the laboratory within 2 h. Pods with uniform size and absence of diseases were selected and then randomly divided into five groups, with ~150 fruit (30 pods were used for appearance observation, and 120 pods were used for the determination of indexes every three days.) each. Treatments designated as CK (control, distilled water), 0.75 $\mu L \cdot L^{-1}$, 1.0 $\mu L \cdot L^{-1}$, 1.25 $\mu L \cdot L^{-1}$, and 1.5 $\mu L \cdot L^{-1}$ of 1-MCP (EthylBloc, Rohm and Haas China, Inc.) were conducted. Yardlong beans were placed in a 38 L container and fumigated with the different concentrations of 1-MCP at 25°C for 12 h. The yardlong beans were then placed in polyethylene bags (Xin Feng Company, China) and transferred to 8°C and 85 ± 5% RH and stored for 21 days. Three replicates of fifteen pods were sampled at every third day to determine physicochemical characteristics.

2.2. Weight Loss, Good Fruit Rate, and Firmness. Weight loss was determined for 30 pods from each treatment group [21]. Pods were weighed individually before packing and during storage. Good fruit rate (%) was evaluated by determining the percentage of surface area showing healthy status and measured based on the method reported in [22]. The sensory quality, in terms of changes in visual appearance and acceptability, was rated on a nine-point Hedonic Scale: 9, excellent (fully characteristic of the product, color, freshness, hardness, and juiciness; like very much); 7, very good (faint loss of sepal greenness, freshness, hardness, and juiciness; like moderately); 5, good (further loss of freshness, hardness, and juiciness; like, limited marketability); 3, fair (faint tissue disruption; neither like nor dislike, limited edible quality), and 1, poor (distinct tissue disruption; dislike very much, inedible quality). Firmness was measured using a firmness tester (FMH-1, Takemura Motor Manufacturing, Matsumoto, Japan). The measurement was conducted with the penetration depth of 10 mm, and five equally position of single side were performed. Three replicates with three pods were performed, and the results were expressed in Newton (N).

2.3. Determination of Color and Chlorophyll Content. Pod color parameters of L^*, a^*, and b^* were evaluated using a colorimeter (Konica Minolta, CM-700d, Osaka, Japan) with an 8 mm aperture and a d65 illuminant setting. Chlorophyll was determined using a colorimetric method [23]. Samples from pods of approximately 0.5 g were grounded in a mortar with a small amount of quartz sand, calcium carbonate powder, and 5 mL of 80% acetone to produce a homogenate, and then 10 mL of 80% acetone was added until the mixture became white. The mixture was left to stand for about 3–5 min. The homogenate was then filtered into 50 mL brown volumetric flasks through filter paper. The mortar, pestle, and the residue were rinsed several times with 80% acetone until the filter paper and residue showed no green coloration. The filtrate was diluted with 80% acetone to a final volume of 25 mL. The supernatant was used in the chlorophyll assay using colorimetric determination with a T6-spectrophotometer (model: T6, Beijing Purkinje General Instrument Company, Beijing, China) at 440 nm, 645 nm, and 663 nm colorimetric wavelengths, with three replicates of each sample.

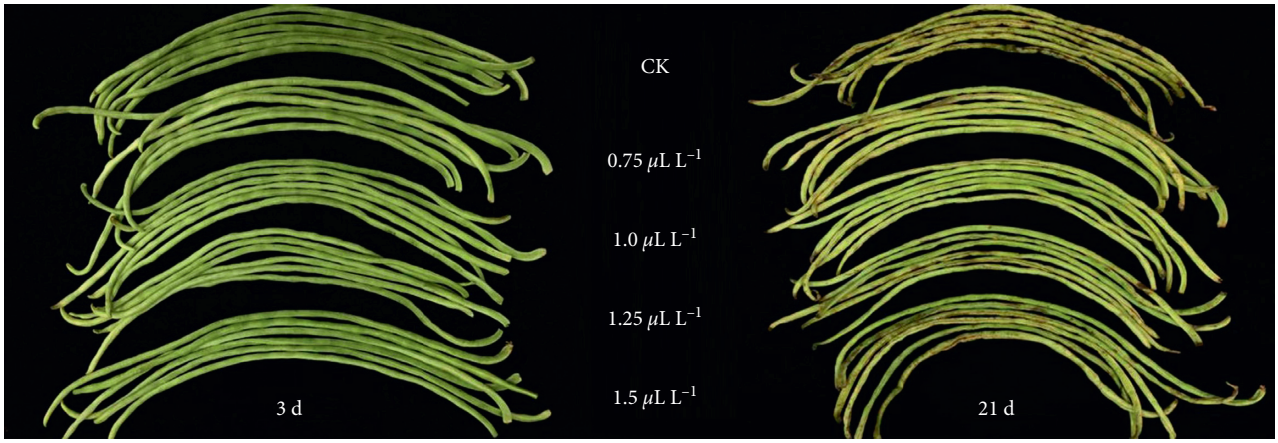
2.4. Determination of Vc Content. The contents of Vc were measured by 2,6-dichlorophenolindophenol titration [17, 24]. Vc concentration was calculated according to the titration volume of 2,6-dichlorophenolindophenol and expressed as $mg \cdot 100\ g^{-1}$. Samples from pods of approximately 2.0 g were grounded in a mortar with a small amount of quartz sand and 5 mL of 2% oxalic acid to produce a homogenate. After centrifugation, the supernatant was extracted with oxalic acid to 50 mL, and the 10 mL sample was titrated with 2,6-dichlorophenolindophenol. The solution was slightly red in color and not fading in 30 s. The amount of the solution was measured.

2.5. Determination of MDA Content. The pod MDA content was determined using the thiobarbituric acid (TBA) reaction [25, 26]. A 2 g sample of pod tissue was homogenized in 10% trichloroacetic acid. The homogenate was centrifuged at 12,000 g for 20 min at 4°C. Then 3 mL supernatant and 3 mL 0.6% thiobarbituric acid were added to a 10 mL test tube, the mixture was heated in a boiling water bath for 20 min, and cooled immediately. Absorbance was then measured at 450 nm, 532 nm, and 600 nm, using distilled water as a blank. Data were expressed as $mmol\ g^{-1}$.

2.6. Assay of SOD Activity. SOD activity in pod tissue was estimated using the method discussed in [27]. Approximately 1.0 g samples of pod tissue were weighed and grounded with a pestle in an ice-cold mortar with 5 mL extracting solution in 50 mM sodium phosphate (pH 7.8). The reaction mixture (5 mL) contained 50 mM sodium phosphate buffer (pH 7.8), 130 mM methionine, 750 μM nitroblue tetrazolium (NBT), 100 μM EDTA-Na_2, 20 μM riboflavin, and 0.1 mL enzyme extract. The mixture was illuminated by light ($60\ mol\ m^{-2}\ s^{-1}$) for 20 min, and the absorbance was then determined at 560 nm. Identical solutions kept in the dark served as blanks. SOD activity was expressed as $U\ g^{-1}$, where one unit was defined as the amount of enzyme that caused a 50% decrease of the SOD inhabitable NBT reduction per mass of fruit pulp per hour.

2.7. Assay of POD Activity. POD activity in pod tissue was estimated by using the method reported in [28]. Approximately 1.0 g samples (pod) were weighed and grounded with a pestle in an ice-cold mortar with 5 mL extracting solution

Table 1: Effects of 1-MCP treatment on weight loss, good fruit, and firmness of yardlong bean during storage at 8°C. Firmness values are mean ± standard error from three replicates. Photograph showing the effects of 1-MCP treatment on yardlong bean fruit after 21 days of storage at 8°C.



1-MCP treatments	Weight loss (%)		Good fruit (%)		Firmness (N)	
	3 d	21 d	3 d	21 d	3 d	21 d
Control	1.9a[Z]	26.0a	75.0d	32.0	7.2 ± 0.001e	3.3 ± 0.004e
0.75 μL·L^{-1}	1.6b	17.1d	90.0a	47.0b	7.3 ± 0.05d	4.0 ± 0.014b
1.0 μL·L^{-1}	0.8c	16.7e	89.0b	51.0a	7.4 ± 0.015c	4.2 ± 0.001a
1.25 μL·L^{-1}	0.9c	18.3c	80.0c	43.0c	7.5 ± 0.016b	3.8 ± 0.007c
1.5 μL·L^{-1}	1.6b	22.6b	80.0c	36.0d	7.6 ± 0.001a	3.5 ± 0.014d

[Z]Mean values (weight loss; good fruit $n = 30$; firmness $n = 9$; ±SE) in each column followed by different lower case letters are significantly different at $P \leq 0.05$ using Tukeys HSD.

(1 mmol PEG, 4% PVPP and 1% Triton X-100). The homogenates were centrifuged at 12,000 g for 30 min at 4°C. The resulting supernatants were used to determine enzymatic activities. Crude enzyme extraction solution was assayed using 3 mL of 25 mM guaiacol as the substrate. Enzyme extraction solution (30 μL) was added to 50 μL of 30% H_2O_2; the reaction started by rapid mixing. At start time, the reaction mixture was transferred into a cuvette and placed in the spectrophotometer sample chamber. With distilled water as a reference, the absorbance values were recorded at 470 nm per 15 s, and one unit of POD was defined as the enzyme activity changes of 0.01 in the 470 nm absorbance in one minute. Results were expressed as U g^{-1} min^{-1}.

2.8. Statistical Analysis. All data were expressed as mean values ± standard error and analysed using SPSS version 17.0 (SPSS, China, Zhejiang University). Data at each time point were subjected to one-way analysis of variance (ANOVA), and differences between pairs of means were measured using Tukeys HSD. $P < 0.05$ was considered as a significant difference.

3. Results and Discussion

3.1. Effect of 1-MCP Treatment on the Weight Loss, Good Fruit Rate, and Firmness. Weight loss, good fruit rate, and firmness were recognized as quality attributes of postharvest yardlong bean that affect pod texture and freshness. As shown in Table 1, 1-MCP treatments exhibited significantly ($P < 0.05$) lower losses of weight than control group after 21 days storage, and 1.0 μL·L^{-1} concentration of 1-MCP exhibited the most retarded effect. Compared with control, weight loss of 1.0 μL·L^{-1} 1-MCP decreased from 1.90% to 0.8% at 3 d and from 26.0% to 16.7% at 21 d. 1-MCP treatment delayed the softening and maintained the healthy characteristic of yardlong bean during 21 days of storage. After being stored at 8°C for 21 days, significant differences were observed between control and 1-MCP-treated beans, and highest values were found in the group treated with 1.0 μL·L^{-1} concentration of 1-MCP, with 51% of good fruit rate and 4.2 N of firmness at 21 day, respectively (Table 1). Thus, 1.0 μL·L^{-1} 1-MCP treatment could effectively reduce the rate of weight loss and suppress the decline of the good fruit and firmness. Similar results have also been reported in avocado [29], Chinese chive scape [30], and eggplant fruit [16] that 1-MCP treatment effectively reduced weight loss, maintained higher firmness, and good fruit rate.

3.2. Effect of 1-MCP Treatment on the Color and Chlorophyll Content. Skin color is one of the important indicators of vegetable quality. 1-MCP significantly delayed ($P < 0.05$) the increase of a^* and b^* values and the decrease of L^* value as compared to the pods in the control group (Figures 1(a)–1(c)). On day 21, a^* and b^* values of 1.0 μL·L^{-1} 1-MCP-treated pods were higher by 1.06 and 1.26 than those of controls, respectively (Figures 1(b), and 1(c)). A sharp decrease in chlorophyll content was observed in the untreated pods after

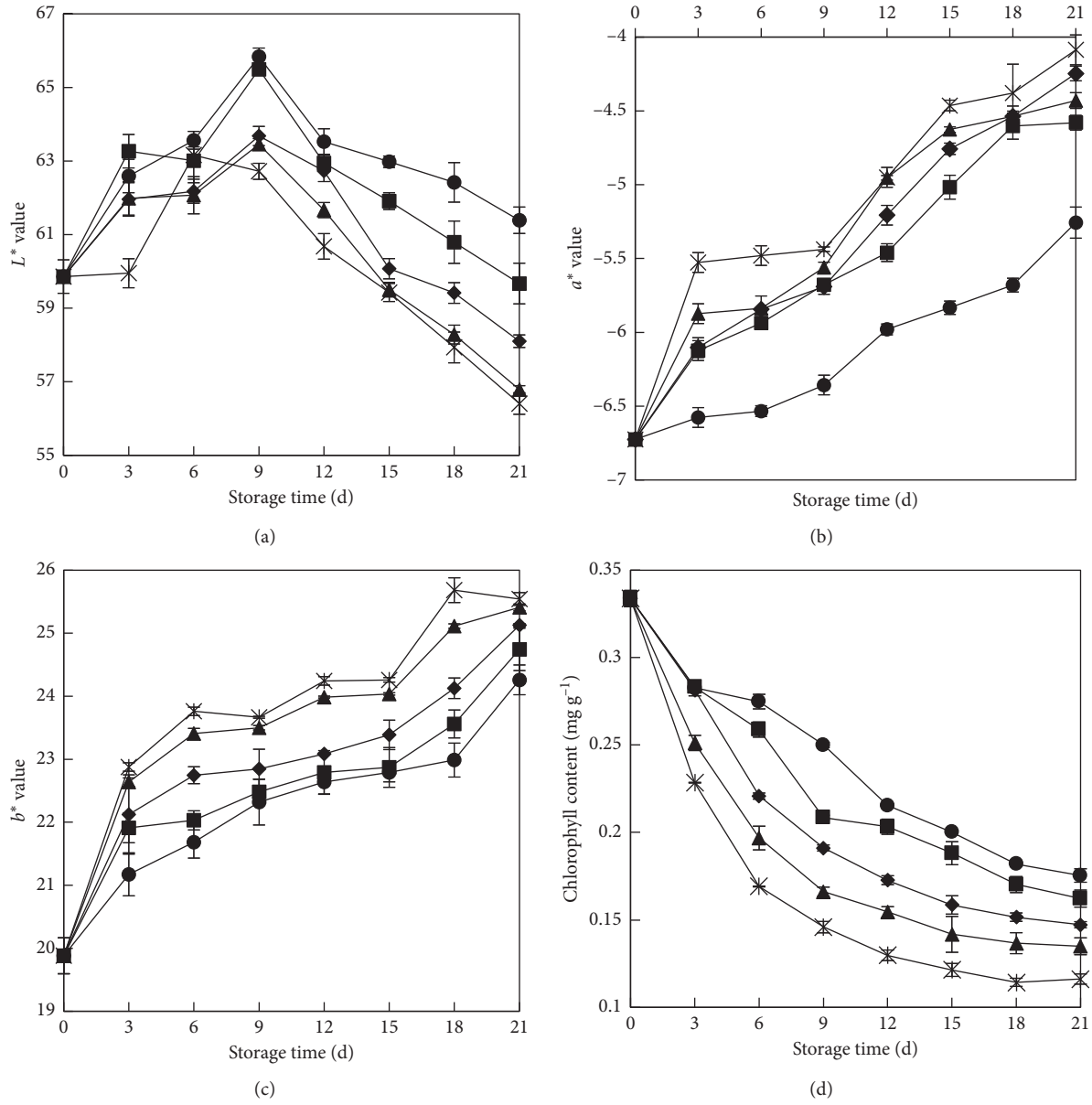


Figure 1: Effects of 1-MCP treatment on L^* (a), a^* (b), b^* (c) values, and chlorophyll content (d) of yardlong bean during storage at 8°C. Each data point is the average of nine pods. Error bars represent the standard error of the means. Control (✻), 0.75 μL·L^{-1} (■), 1.0 μL·L^{-1} (●), 1.25 μL·L^{-1} (◆), and 1.5 μL·L^{-1} (▲).

21 days of storage (Figure 1(d)). After 21 days of storage, yardlong bean treated with 1.0 μL·L^{-1} 1-MCP had the highest chlorophyll content (0.18 mg g^{-1}). These results were in agreement with that of the previous studies that 1-MCP treatment delayed the color change of broccoli floret [18, 31], Strawberry [15], and eggplant [16].

3.3. Effect of 1-MCP Treatment on the Vc Content. One of the nutritional importance of yardlong bean focused on vitamin C (Vc). A shape decrease of Vc content of all the five treated yardlong beans were observed over the storage time (Figure 2). 1-MCP treatment retarded the Vc content decline on 5% level as compared to the control (Figure 2). 1.0 μL·L^{-1} concentration of 1-MCP treatment obviously restrained the Vc decreasing rate, as the Vc decreasing rate at 1.0 μL·L^{-1} 1-MCP-treated beans was 58.2%, which was remarkably lower than that (93.3%) of the controls after 21 days storage. Thus, the results indicated that 1-MCP treatment had the positive role in maintaining Vc content of yardlong bean, especially treated with 1.0 μL·L^{-1} 1-MCP. This result was in agreement with previous studies that Vc content lost more than half in broccoli during postharvest storage, and 1-MCP treatment maintained significantly better retention of Vc [18, 32].

3.4. Effect of 1-MCP Treatment on the MDA Content. MDA is a major product of membrane lipid peroxidation, reflecting cellular membrane integrity. MDA content in both control and 1-MCP-treated yardlong beans increased

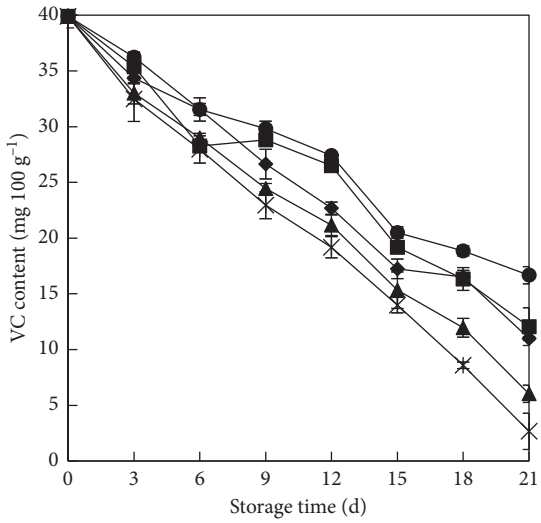


Figure 2: Effects of 1-MCP on Vc content of yardlong bean fruit during storage at 8°C. Error bars represent standard error of the means. Control (✻), 0.75 μL·L^{-1} (■), 1.0 μL·L^{-1} (●), 1.25 μL·L^{-1} (◆), and 1.5 μL·L^{-1} (▲).

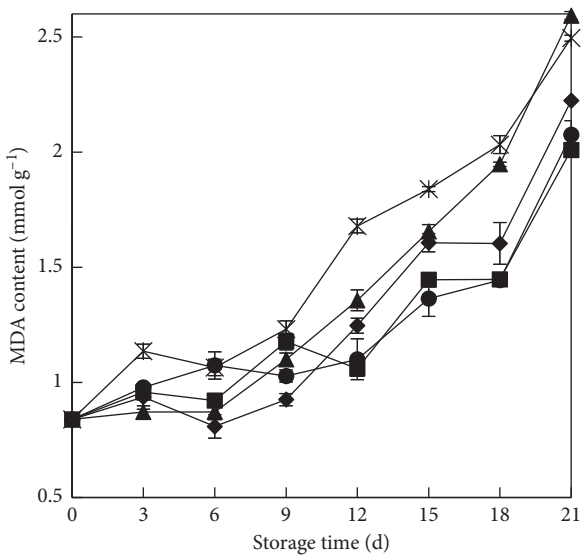


Figure 3: Effects of 1-MCP treatment on MDA content of yardlong bean during storage at 8°C. Each data point is the average of nine fruits. Error bars represent the standard error of the means. Control (✻), 0.75 μL·L^{-1} (■), 1.0 μL·L^{-1} (●), 1.25 μL·L^{-1} (◆), and 1.5 μL·L^{-1} (▲).

progressively, with a lower level of 1-MCP-treated pods than in the control after 21 days storage (Figure 3). MDA content of untreated fruit increased remarkably after 9 days of storage (Figure 3). In the middle of the storage period (12 days), MDA content of the control beans was higher by 0.83, 0.72, 0.54, and 0.46 mmol g^{-1} than that in 0.75, 1.0, 1.25, and 1.5 μL·L^{-1} 1-MCP-treated beans, respectively. Hong et al. [13] found that 1-MCP treatment inhibited the increase of MDA content in early storage, but the effect was not significant at the latter period of storage, which is similar to the conclusions of this study. However, no effect of 1-MCP treatment on MDA was reported in field-grown cotton [33].

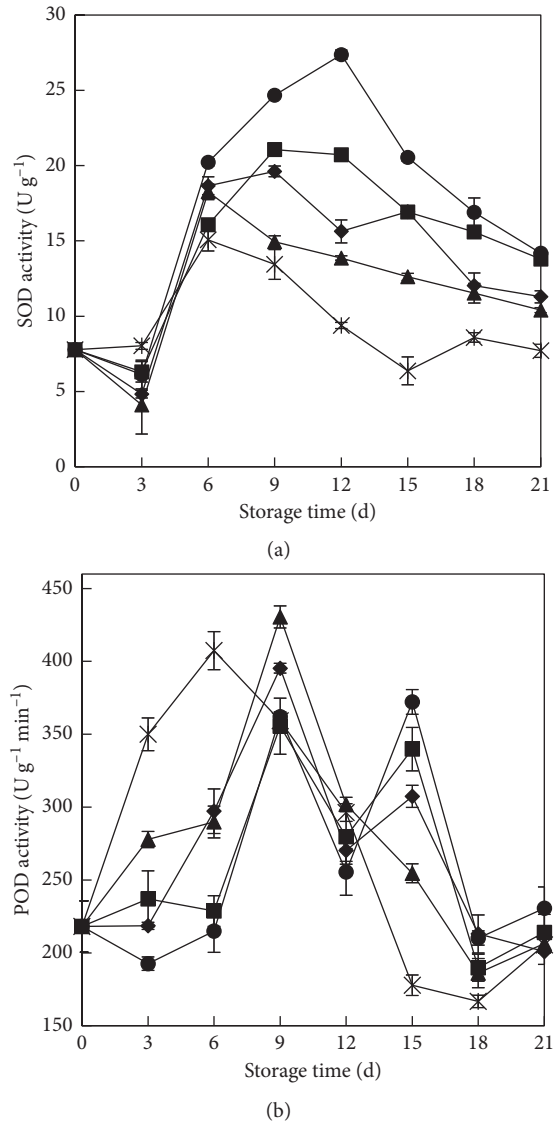


Figure 4: Effects of 1-MCP treatment on SOD (a) and POD (b) activities of yardlong bean during storage at 8°C. Each data point is the average of nine fruits. Error bars represent the standard error of the means. Control (✻), 0.75 μL·L^{-1} (■), 1.0 μL·L^{-1} (●), 1.25 μL·L^{-1} (◆), and 1.5 μL·L^{-1} (▲).

3.5. Effect of 1-MCP Treatment on the SOD and POD Activities. SOD and POD are important enzymes related to fruit and vegetable senescence and defense responses, protecting cells from oxidative damage by scavenging reactive oxygen species [34, 35]. The SOD activity decreased in the first three days of storage, then increased rapidly, and peaked at day 6 (control and 1-MCP at 1.5 μL·L^{-1}), day 9 (1-MCP at 0.75 and 1.25 μL·L^{-1}), and day 12 (1-MCP at 1.0 μL·L^{-1}). The 1-MCP-treated samples exhibited higher SOD activities than those in the controls after 3 days of storage (Figure 4(a)). POD activity in the control beans increased and peaked at the 6th day and then declined. POD activities in treated beans showed two fluctuation and peaked at the 9th day and 15th day. The treated beans had higher POD activities after 9 days of storage, and POD

activity in 1.0 $\mu L \cdot L^{-1}$ 1-MCP-treated bean was the highest of all the treatments (Figure 4(b)). The results suggested that 1-MCP treatment could significantly induce SOD and POD activities, and 1-MCP at 1.0 $\mu L \cdot L^{-1}$ had the significant effect on improving the activities of SOD and POD. The similar enzyme activities enhanced by 1-MCP were reported in green asparagus [36], Chinese chive scape [30], and eggplant [16].

4. Conclusion

Positive effects of 1-MCP treatments were reported in this study on physicochemical quality of yardlong bean during cold storage. We demonstrated that the application of 1-MCP suppressed the change in skin color and the decrease in firmness and reduced the increase in weight loss, the degradation in chlorophyll, and Vc content of yardlong beans. In addition, 1-MCP improved activities of antioxidant enzymes, such as SOD and POD, and reduced the accumulation of MDA in yardlong beans. 1-MCP at a concentration of 1.0 $\mu L \cdot L^{-1}$ proved to be the most suitable concentration of all the treatments. Our results suggest 1-MCP at 1.0 $\mu L \cdot L^{-1}$ maintains postharvest quality of yardlong beans and has the potential for commercial application in the future.

Data Availability

The data used to support the findings of this study are available from the corresponding author upon request.

Conflicts of Interest

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

Authors' Contributions

Zitao Jiang, Jiaoke Zeng, and Wen Li contributed equally to this study.

Acknowledgments

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Supplementary Materials

The supplementary file is the experimental data used to support the findings of this study. (*Supplementary Materials*)

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Research Article

Free Radical-Scavenging, Anti-Inflammatory, and Antibacterial Activities of Water and Ethanol Extracts Prepared from Compressional-Puffing Pretreated Mango (*Mangifera indica* L.) Peels

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During the processing of mango, a huge amount of peel is generated, which is environmentally problematic. In the present study, a compressional-puffing process was adopted to pretreat the peels of various mango cultivars, and then the bioactive compounds of mango peels were extracted by water or ethanol. The phenolic compound compositions as well as the free radical-scavenging, anti-inflammatory, and antibacterial activities of water extract (WE) and ethanol extract (EE) from nonpuffed (NP) and compressional-puffed (CP) mango peels were further evaluated. It was found that compressional-puffing could increase the yield of extracts obtained from most mango varieties and could augment the polyphenol content of extracts from Jinhwang and Tainoung number 1 (TN1) cultivars. The WE and EE from TN1 exhibited the highest polyphenol content and the greatest free radical-scavenging activities among the mango cultivars tested. Seven phenolic compounds (gallic acid, pyrogallol, chlorogenic acid, *p*-hydroxybenzoic acid, *p*-coumaric acid, ECG, and CG) were detected in CPWE (compressional-puffed water extract) and CPEE (compressional-puffed ethanol extract) from TN1, and antioxidant stability of both CPWE and CPEE was higher than that of vitamin C. Further biological experiments revealed that CPEE from TN1 possessed the strongest anti-inflammatory and antibacterial activities, and thus it is recommended as a multibioactive agent, which may have applications in the food, cosmetic, and nutraceutical industries.

1. Introduction

Mango (*Mangifera indica* L.) is recognized as one of the most economically productive fruits in tropical and subtropical areas throughout the globe. Mango has excellent nutritional value and health-promoting properties. A variety of studies have been performed showing high concentrations of antioxidants including ascorbic acid, carotenoids, and phenolic compounds in mango [1]. Mango fruit is the main edible part and is usually processed into various products such as puree, nectar, jam, leather, pickles, chutney, frozen mango, dehydrated products, and canned slices. During the processing of mango, a huge amount of peel is generated, which constitutes approximately 15–20% of the mango fruit [2]. Mango peel is a waste by-product, and its disposal may have a substantial impact on the environment. Previous studies reported that mango peel contains a variety of valuable compounds such as polyphenols, carotenoids, enzymes, and dietary fiber [2]. Extracts from mango peel also exhibit antioxidant activity [3], anti-inflammatory activity [4], protection against membrane protein degradation and morphological changes in rat erythrocytes caused by hydrogen peroxide (H_2O_2) [5], antibacterial activity [6], and anticancer activity [7]. Hence, the utilization of mango peels may be an economical means of ameliorating the problem of waste disposal from mango production factories, as well as converting a by-product into material for food, cosmetic, and pharmaceutical industrial usages.

Free radicals, including superoxide anion radical ($O_2^{\bullet -}$), hydroperoxyl radical ($HO_2^{\bullet}$), hydroxyl radical ($HO^{\bullet}$),

peroxyl radical ($ROO^{\bullet}$), and alkoxyl radical ($RO^{\bullet}$), are defined as any molecules or atoms with one or more unpaired electrons and are often involved in human diseases [8]. Many studies have shown that free radicals in living organisms cause oxidative damage to different molecules such as lipids, proteins, and nucleic acids and these are involved in the interaction phases of many diseases such as cancer, atherosclerosis, respiratory ailments, and even neuronal death [9]. Antioxidants are substances that delay or prevent the oxidation of cellular oxidisable substrates. They exert their effect by scavenging reactive oxygen species (ROS) or preventing the generation of ROS [10]. Synthetic antioxidant compounds such as butylated hydroxytoluene (BHT) and butylated hydroxyanisole (BHA) have potent antioxidant activity and are commonly used in processed foods. However, they have been restricted because of their carcinogenicity and other toxic properties [11, 12]. Thus, in recent years, there has been considerable interest in natural antioxidants derived from biological materials because of their presumed safety and potential nutritional and therapeutic value.

A large number of publications have suggested that fruit polyphenols are related to immunomodulatory and anti-inflammatory properties via *in vitro* and animal studies [13]. Inflammation is a complicated physiological phenomenon that occurs when the immune system in the body is activated to counter threats such as injury, infection, and stress. Macrophages often play a unique role in the immune system because they not only elicit an innate immune response but also act as effector cells in inflammation and infection. When macrophages encounter bacterial endotoxin lipopolysaccharide (LPS), they can be stimulated to generate a variety of inflammatory mediators such as nitric oxide (NO), tumor necrosis factor-α (TNF-α), interleukin-1β (IL-1β), IL-6, prostaglandin E2 (PGE2), and adhesion molecules to help eradicate the bacterial assault [14]. Generally, substances with inhibitory effects on the expression and activity of enzymes (e.g., inducible NO synthase (iNOS)) involved in the generation of inflammatory mediators such as NO in the mouse macrophage-like cell line RAW 264.7 are considered to possess immunomodulatory activity [15]. Since a variety of polyphenols exist in mango peels, further research on the use of mango peel extracts as immunomodulatory or anti-inflammatory agents is warranted.

Antibacterial agents are the synthetic or natural compounds that interfere with the growth and division of bacteria. A number of studies have shown that pathogenic microorganisms in humans and various animal species have developed resistance to drugs. This drug resistance is due to the random or otherwise inappropriate usage of commercial antimicrobial agents. As such, there is an urgent need for new antibacterial agents. In addition, synthetic antibiotics have been known to induce side effects such as the appearance of resistant bacteria, skin irritation, organ damage, and immunohypersensitivity [16]. Accordingly, many studies have attempted to develop new agents with high antibacterial activity but with fewer or possibly even no side effects. There is a particular demand for antibacterial compounds from natural resources [17]. Plants produce a range of antimicrobial compounds in various parts such as bark, stalk, leaves, roots, flowers, pods, seeds, stems, hull, latex, and fruit rind [6]. Fruit peel is the outer covering of a fruit, which functions as a physical barrier. It also serves as a chemical barrier by virtue of the presence of many antimicrobial constituents, which protect the fruit from exposure to external pathogens or other factors that may tend to decrease the quality of the fruit. Therefore, fruit peels are good sources for obtaining natural antibacterial agents.

Bioactive compounds in mango peel are generally extracted via the following methods: extraction with 80% ethanol by sonication for 3 days at room temperature [18]; extraction performed three times with methanol, for 3 h per time [19]; extraction with 95% ethanol three times, 72 h per time [20]; extraction with acetone or ethyl acetate for up to 20 h [21–23]; extraction by microwave-assisted method [24, 25]; or extraction with supercritical CO_2, followed by pressurized ethanol [26]. However, these methods generally involve the use of a large volume of solvents, require a long extraction time, consume a lot of energy, are costly, and sometimes are not eco-friendly. The present study builds upon on the research reported in our previous investigation [27]. In brief, we previously developed a compressional-puffing process that has been successfully implemented to increase the extraction yield of fucoidan from brown seaweed [27, 28] and augment the extraction yields of total phenolics and total flavonoids from pine needles [29, 30]. Compressional-puffing can be utilized as a pretreatment step to disrupt the cellular structure of samples, thereby better enabling the release of bioactive compounds by solvent extraction [27]. In this study, compressional-puffing was utilized for pretreatment of mango peels, and water extract (WE) and ethanol extract (EE) extracted from nonpuffed (NP) and compressional-puffed (CP) mango peels were compared. The phenolic compound composition and the free radical-scavenging, anti-inflammatory, and antibacterial activities of WE and EE from mango peels were also evaluated. To the best of the authors' admittedly limited knowledge, this is the first study to elucidate the free radical-scavenging, anti-inflammatory and antibacterial activities of WE and EE extracted from compressional-puffed mango peels. The recovered WE and EE are expected to possess multifunctional activities providing a wide range of benefits. The utilization of mango peel will also help to play a role in minimizing the generation of waste worldwide.

2. Materials and Methods

2.1. Materials. Folin-Ciocalteu's phenol reagent, gallic acid, protocatechuic acid, chlorogenic acid, *p*-hydroxybenzoic acid, pyrogallol, caffeic acid, mangiferin, epicatechin, *p*-coumaric acid, ferulic acid, epicatechin gallate (ECG), catechin gallate (CG), ellagic acid, rutin, quercetin, kaempferol, homogentisic acid, tannic acid, vanillic acid, 2,2-diphenyl-1-picrylhydrazyl (DPPH), sodium nitrite, LPS, dimethyl sulfoxide (DMSO), and 2,2′-azino-bis(3-ethylbenzothiazoline-6-sulphonic acid) diammonium salt (ABTS) were purchased from Sigma-Aldrich (St. Louis, MO, USA). 3-(4, 5-Dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide

Figure 1: Appearance of various Taiwanese mango varieties. (1) Haden; (2) Tainoung number 1; (3) Tu; (4) Jinhwang; (5) Yuwen; (6) Irwin.

(MTT) was purchased from Calbiochem (San Diego, CA, USA). Dulbecco's modified Eagle's medium (DMEM), trypsin/EDTA, fetal bovine serum (FBS), penicillin, and streptomycin were purchased from Gibco Laboratories (Grand Island, NY, USA). Methanol, acetic acid, and potassium persulfate were obtained from Nihon Shiyaku Industries, Ltd. (Tokyo, Japan). All other reagents if not declared were purchased from Sigma-Aldrich (St. Louis, MO, USA) and were all of analytical grade.

2.2. Mango Fruits and Peels. Six mango cultivars, produced in Tainan City, Taiwan, were utilized in this study. The varieties, namely, Jinhwang, Tainoung number 1 (TN1), Irwin, Yuwen, Haden, and Tu (Figure 1), were collected from a local grocery market in Xinhua District, Tainan City, Taiwan. The fruits were used after they had completed ripening. Samples of peels were separated manually from six varieties of mango fruits and were then oven-dried and stored in aluminum bags at 4°C until use.

2.3. Compressional-Puffing Procedure. A compressional-puffing method [27, 28, 31] with minor modification was adopted to pretreat mango peels. In brief, the dried peel samples were crumbled and sieved using a 20-mesh screen. The portion retained by the screen was collected and then compressional-puffed using a continuous compressional-puffing machine with the temperature set at 220°C. The corresponding mechanical compression pressure and steam pressure levels inside the chamber are listed in Table 1. After the compressional-puffing, the peel samples were ground into fine particles and stored at 4°C for further extraction experiments.

2.4. Extraction Procedure. We followed the methods of Yang et al. (2017) [28]. Briefly, the nonpuffed and compressional-puffed peel samples were pulverized and sieved using a 20-mesh screen. The portion passed through the screen was collected and extracted by 95% ethanol (w/v = 1 : 10) for 4 h at 25°C with shaking. The resultant solution was then centrifuged at 9,170 ×g for 10 min and the supernatant was collected. NPEE (nonpuffed ethanol extract) and CPEE (compressional-puffed ethanol extract) were thus obtained after oven-drying the supernatant at 40°C. In addition, the precipitates after 95% ethanol extraction were further extracted by double-distilled water (w/v = 1 : 10) for 1 h at 70°C with shaking. Then the mixture was centrifuged at 9,170 ×g for 10 min and the supernatant was collected. NPWE (nonpuffed water extract) and CPWE (compressional-puffed water extract) were obtained after oven-drying the supernatant at 50°C. All dried extracts were milled to fine particles and stored at 4°C for further analyses. The combined compressional-puffing pretreatment and extraction process is depicted in detail in Figure 2. The extraction yield was calculated using the following equation:

$$\text{extraction yield } (\%) = \left(g_A / g_B\right) \times 100, \quad (1)$$

where g_A represents the dry mass weight of the extract and g_B is the weight of the mango peel sample on a dry basis.

2.5. Determination of Polyphenol Content. Polyphenol content was estimated by the Folin-Ciocalteu colorimetric method based on the procedure of Singleton and Rossi (1965) [32] and using gallic acid as the standard agent.

2.6. High-Performance Liquid Chromatography (HPLC) Analysis of Total Phenolic Compound Composition. The separation of total phenolic compounds was performed by the method of Schieber et al. (2000) [33] and using a Shimadzu HPLC system (Shimadzu, Kyoto, Japan) equipped with a UV-vis detector. A reversed-phase Inspire C18 column (250 mm × 4.6 mm, id 5 μm) purchased from Dikma Technologies (USA) was used for all chromatographic separations. The column was operated at 25°C. The mobile phase consisted of 2% (v/v) acetic acid in water (eluent A), 0.5% acetic acid in water, and acetonitrile (50 : 50, v/v; eluent B). The gradient program was as follows: 20–55% B (50 min), 55–100% B (10 min), and 100–20% B (5 min). The injection volume of all samples was 20 μl. The spectra were monitored at 280 nm and performed at a flow rate of 1 ml/min. Gallic acid, pyrogallol, protocatechuic acid, chlorogenic acid, *p*-hydroxybenzoic acid, caffeic acid, mangiferin, epicatechin, *p*-coumaric acid, ferulic acid, ECG, CG, ellagic acid, rutin, quercetin, kaempferol, homogentisic acid, tannic acid, and vanillic acid were used as standards for HPLC analyses.

2.7. DPPH Radical-Scavenging Activity. The scavenging activity of the DPPH radical in the samples was determined

Table 1: Process variables for compressional-puffing and extraction and extraction yields for various Taiwanese mango peel extracts.

Operational variables		NPWE	CPWE	NPEE	CPEE
Mechanical compression	Pressure (kg/cm^2)	0	5	0	5
	Number of compression times	0	3	0	3
Puffing	Temperature (°C)	0	220	0	220
	Pressure (kg/cm^2)	0	11	0	11
	Time (sec)	0	10	0	10
Pretreatment	Solvent	95% EtOH	95% EtOH	NA*	NA
	Temperature (°C)	25	25	NA	NA
	Time (h)	4	4	NA	NA
Extraction	Solvent	ddH_2O	ddH_2O	95% EtOH	95% EtOH
	Temperature (°C)	70	70	25	25
	Time (h)	1	1	4	4
Extraction yield of extract (%)**		NPWE	CPWE	NPEE	CPEE
Jinhwang cultivar		$33.5 \pm 0.4^{cBC***}$	36.6 ± 2.3^{bC}	23.4 ± 1.2^{bA}	30.2 ± 1.0^{cB}
Tainoung number 1 cultivar		29.5 ± 1.2^{bA}	34.8 ± 1.0^{bB}	29.2 ± 0.7^{dA}	33.7 ± 0.9^{dB}
Irwin cultivar		30.9 ± 0.9^{bcB}	40.0 ± 2.2^{bC}	22.6 ± 0.3^{bA}	29.6 ± 0.4^{cB}
Yuwen cultivar		31.2 ± 1.4^{bcB}	37.0 ± 1.8^{bC}	26.3 ± 0.9^{cA}	37.4 ± 1.0^{eC}
Haden cultivar		25.5 ± 1.5^{aB}	28.6 ± 2.7^{aB}	18.8 ± 0.8^{aA}	20.4 ± 0.5^{aA}
Tu cultivar		25.9 ± 0.3^{aB}	29.1 ± 0.1^{aD}	22.9 ± 0.5^{bA}	27.0 ± 0.1^{bC}

*NA: not applicable. **Extraction yield of extract (%) = ($g_{\text{solid extract, dry basis}}/g_{\text{mango peel sample, dry basis}}$) × 100. ***Values are mean ± SD ($n = 3$); values in the same column with different letters (in a, b, c, d, and e) and in the same row with different letters (in A, B, C, and D) are significantly different ($p < 0.05$).

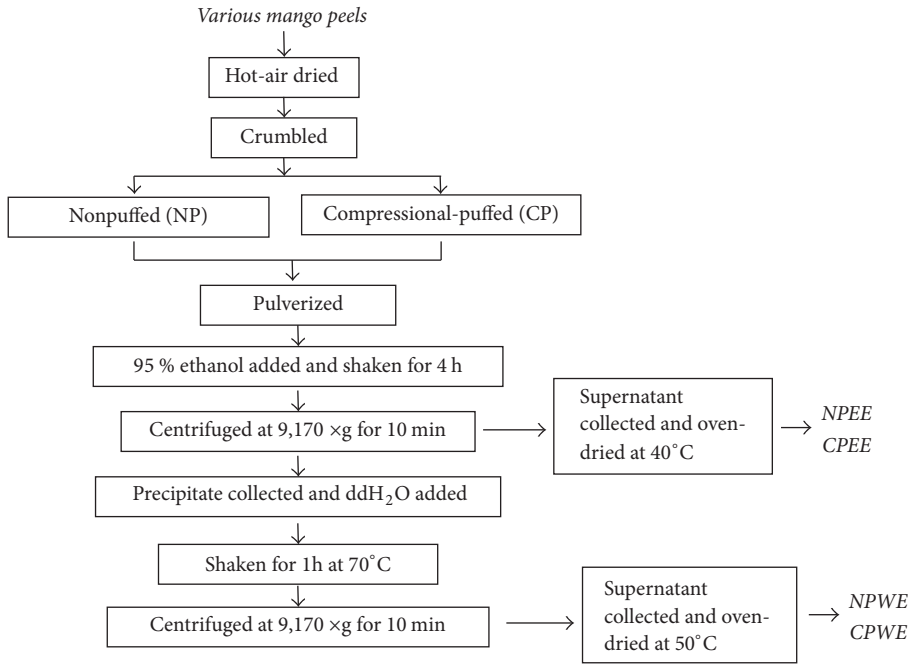

Figure 2: Flowchart of the compressional-puffing process and extraction methods for NPEE, CPEE, NPWE, and CPWE.

using the method described previously [28, 34]. In brief, 50 μl of mango peel extract (concentrations ranging from 0 to 300 μg/ml for Tainoung number 1 and Haden cultivars; 0–600 μg/ml for Jinhwang and Tu cultivars; and 0–900 μg/ml for Irwin and Yuwen cultivars) was added to 200 μl 0.1 mM DPPH solution (in methanol). The mixture was shaken vigorously for 1 min and left to stand for 30 min in the dark at room temperature. After the reaction, the absorbance of all sample solutions was then measured at 517 nm using an ELISA reader (PowerWave 340, BioTek Instruments, Winooski, VT, USA). The radical-scavenging activity was calculated as the percentage inhibition using the following equation:

$$\text{DPPH}_{\text{radical-scavenging}}\,(\%) = \left[1 - \frac{A_{\text{sample}}}{A_{\text{control}}}\right] \times 100, \quad (2)$$

where A_{sample} is the absorbance of the methanol solution of DPPH with tested samples and $A_{control}$ represents the absorbance of the methanol solution of DPPH without the sample.

2.8. ABTS Radical Cation-Scavenging Activity. The ABTS radical cation-scavenging activity was performed according to the method described previously [28, 34]. The $ABTS^{\bullet+}$ solution was produced by mixing 5 ml of 7 mM ABTS solution with 88 μl of 140 mM potassium persulfate and allowing the mixture to stand in the dark for 16 h at room temperature before use. The $ABTS^{\bullet+}$ solution was diluted with 95% ethanol so that its absorbance at 734 nm was adjusted to 0.70 ± 0.05. To determine the scavenging activity, 100 μl diluted $ABTS^{\bullet+}$ solution was mixed with 100 μl of mango peel extract (concentrations ranging from 0 to 100 μg/ml for Tainoung number 1 and Haden cultivars; 0–300 μg/ml for Irwin, Yuwen, and Tu cultivars; and 0–500 μg/ml for Jinhwang cultivar) and the mixture was allowed to react at room temperature for 6 min. After the reaction, the absorbance of all sample solutions was then measured at 734 nm using an ELISA reader (PowerWave 340, BioTek Instruments, Winooski, VT, USA). The blank was prepared in the same manner, except that distilled water was used instead of the sample. The scavenging activity of $ABTS^{\bullet+}$ was calculated using the following equation:

$$\text{ABTS}_{\text{radical cation-scavenging}}\,(\%) = \left[1 - \frac{A_{\text{sample}}}{A_{\text{control}}}\right] \times 100, \quad (3)$$

where A_{sample} is the absorbance of ABTS with tested samples and $A_{control}$ represents the absorbance of ABTS without the sample.

2.9. Cell Line and Culture. Murine macrophage cell lines RAW 264.7 were obtained from the Bioresource Collection and Research Center, the Food Industry Research and Development Institute (FIRDI, Hsinchu, Taiwan). The cells were grown in DMEM supplemented with 10% FBS and 100 U/ml penicillin-streptomycin solution at 37°C in a humidified chamber with 5% CO_2. The medium was changed every two days.

2.10. Measurement of Cell Viability. The MTT assay was used to evaluate cell viability. Briefly, RAW 264.7 cells (2×10^5/ml in a 96-well plate) were plated with culture medium and incubated for 24 h at 37°C, with 5% CO_2 in a humidified atmosphere. The medium was removed and fresh serum-free medium containing different concentrations of mango peel extracts (concentrations ranging from 0 to 25 μg/ml for CPEE of TN1 and CPWE of TN1) was added. After 24 h of incubation at 37°C, with 5% CO_2, the MTT reagent (0.1 mg/ml) was added. After incubating at 37°C for 4 h, the MTT reagent was removed and DMSO (100 μl) was added to each well and thoroughly mixed by pipetting to dissolve the MTT-formazan crystals. The absorbance was then determined by an ELISA reader (PowerWave 340, BioTek Instruments, Winooski, VT, USA) at a wavelength of 570 nm. The cell viability (%) was calculated using the following equation:

$$\text{Cell viability}\ (\%) = \left(\frac{T}{C}\right) \times 100, \quad (4)$$

where T is the absorbance in the test and C is the absorbance for the control.

2.11. Measurement of Nitrite Oxide in Culture Media. RAW 264.7 cells (2×10^5 cells/ml) were seeded in a 96-well flat bottom plate for 24 h at 37°C with 5% CO_2. The culture medium was removed and replaced with fresh medium containing tested samples at various concentrations prior to challenging with 1 μg/ml of LPS. The nitrite concentration was measured in the culture supernatant after 24 h of coincubation. In brief, 50 μl of the cultured supernatants was added in the 96-well plate and 100 μl of Griess reagent was added to each well and allowed to stand for 10 min at room temperature. The absorbance at 540 nm was measured using an ELISA reader (PowerWave 340, BioTek Instruments, Winooski, VT, USA), and the quantification of nitrite was standardized with $NaNO_2$ at 0–100 μM concentrations [35].

2.12. Zone of Inhibition. Five bacteria were tested for antibacterial activity of mango peel extracts. These were three Gram-negative bacteria (*Escherichia coli* ATCC 11775, *Salmonella typhimurium* ATCC 13311, and *Vibrio parahaemolyticus* ATCC 17802) and two Gram-positive bacteria (*Staphylococcus aureus* ATCC 12600 and *Bacillus cereus* ATCC 14579), which were obtained from the Culture Collection and Research Center of the Food Industry Research and Development Institute, Hsinchu, Taiwan. Antibacterial activity was measured using the standard method of diffusion disc plates on agar [36]. In brief, *E. coli*, *S. typhimurium*, *S. aureus*, and *B. cereus* were grown in tryptic soy broth (TSB) medium (Difco Laboratories, Detroit, MI, USA) and *V. parahaemolyticus* was grown in TSB medium + 3% NaCl for 24 h at 37°C, and 0.1 ml of each culture of bacteria at proper cell density was spread on tryptic soy agar (TSA, Difco Laboratories, Detroit, MI, USA) plate surfaces (3% NaCl was added to TSA for *V. parahaemolyticus*). Paper disc (8 mm in diameter) was placed on the agar medium to load 50 μl containing 2 mg of mango peel extract (4%, w/v, in 0.05 M acetate buffer, pH 6.0). Control paper discs were prepared by infusing with 50 μl Antibiotic-Antimycotic Solution (containing 10,000 units/ml penicillin, 10 mg/ml streptomycin, and 25 μg/ml amphotericin) (Corning, Corning, NY, USA) or 50 μl 0.05 M acetate buffer. The plates were incubated at 37°C for 24 h. After 24 h, antibacterial activity of the extracts against the test bacteria was observed by growth-free zone of inhibition near the respective disc and the inhibition diameters were measured.

2.13. Statistical Analysis. Experiments were performed at least three times. Values represent the means ± standard deviation (SD). Statistical analyses were done using the Statistical Package for the Social Sciences (SPSS). The results obtained were analyzed using one-way analysis of variance (ANOVA), followed by Duncan's Multiple Range tests. $p < 0.05$ was considered statistically significant. Correlation analyses were performed using the square of Pearson's correlation coefficient (R^2).

3. Results and Discussion

3.1. Effects of Mango Varieties, Compressional-Puffing, and Extraction Methods on Extraction Yields of Peel Extracts. Six varieties of mango fruits, namely, Jinhwang, Tainoung number 1 (TN1), Irwin, Yuwen, Haden, and Tu, were collected from a local grocery market in Xinhua District, Tainan City, Taiwan. Samples of peels were separated manually and the peels were oven-dried till the moisture content reached 4–7% (wet basis). The dried peel samples were crumbled and sieved using a 20-mesh screen, and the portion retained by the screen was collected and compressional-puffed according to the technique developed previously [27]. Compressional-puffing applies a mechanical compression force of approximately 5 kg/cm^2 to the sample three times before puffing, which can account for the difference between compressional-puffing and the conventional puffing gun process. The puffing temperatures were set at 220°C, and the corresponding pressure level inside the chamber was found to be 11 kg/cm^2 (Table 1). The NP and CP peel samples were ground and sieved using a 20-mesh screen. The portion passing through the screen was collected and then the bioactive compounds were extracted by either ethanol or hot water as shown in Figure 2. In the preliminary experiment, we extracted puffed peel sample directly using 70°C hot water and found that the extract, after being dried, exhibited a stone-like hard structure, which stuck tightly to the inner surfaces of the container and was difficult to dislodge. Thus, the 70°C hot water extraction condition was not adopted in the present study. After extraction, four peel extracts, namely, NPWE (nonpuffed water extract), CPWE (compressional-puffed water extract), NPEE (nonpuffed ethanol extract), and CPEE (compressional-puffed ethanol extract), were obtained according to their puffing pretreatments and extraction methods for each mango cultivar (Figure 2). The yields of these extracts are indicated in Table 1. In the comparison of extraction yields among different mango varieties for these four extracts, it was found that the yields of extracts for the tested mango cultivars were similar, except that Haden and Tu cultivars had relatively lower extraction yields. Thus, the peels of Jinhwang, TN1, Irwin, and Yuwen cultivars with higher yields of extracts would have advantages for further commercial production. It was reported that compressional-puffing could primarily rupture the structure of the puffed samples and then augment the extraction yield of crude fucoidan from brown algae [27, 28] and increase the extraction yields of total phenolics and total flavonoids from pine needles [29, 30]. In the present study, we also found that compressional-puffing could rupture the structure of mango peel (data not shown) and increase the extraction yields in both CPWE and CPEE as compared to NPWE and NPEE, respectively (Table 1). Therefore, compressional-puffing can also be effectively used in mango peels to facilitate the release of bioactive compounds by simple extraction operations. A comparison of the extraction yields between water and ethanol extractions revealed that water extraction tended to have higher yields of extracts as compared to ethanol extraction. A higher yield of extract has the potential for commercialized production. In addition, previous reports revealed that the composition of mango peel extract is complicated, and it may contain polyphenols, flavonoids, carotenoids, vitamin E, vitamin C, pectin, unsaturated fatty acids, and other biologically active components that positively influence health [25, 37–39]. Mango peel extract has also exhibited biological functions such as antioxidant properties [25, 39] and inhibition of HeLa human cervical carcinoma cell proliferation [38]. Generally, phenolic compounds are the major bioactive components of mango peels [18] and these have exhibited antioxidant activity and an antiproliferative effect on HeLa cells [25, 37–39]. Thus, the phenolic compound composition in our mango peel extracts and their effects on biological functions warrant further examination. Taken together, peel extracts from Jinhwang, TN1, Irwin, and Yuwen cultivars had higher extraction yields than those of Haden and Tu cultivars. Compressional-puffing pretreatment resulted in a worthwhile incremental increase in the extraction yields of mango peel extracts. Water extraction tended to have higher yields of extracts as compared to ethanol extraction, which would be beneficial in commercialized production. The phenolic compound composition and biological functions of mango peel extracts require further characterization.

3.2. Polyphenol Contents and Free Radical-Scavenging Activities of Peel Extracts from Various Mango Cultivars. Phenolic compounds are reported to be the major bioactive components that exist in mango peels [18]. In the present study, four peel extracts (NPWE, CPWE, NPEE, and CPEE) from six mango cultivars were utilized to determine their polyphenol contents by the Folin-Ciocalteu colorimetric method. The results presented in Table 2 suggest that peel extracts from the TN1 cultivar possessed the highest amount of total phenolic compounds as compared to other peel extracts. Thus, it is reasonable to postulate that peel extracts of the TN1 may exhibit high biological activities, and therefore further investigation is warranted. Moreover, a comparison of the polyphenol contents between NPWE and CPWE in all mango cultivars revealed that polyphenol content of CPWE was higher than that of NPWE (Table 2), indicating that compressional-puffing could increase the polyphenol content of water extracts in all mango cultivars. However, in the case of ethanol extracts, only CPEEs from Jinhwang and TN1 had higher polyphenol contents than those of NPEEs from Jinhwang and TN1 (Table 2). Moreover, for all mango cultivars, polyphenol contents of ethanol extracts were higher than those of water extracts (Table 2), indicating that ethanol extraction was effective in the extraction of polyphenols. Polyphenols are well known to exhibit antioxidant activity due to their ability to scavenge free radicals via hydrogen donation or electron donation and the reactivity of the phenol moiety [40]. Accordingly, the antioxidant capacities of NPWE, CPWE, NPEE, and CPEE of six mango peels were characterized using DPPH and ABTS radical-scavenging assays. DPPH is a stable free radical and is widely used to evaluate the antioxidant activity in a relatively short time compared to other methods [41]. The SC_{50} values (concentration of mango peel extract capable of scavenging 50% of DPPH radical) of the peel extracts (NPWE, CPWE, NPEE, and CPEE) from six mango cultivars for DPPH

Table 2: Polyphenol content, DPPH radical-scavenging activity, and ABTS radical cation-scavenging activity of extracts from various Taiwanese mango peels.

Polyphenols (%)*	NPWE	CPWE	NPEE	CPEE
Jinhwang cultivar	$1.40 \pm 0.11^{aA****}$	2.11 ± 0.24^{aB}	5.31 ± 0.25^{aC}	9.13 ± 0.16^{cD}
Tainoung number 1 cultivar	15.9 ± 0.9^{eA}	16.6 ± 1.1^{eA}	23.5 ± 0.4^{eB}	28.5 ± 0.7^{eC}
Irwin cultivar	3.09 ± 0.18^{bA}	2.92 ± 0.19^{aA}	7.06 ± 0.29^{bC}	5.07 ± 0.11^{aB}
Yuwen cultivar	2.36 ± 0.25^{bA}	4.63 ± 0.90^{bB}	7.21 ± 0.05^{bD}	6.26 ± 0.05^{bC}
Haden cultivar	6.41 ± 0.20^{dA}	7.31 ± 0.19^{cB}	18.9 ± 0.3^{dD}	13.4 ± 0.3^{dC}
Tu cultivar	5.25 ± 0.27^{cA}	10.1 ± 1.6^{dB}	14.7 ± 0.2^{cD}	13.0 ± 0.5^{dC}
DPPH, SC_{50} values (μg/ml)**	NPWE	CPWE	NPEE	CPEE
Jinhwang cultivar	499 ± 7^{fD}	368 ± 13^{fC}	197 ± 12^{dA}	251 ± 0^{fB}
Tainoung number 1 cultivar	57.0 ± 2.2^{aC}	67.0 ± 2.2^{aD}	46.0 ± 1.4^{aB}	41.7 ± 1.3^{aA}
Irwin cultivar	368 ± 11^{eC}	255 ± 2^{dB}	195 ± 9^{dA}	222 ± 8^{eA}
Yuwen cultivar	324 ± 3^{dD}	303 ± 5^{eC}	165 ± 5^{cA}	206 ± 4^{dB}
Haden cultivar	124 ± 3^{bD}	101 ± 5^{bC}	69 ± 5^{bA}	86 ± 4^{bB}
Tu cultivar	183 ± 2^{cD}	158 ± 5^{cC}	78.3 ± 4.9^{bA}	96.7 ± 4.7^{cB}
Vitamin C	11.3 ± 0.1			
ABTS, SC_{50} values (μg/ml)***	NPWE	CPWE	NPEE	CPEE
Jinhwang cultivar	186 ± 0^{eD}	139 ± 0^{eC}	70.0 ± 0.0^{fB}	54.0 ± 3.3^{cA}
Tainoung number 1 cultivar	28.2 ± 3.8^{aC}	23.3 ± 0.5^{aB}	15.7 ± 0.9^{aA}	13.0 ± 0.8^{aA}
Irwin cultivar	113 ± 7^{cC}	101 ± 6^{dC}	59.0 ± 0.8^{eA}	76.3 ± 2.8^{eB}
Yuwen cultivar	137 ± 2^{dC}	102 ± 2^{dB}	52.0 ± 0.9^{dA}	62.0 ± 1.7^{dA}
Haden cultivar	55.3 ± 1.7^{bC}	37.3 ± 2.4^{bB}	27.3 ± 0.9^{cA}	30.7 ± 1.7^{bA}
Tu cultivar	115 ± 5^{cD}	77.3 ± 3.4^{cC}	24.7 ± 1.3^{bA}	34.0 ± 1.6^{bB}
Vitamin C	3.58 ± 0.07			

*Polyphenols (%) = $(g/g_{\text{solid extract, dry basis}}) \times 100$. **$SC_{50}$ values (concentration of mango peel extract capable of scavenging 50% of DPPH radical) for DPPH radical-scavenging of different mango peel extracts. ***SC_{50} values (concentration of mango peel extract capable of scavenging 50% of ABTS cation radical) for ABTS radical cation-scavenging of different mango peel extracts. ****Values are mean ± SD ($n = 3$); values in the same column with different letters (in a, b, c, d, e, and f) and in the same row with different letters (in A, B, C, and D) are significantly different ($p < 0.05$).

radical-scavenging activity are presented in Table 2. As shown in Table 2, all peel extracts from TN1 exhibited the most DPPH radical-scavenging activity as compared to other mango cultivars, and the most potent was CPEE of TN1 with an SC_{50} value of 41.7 ± 1.3 μg/ml. Kim et al. (2010) reported that the SC_{50} value of the DPPH radical-scavenging activity of Irwin mango peel ethanol extract was about 40 μg/ml [18], which was similar to the SC_{50} value of CPEE of TN1 reported here. A comparison of the DPPH radical-scavenging activities of the CPWE group with those of the NPWE group revealed that compressional-puffing could increase the DPPH radical-scavenging activities of peel extracts (Table 2). Moreover, DPPH radical-scavenging activity of all EE groups (including NPEE and CPEE) was greater than that of the WE groups (NPWE and CPWE), which appeared to be positively correlated with the higher polyphenol amount in the EE groups as shown in Table 2. Regarding the scavenging activity of $ABTS^{\bullet+}$, the relatively long-lived $ABTS^{\bullet+}$ was decolorized during the reaction with hydrogen-donating antioxidant [42]. The SC_{50} values (concentration of mango peel extract capable of scavenging 50% of ABTS radical cation) of the peel extracts (NPEE, CPEE, NPWE, and CPWE) from six mango cultivars for ABTS radical cation-scavenging activity are also presented in Table 2. The results show that, among the extracts from six mango cultivars, peel extracts from the TN1 exhibited the most ABTS radical cation-scavenging activity, and the SC_{50} value for the most potent CPEE of TN1 was 13.0 ± 0.8 μg/ml. Kim et al. (2010) reported that the SC_{50} value of the ABTS radical cation-scavenging activity for Irwin mango peel ethanol extract was about 200 μg/ml [18], which was less effective in ABTS radical cation-scavenging capacity as compared to our CPEE of TN1. Regarding NPWE and CPWE, compressional-puffing could increase the ABTS radical cation-scavenging activity in CPWE of mango cultivars, which was similar to the finding for DPPH radical-scavenging activity. All EEs (including NPEE and CPEE) had greater ABTS radical cation-scavenging activity compared to WEs (including NPWE and CPWE) (Table 2). To better understand the relationship between polyphenol contents and free radical-scavenging activities of peel extracts, a correlation plot was performed and the results are shown in Figure 3. A high correlation between the polyphenol contents of peel extracts and their corresponding free radical-scavenging activities (DPPH and ABTS radical-scavenging activities) was found in NPWE, CPWE, NPEE, and CPEE, which was also consistent with previously reported observations [43]. In summary, peel extracts from the TN1 had the highest amount of total phenolic compounds and possessed the most DPPH and ABTS free radical-scavenging activities. For all water extracts, compressional-puffing had a tendency to increase the contents of total phenolic compounds in CPWEs and resulted in an incremental increase in free

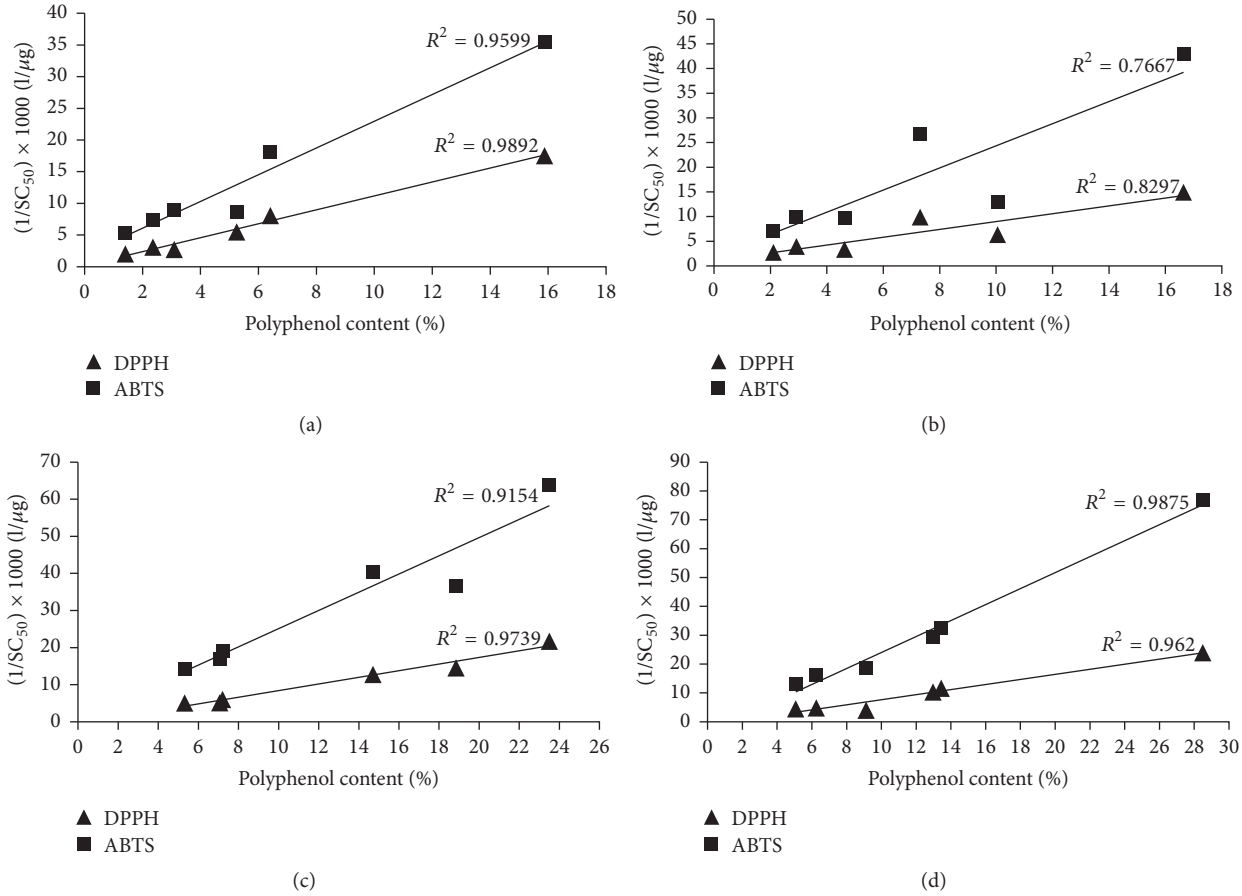

Figure 3: Association between polyphenol content and DPPH/ABTS radical-scavenging activities of mango peel extracts. (a) NPWE; (b) CPWE; (c) NPEE; (d) CPEE. SC_{50}: concentration for scavenging 50% of DPPH or ABTS free radicals.

radical-scavenging activities as compared to NPWEs. For all ethanol extracts, only CPEE of TN1 had a higher content of total phenolic compounds and possessed higher free radical-scavenging activities as compared to NPEE of TN1. Moreover, for all extracts, ethanol extracts generally had a higher amount of total phenolic compounds and caused greater free radical-scavenging activities as compared to water extracts. Therefore, in summary, both CPWE and CPEE of the TN1 cultivar warrant further analyses of the phenolic compound composition and storage stability of their antioxidant capacity, as well as their anti-inflammatory and antibacterial activities.

3.3. Analysis of Phenolic Compound Composition, Storage Stability of Antioxidant Capacity, Anti-Inflammatory Activity, and Antibacterial Activity in CPWE and CPEE of TN1 Cultivar. Peel extracts of TN1 cultivar have the highest amount of total phenolic compounds and the most free radical-scavenging activities. Moreover, CPWE and CPEE from TN1 had higher extraction yields and greater polyphenol contents as compared to NPWE and NPEE from TN1. Therefore, the phenolic compound composition of CPWE and CPEE from TN1 was analyzed by RP-HPLC coupled with UV-vis detector. The results are shown in Figure 4 and Table 3. In Figure 4, it can be seen that seven phenolic compounds, namely, gallic acid, pyrogallol, chlorogenic acid, *p*-hydroxybenzoic acid, *p*-coumaric acid, ECG, and CG, were tentatively identified in CPWE and CPEE of TN1 by HPLC analysis. Table 3 shows the quantitative data of phenolic compound composition in the CPWE and CPEE of TN1. It was found that both CPWE and CPEE of TN1 contained large amounts of *p*-hydroxybenzoic acid, gallic acid, and pyrogallol and smaller amounts of chlorogenic acid, CG, *p*-coumaric acid, and ECG. A comparison of the phenolic compound composition in

Table 3: Phenolic compound composition in the CPWE and CPEE of Tainoung number 1 cultivar.

Compound	Tainoung number 1 cultivar	
	CPWE (mg/100 g)*	CPEE (mg/100 g)
p-Hydroxybenzoic acid	1863 ± 318	3313 ± 2
Gallic acid	579 ± 72	1052 ± 1
Pyrogallol	566 ± 55	930 ± 90
Chlorogenic acid	125 ± 8	245 ± 7
Catechin gallate (CG)	125 ± 43	189 ± 52
p-Coumaric acid	68.9 ± 9.4	131 ± 0
Epicatechin gallate (ECG)	32.0 ± 3.9	50.8 ± 7.0

*The concentration of phenolic compound is expressed as mg/100 g peel weight, dry basis.

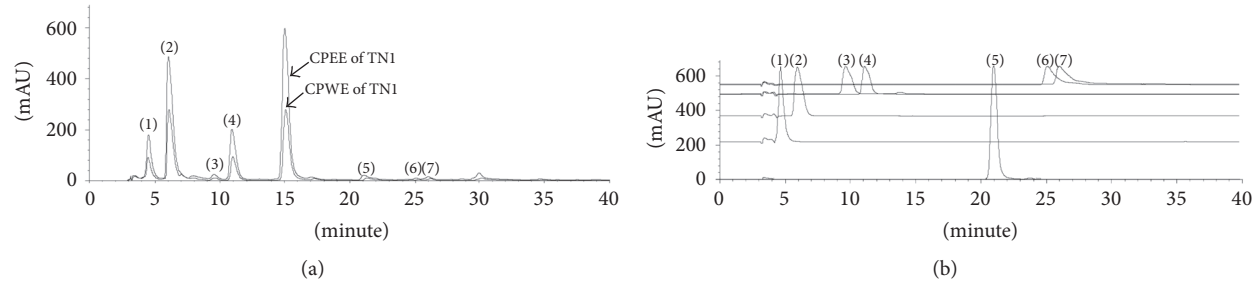

Figure 4: (a) High-performance liquid chromatography of peel extracts (CPWE and CPEE) of Tainoung number 1 cultivar; (b) high-performance liquid chromatography of polyphenol standards: gallic acid (1), pyrogallol (2), chlorogenic acid (3), *p*-hydroxybenzoic acid (4), *p*-coumaric acid (5), ECG (6), and CG (7).

CPWE and CPEE revealed that CPEE of TN1 had greater amounts of *p*-hydroxybenzoic acid, gallic acid, pyrogallol, chlorogenic acid, CG, *p*-coumaric acid, and ECG than those of CPWE (Table 3). These results are consistent with the data shown in Table 2, which illustrates that CPEE of TN1 has higher total phenolic compounds compared to CPWE of TN1. We found that *p*-hydroxybenzoic acid was the predominant phenolic compound detected (up to 3313 ± 2 mg/100 g peel weight, dry basis) in CPEE of TN1, and the results were also supported by other studies reporting that *p*-hydroxybenzoic acid could be detected in the extract of mango cultivar [44]. The concentrations of gallic acid for CPWE and CPEE of TN1 were recorded as 579 ± 72 and 1052 ± 1 mg/100 g peel weight, dry basis, respectively. These data are comparably higher than those reported previously for the ethanol extract of mango peel, with an average gallic acid concentration of 152.20 ± 0.14 mg/100 g mango peel, dry weight [45]. Previous studies suggested that pyrogallol can be detected in the ethanolic extract of mango kernel (the mango tested was purchased from an Egyptian local market) with a concentration of 1337.9 ± 0.31 mg/100 g mango kernel, dry weight, but it was absent in the ethanolic extract of mango peel [45]. However, we found that pyrogallol could be detected in CPWE and CPEE of TN1 with a concentration of 566 ± 55 and 930 ± 90 mg/100 g peel weight, dry basis, respectively. We speculate the reason may be due to differences between the tested mango varieties. Structurally, *p*-hydroxybenzoic acid, gallic acid, and pyrogallol are monophenolic compounds, which exhibit antioxidant activity owing to their hydrogen-donating or electron-donating properties [46]. Therefore, the high free radical-scavenging activities of CPWE and CPEE of TN1 may be attributed to the high contents of *p*-hydroxybenzoic acid, gallic acid, and pyrogallol. Besides phenolic compounds, previous studies reported that a synergistic effect of combinations of phytochemicals may also result in beneficial biological functions such as inhibition of proliferation of human cancer cells [38, 47]. Thus, the synergistic effects of constituents in CPWE and CPEE of TN1 with respect to their effects on biological functions warrant further investigation. The storage stability of antioxidant agent is important with respect to its potential industrial application. Here, we evaluated the storage stability of vitamin C, CPWE of TN1, and CPEE of TN1 by DPPH radical-scavenging assay. The test sample powders were redissolved in double-distilled water at various concentrations and the sample solutions were stored at room temperature for 1, 2, 4, and 8 hours, and then the corresponding DPPH radical-scavenging activities were determined. The data presented in Figure 5(a) suggest that the well-known natural antioxidant vitamin C would dramatically reduce its DPPH radical-scavenging activity after 1–8 hours' storage. However, the DPPH radical-scavenging activities in either CPWE of TN1 or CPEE of TN1 were not obviously changed after 1–8 hours' storage (Figures 5(b) and 5(c)). These findings clearly indicate that the peel extracts of mango exhibited a high storage stability in terms of antioxidant activity. Fruit polyphenols have been reported to be related to immunomodulatory and anti-inflammatory properties via *in vitro* and animal studies [13]. NO is an inflammatory mediator induced by inflammatory cytokines or bacterial LPS in various cell types including macrophages [48]. Samples with NO inhibitory activity thus have the potential to possess anti-inflammatory activity. CPEE and CPWE from TN1 were tested for their anti-inflammatory activities by investigating their effects on NO production in LPS-induced RAW264.7 macrophages. Neither CPEE nor CPWE obviously affected the viability of RAW264.7 cells at the 6.25–25 μg/ml concentrations that were tested, in the presence of 1 μg/ml LPS (Figure 6(a)). As shown in Figure 6(b), when RAW264.7 cells were treated with 1 μg/ml LPS, the NO production was increased from 3.11 ± 0.25 μM to 12.8 ± 0.1 μM. Moreover, when RAW264.7 cells were treated with 1 μg/ml LPS in the presence of various concentrations of CPEE, it was found that NO production was significantly decreased from 12.8 ± 0.1 μM to 9.54 ± 0.08 μM, whereas in the presence of various concentrations of CPWE, NO production was only slightly reduced. These results indicate that CPEE of TN1 had apparent anti-inflammatory activity, and thus it may have potential as a natural and safe agent in the protection of human health by modulating the immune system. Previous studies demonstrated that extracts with high polyphenol content exhibited high antibacterial activity [49]. As such, we evaluated the antibacterial activity of CPEE and CPWE of TN1 by the diffusion disc method. Five bacteria, three Gram-negative bacteria (*E. coli*, *S. typhimurium*, and *V. parahaemolyticus*) and two Gram-positive bacteria (*S. aureus* and *B. cereus*), were adopted to assess the antibacterial properties. As can be seen in Figures 7(a)–7(f), both CPEE and CPWE of TN1 exhibited antibacterial activities against the

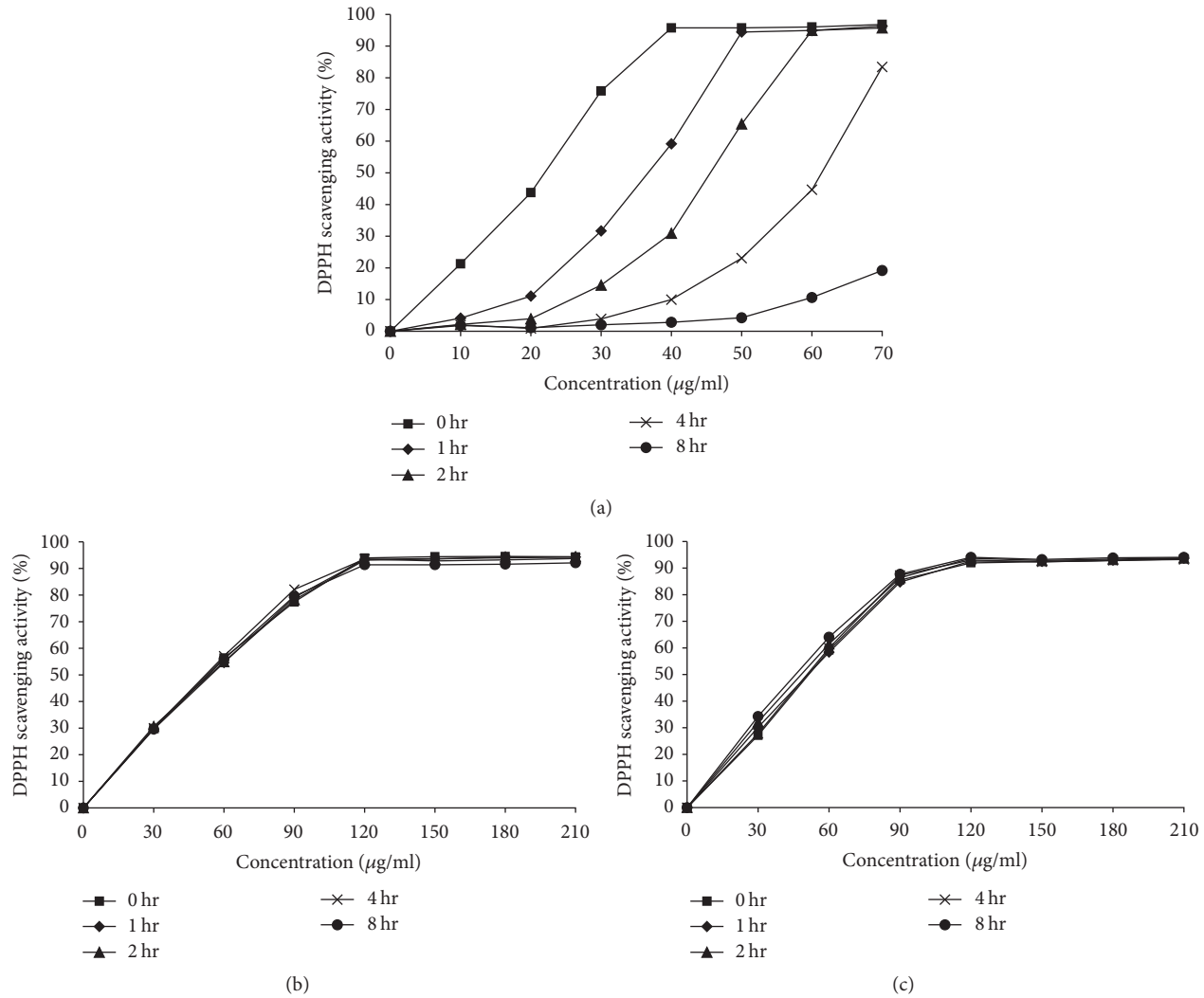

Figure 5: DPPH scavenging activities of vitamin C, CPWE of TN1, and CPEE of TN1 under different storage times. (a) Vitamin C; (b) CPWE of TN1; (c) CPEE of TN1.

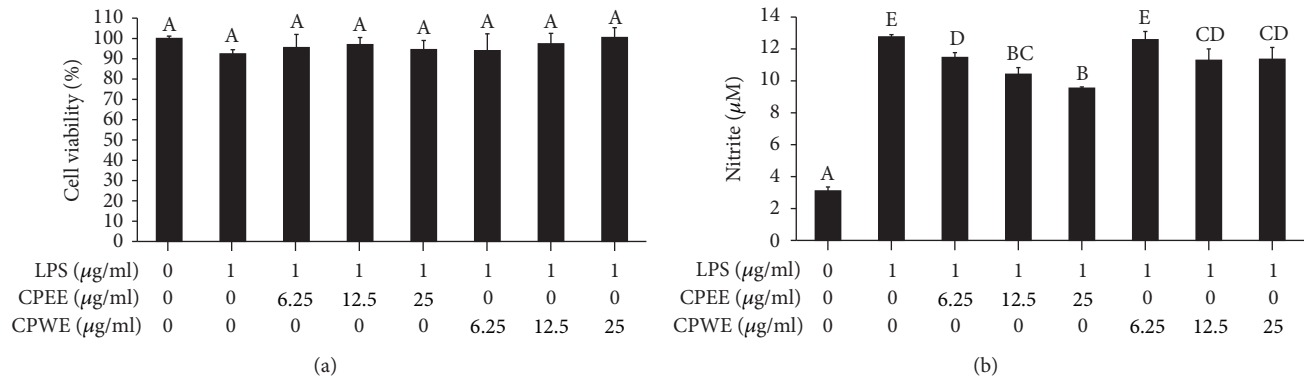

Figure 6: (a) Effects of CPEE of TN1, CPWE of TN1, and LPS on cell viability of RAW 264.7 cells. (b) Effects of CPEE of TN1, CPWE of TN1, and LPS on NO secretion in RAW 264.7 cells. The data are the means ± SD of triplicate samples. Bars with different letters are significantly different ($p < 0.05$).

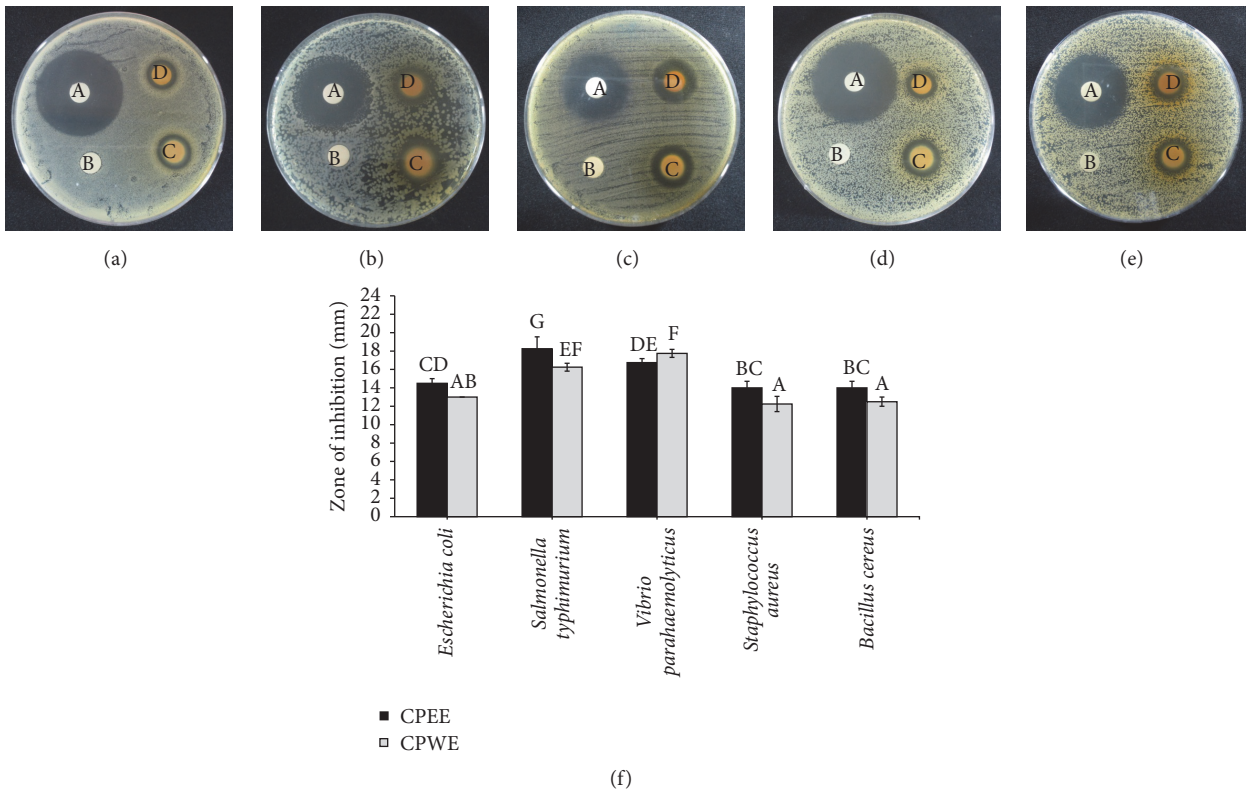

Figure 7: Zone of inhibition of CPEE of TN1 and CPWE of TN1 at concentration of 4%, w/v, in 0.05 M acetate buffer, pH 6.0, against (a) *Escherichia coli*, (b) *Salmonella typhimurium*, (c) *Vibrio parahaemolyticus*, (d) *Staphylococcus aureus*; and (e) *Bacillus cereus*. In each dish, A, B, C, and D represent antibiotic, acetate buffer, CPEE of TN1, and CPWE of TN1, respectively. (f) The bar graph summarizes the four separate antibacterial experiments and shows the zone of inhibition according to treatments. Values are expressed as the mean ± SD ($n = 4$). The means that have at least one common letter do not differ significantly ($p > 0.05$).

five bacteria tested. The Gram-negative bacteria were more sensitive than Gram-positive ones to CPEE and CPWE of TN1 (Figure 7(f)). In addition, for these five bacteria, except *V. parahaemolyticus*, CPEE exhibited higher antibacterial activity compared to CPWE. These results may be attributed to the higher polyphenol content detected in CPEE (Table 2), which is also consistent with previous findings [50]. Interestingly, for *V. parahaemolyticus*, CPEE had less antibacterial activity compared to CPWE. We speculate the reason may be due to the presence of 3% NaCl in the medium of *V. parahaemolyticus*. However, further experimental studies are needed to elucidate the mechanism of action. In summary, the present study demonstrated that CPEE and CPWE from TN1 had high amounts of phenolic compounds, possessed good and stable free radical-scavenging activities, and exhibited anti-inflammatory and antibacterial activities. CPEE of TN1 exhibited the most antioxidant, anti-inflammatory, and antibacterial properties and thus has potential for use in the food, cosmetics, and nutraceutical industries.

4. Conclusion

In this study, we employed a compressional-puffing pretreatment process and two extraction methods to extract bioactive compounds from six Taiwanese mango peels. The compressional-puffing process increases the extraction yields and polyphenol contents of peel extracts. Ethanol extracts of peels had higher amounts of total phenolic compounds and greater free radical-scavenging activities as compared to water extracts of peels. The polyphenol contents of extracts positively correlated to the free radical-scavenging activities of extracts. Among these extracts, CPEE of TN1 exhibited the most antioxidant, anti-inflammatory, and antibacterial properties. Thus it is suggested as a natural, safe, and stable antioxidant agent with anti-inflammatory and antibacterial properties, which may have a wide range of applications in food, cosmetics, and nutraceuticals. Future studies on the polyphenol composition and biological activities of mango peel extracts after an *in vitro* digestion as well as investigations of the *in vivo* biological activities of mango peel extracts are warranted.

Conflicts of Interest

The authors have no conflicts of interest to declare.

Authors' Contributions

Chun-Yung Huang and Chia-Hung Kuo contributed equally to this work.

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Research Article

Chemical Composition and Antioxidant Activities of Five Samples of *Prunus mume* Umezu from Different Factories in South and East China

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This paper investigated chemical composition and antioxidant activities of umezu, pickling liquid of *Prunus mume*, from different factories in South and East China. The organic acid and phenolic acid profiles were also analyzed. Results showed that umezu was rich in organic acids and extremely sour as *P. mume* fruit in addition to its high NaCl level (≥20%). Total acid in umezu was more than 43.78 g/L in which main organic acids were citric acid and malic acid. Umezu contained more than 250.54 mg GAE/L total phenolic in which dominant phenolic acids were hydroxycinnamic acid derivatives. Umezu exhibited powerful antioxidant activities in ORAC, ABTS, DPPH, and FRAP assays. Reducing sugar, phenolic compounds, and antioxidant activities of umezu were affected by sample origins and fruit cultivars. Given its rich flavor components and high antioxidant activity, umezu could serve as a new dietary supplement or a natural preservative in food industry.

1. Introduction

Prunus mume, also known as Mei or green plum in China, mume or Japanese apricot in Japan, and Maesil in Korea, is a deciduous tree of the Rosaceae family [1]. The fruit of *P. mume*, weighing 12.0–30.0 g freshly, green in immature and yellow in mature stage as well as rich in organic acids, edible fibre, minerals, and phenolic compounds, is known to have good medicinal effects on stomach and intestine disorders [2]. Unlike many other fruits which are mainly consumed freshly, most *P. mume* fruits should be processed before consumption, due to presence of cyanoglycoside, prunasin, and amygdalin and very high organic acid content [3].

Traditionally, *P. mume* fruit was firstly pickled with salt or smoked to dry and then processed to preserved fruit or used as medicinal material [4]. For example, the pickled *P. mume* fruit (named umeboshi in Japan) has been widely used as a food garnish, sauce, juice, and liquor in China, Japan, Korea, and other Southeast Asian countries. Besides, smoked and dried immature *P. mume* fruit, *Fructus mume* (Chinese name "Wu Mei"), has been traditionally used as a medical alternative for its antitussive, expectoration, antiemetic, antidiarrheal, anthelmintic, and antipyretic actions [5]. In addition, in many other Asian countries, mainly in Japan, a few of these processed products of *P. mume* fruit, such as umeboshi and *bainiku ekisu* (concentrated juice of *P. mume* fruit), had been used as a folk remedy for fatigue, diarrhea, and fever [6].

Even though a growing number of new technologies have been developed, salt-pickling remains the dominant method for *P. mume* fruit processing. During pickling of *P. mume* with salt, a large amount of liquid named Meilu in China and umezu in Japan is produced. Owing to its extreme salty and sour taste, umezu is mostly disposed as sewage, causing environment pollution. Umezu, composed of salt and liquid from *P. mume* fruit, is a natural source with great potential application. To realize the industrial utilization of umezu, its nutritional values, functional properties, and potential disadvantages should be characterized. To the best of our knowledge, there are no previous reports on umezu compositional characteristics, as well as further development. In this paper, five *P. mume* pickling liquid (umezu) samples

were collected from different factories in the South and East China, and their chemical constituents, including the content and proportion of organic acids and phenolic acids, and antioxidant activities were investigated.

2. Materials and Methods

2.1. Chemicals and Reagents. Folin-Ciocalteu phenol reagent, Trolox® (6-hydroxy-2,5,7,8-tetramethylchroman-2-carboxylic acid), 2,4,6-tripyridyl-s-triazine (TPTZ), 2,2′-azinobis (3-ethylbenzothiazoline-6-sulfonic acid) diammonium salt (ABTS), 2,2′-azobis-(2-amidinopropane) dihydrochloride (ABAP), and 3′,6′-dihydroxyspiro[isobenzofuran-1(3H), 9′-(9H)-xanthene]-3-one disodium salt (FL) were purchased from Sigma Chemical Co. (St. Louis, MO, USA). Gallic, chlorogenic, neochlorogenic, cryptochlorogenic, caffeic, p-coumaric, protocatechuic, vanillic, and ferulic acids, catechin, and epicatechin were purchased from Biopurify Phytochemicals Ltd (Chengdu, China). HPLC-grade acetic acid and acetonitrile were obtained from Fisher (Suwanee, GA, USA). All other chemicals used were of analytical grade or above.

2.2. Umezu Samples. Two umezu samples (umezu of white mume and green mume) were acquired from Zhaoan (Fujian Province, China), while the other three samples were from Puning, Raoping, and Luhe (Guangdong Province, China), respectively, in May 2015. Both Baifen Mei and Qingzhu Mei were two dominating cultivars of *P. mume* planted in China. All umezu samples were stored in pots made of polyethylene terephthalate (PET) under 4°C until test.

2.3. Proximate Composition. Umezu samples were centrifuged at 3130 ×g for 10 min before test. A benchtop refractometer (RFM340, Bellingham and Stanley, UK) was used to acquire soluble solids content (SSC). The pH value was assayed with a pH meter (PB -20, Sartorius, Göttingen, Germany). The NaCl content was determined following the Chinese National Standard (GB/T 12457-2008). TA content was determined according to AOAC method 942.15 [7]. Aliquot of 2.0 mL sample was diluted to 100 mL with ultrapure water, mixed, and then titrated with 0.1 M NaOH to an end point of pH 8.2. TA was calculated as % citric acid. The total phenolic content was analyzed by the Folin-Ciocalteu (FC) colorimetric method [8]. Briefly, 0.3 mL neutralized umezu was mixed with 3.7 mL of distilled water and 2.0 mL of FC reagent for 6 min. A 2.0 mL of 7% aqueous sodium carbonate solution was added to make up a total volume of 8.0 mL. The mixture was incubated for 90 min at 25°C. The absorbance was detected at 760 nm using a Shimadzu UV-1800 spectrometer (Shimadzu Inc., Kyoto, Japan) using gallic acid as the standard. Total phenolic content was expressed as mg gallic acid equivalents (GAE) per 1000 mL sample. All the measurements were done in triplicate, and the average values were used.

2.4. Analysis of Organic Acids by HPLC. Determination of organic acid profile of umezu was conducted by high performance liquid chromatography (HPLC) as previously described [9]. An Agilent 1200 series HPLC system (Agilent Technologies, Palo Alto, California, USA) equipped with VWD detector was used to analyze organic acids. The system was run at a flow rate of 0.1 mL/min. The samples were analyzed on an Aminex HPX-87H ion exchange column (300 mm × 7.8 mm) protected with a Bio-Rad guard cartridge (30 mm × 4.6 mm) composed of the same stationary phase and the column (not the guard cartridge) was thermostated at 35°C. Mobile phase was 5.0 mM H_2SO_4 in dH_2O. ChemStation Rev. A.10.02 software (Agilent Technologies) was used for data acquisition, peak integration, and standard calibration. Umezu samples were diluted with mobile phase (1 : 50). The injection volume was 20 μL. Organic acids were detected with the VWD detector at 210 nm. Samples were quantified by comparing the retention times and peak areas with the known authentic standards. The results were expressed as g/L.

2.5. Analysis of Phenolic Compounds by HPLC. Determination of the phenolic compounds was conducted by HPLC as previously described [10]. All samples were analyzed on an Agilent 1260 HPLC system (Agilent Technologies, Palo Alto, California, USA) equipped with DAD detector and autosampler, using a 250 × 4.6 mm, 5 μm Agilent Zorbax SB-C18 column (Palo Alto, CA, USA). The conditions used were as follows: column temperature 30°C; injection volume 20 μL; total HPLC run time 50 min; mobile phase 0.4% aqueous solution acetic acid (solution A); and acetonitrile (solution B). The mobile phase was programmed as follows: 0–40 min, solution B 5–25%; 40–45 min, solution B 25–35%; 45–50 min, solution B 35–50%. The mobile phase was pumped at a constant flow rate of 1.0 mL/min. Detection was set at 280 nm. Before analysis, all samples were filtered through a 0.25 μm membrane filter (Millipore, Billerica, MA, USA). Samples were quantified by comparing the retention times and peak areas with authentic standards. The results were expressed as mg/L.

2.6. Antioxidant Activity Determined by ORAC Assay. The oxygen radical absorbance capacity (ORAC) assay was carried out according to a modified method from our laboratory (Zhang et al., 2010). The assay was performed using black-walled 96-well plates (Corning Scientific, Corning, NY, USA). The diluted umezu was prepared with 75 mM phosphate buffer (pH 7.4). The final reaction consisted of 20 μL of umezu or 20 μL of Trolox standard (range = 6.25–50 μM) and 200 μL of fluorescein (final concentration 0.96 μM). The mixture was incubated at 37°C for 10 min. Twenty μL of 119 mM ABAP was then added to each well using a multichannel pipette. Fluorescence intensity was measured using a Fluoroskan Ascent FL plate-reader (Thermo Labsystems, Franklin, MA, USA) at wavelengths of 485 nm for excitation and 538 nm for emission for 35 cycles every 4.5 min. ORAC was expressed as μM Trolox equivalents per 1000 mL.

2.7. Antioxidant Activity Determined by ABTS Assay. The ABTS radical scavenging activity assay was carried out as previously described [11]. Briefly, ABTS was dissolved in distilled water to a concentration of 7 mM. The ABTS stock solution was then obtained by mixing 7 mM ABTS water

solution with 2.45 mM potassium persulfate solution and leaving it to stand in the dark at room temperature for 12–16 h before use. The ABTS radical solution was diluted with methanol and adjusted to an absorbance of 0.700 ± 0.020 at 734 nm. The assay was performed using 96-well microplates. 0.10 mL of the diluted umezu was added to 2 mL of the ABTS radical solution for use in each reaction. The absorbance was measured at 734 nm. The ABTS antioxidant activity was expressed as μg/L Trolox equivalents per 1000 mL.

2.8. Antioxidant Activity Determined by FRAP Assay. The FRAP assay was carried out according to the method as previously described [12]. The working solution consisted of 25 mL 300 mM acetate buffer (5.1 g $CH_3COONa{\cdot}3H_2O$ and 20 mL of CH_3COOH pH 3.6), 2.5 mL TPTZ solution (10 mM TPTZ in 40 mM HCl), and 2.5 mL 20 mM $FeCl_3{\cdot}6H_2O$ solution which was incubated 10 min at 37°C before use. A 0.2 mL umezu was reacted with 2.8 mL of the working solution for 30 min in the dark at room temperature. Absorbance was detected at 593 nm using a Shimadzu UV-1800 spectrometer. Methanol was used as the control and the standard curve was established with Trolox. The FRAP antioxidant activity was expressed as mg Trolox equivalents (TE) per 1000 mL umezu of sample.

2.9. Antioxidant Activity Determined by DPPH Assay. The DPPH radical scavenging capacities of umezu were performed according to a previous report [13]. Two hundred μL of six serially diluted umezu or methanol (control) samples were added to 2.8 mL of methanolic solution of 70 μM DPPH. After shaking, the mixtures were placed in the dark at room temperature for 30 min. A spectrometer (Shimadzu UV-1800) was used to read their absorbance values at 515 nm. Antioxidant activity was calculated according to the following equation in which I% stands for inhibition percentage: $I\% = (\mathrm{Abs}_{\mathrm{control}} - \mathrm{Abs}_{\mathrm{sample}}) \times 100/\mathrm{Abs}_{\mathrm{control}}$. Sample concentration at which 50% DPPH scavenging effect was acquired was used to express median effective concentration (EC50) value. EC50 was calculated by constructing the percentage of DPPH scavenging versus log (phenolic concentration expressed as the volume of umezu) curves (mg/mL).

2.10. Statistical Analysis. All the analyses were performed in triplicate and results were presented as mean ± standard deviations of each sample. Different samples were analyzed with ANOVA followed by the SNK-q test to identify differences between values. A value of $p < 0.05$ was considered to be statistically different. All statistical analyses were performed using the SPSS statistical package version 13.0 (SPSS Inc., Chicago, IL, USA).

3. Results and Discussion

3.1. Proximate Composition. Traditional *P. mume* pickling process is practiced in a cubic pool as follows: one layer of fruit by one layer of salt until the pool is full; and then an inert cloth is used to cover the surface and a heavy item, usually stone, is put on the top. Salt is added at a dose of 250–300 kg salt per 1000 kg fresh *P. mume* fruit. It takes more than three months for the pickling process to occur. During pickling, water and soluble substances such as sugar, organic acid, mineral, and bioactive compounds effuse from the fruits while salt is intruded into the fruits until a balance is achieved at the end. The umezu yield is 45–50% of fresh fruits.

The chemical composition could directly reflect the physiochemical property of *P. mume* umezu [4]. Table 1 summarized the proximate composition of umezu samples from five different factories in two provinces in China. As expected, most umezu samples were high in salt and acid but low in sugar. They contain 33.00–34.50° Brix solid matter in which 20.36–22.01% is NaCl. Total titratable acid content is 4.55–5.44%, which makes them very acidic (pH 1.85–1.90). Reducing sugar content is only 5.18–8.56%. The ratio of sugar to titratable acid among those samples is only 1.14–1.57. Noteworthy, the solid and NaCl contents of umezu from different factories were not different indicating similar pickling processing including recipe was used. Among all samples, umezu from Luhe presented the lowest total acid (4.55%) and reducing sugar (5.18%) while that of other four samples had similar total acid and reducing sugar contents. As reported previously, the *P. mume* fruit was an important source of phenolic antioxidant and antimicrobial agent [14–16]. The total phenolic content ranged from 250.54 to 330.74 mg/L in this study. The different phenolic content among the five umezu samples might be ascribed to different fruit cultivars and/or growing conditions as fruit quality was affected by factors including weather, environment, plant management, and so on besides species [17]. Thus, *P. mume* fruit from different species or different growing origins would render umezu samples with different chemical compositions.

3.2. Organic Acid Profile. Organic acids are important flavor regulators for acidity regulation that could confer sour flavor, control microorganism growth, prevent browning, increase solubility of metal ion, improve appetite, promote digestion, and so on [6]. The characteristic sourness of *P. mume* fruits is caused by the presence of free organic acids in this fruit [9, 18]. Generally, the total titratable acid of *P. mume* fruits could be as high as 6.0–7.0% on a fresh weight basis, though its profile is diversified [2]. Organic acids, with high polarity, are hydrophilic compounds and very stable in solution [19]. Chen et al. [18] detected seven organic acids in *P. mume* by HPLC. Pan (2007) purified ten organic acids, including citric acid, malic acid, oxalic acid, glycolic acid, succinic acid, lactic acid, pyroglutamic acid, formic acid, acetic acid, and propionic acid from this fruit. During pickling of *P. mume* fruit with salt, they could easily penetrate the fruit into the pickling liquids, that is, umezu.

Typical organic acid chromatogram of umezu from different factories was presented in Figure 1 and the main organic acid contents were shown in Table 2. Seven organic acids were detected in the umezu samples, among which six organic acids were identified by HPLC. The major organic acids detected in this assay were citric acid (67.5%–74.3% of total acid) and malic acid (12.9%–18.9% of total acid), and other minor organic acids included oxalic, succinic, lactic, and acetic acid. The organic acid profile of umezu is consistent

Table 1: Proximate composition of umezu from different factories.

Umezu samples	pH	Solid content (°Brix)	NaCl (%)	Total acid (%)	Reducing sugar (%)	Total phenolic (mg/L)
Fujian green mume	1.81	34.03 ±0.04^a	22.01 ± 0.54^a	5.44 ± 0.24^a	8.56 ± 0.03^a	300.77 ± 0.37^b
Fujian white mume	1.90	33.69 ± 0.02^a	21.22 ± 0.15^a	5.18 ± 0.11^a	7.05 ± 0.05^b	250.78 ± 0.19^c
Luhe green mume	1.89	31.43 ± 0.11^a	20.36 ± 0.48^a	4.55 ±0.15^b	5.18 ±0.02^c	260.47 ± 0.85^c
Puning green mume	1.85	33.00 ± 0.02^a	20.44 ± 0.45^a	5.05 ± 0.11^a	7.14 ± 0.03^b	250.54 ± 0.56^c
Raoping green mume	1.86	34.50 ± 0.04^a	21.45 ± 0.67^a	5.42 ± 0.17^a	7.38 ± 0.05^b	330.74 ± 0.75^a

Means ± SD (n = 3) within each column followed by the same letter are not significantly different at the 5% level.

Table 2: Predominant organic acids of umezu from different factories (g/L).

Umezu samples	Oxalic acid	Citric acid	Malic acid	Succinic acid	Lactic acid	Acetic acid	Total
Fujian green mume	0.71 ± 0.16^a	36.93 ± 0.34^b	7.60 ± 0.63^b	1.01 ± 0.19^a	4.51 ± 0.17^a	1.30 ± 0.02^a	52.06 ± 1.14^a
Fujian white mume	0.36 ± 0.04^c	34.06 ± 3.54^c	9.54 ± 1.94^a	1.13 ± 0.04^a	4.20 ± 0.22^a	1.15 ± 0.08^b	50.44 ± 5.43^a
Luhe green mume	0.57 ± 0.06^b	30.89 ± 0.73^d	6.42 ± 0.12^c	1.26 ± 0.24^a	3.98 ± 0.20^b	0.66 ± 0.21^c	43.78 ± 0.73^b
Puning green mume	0.46 ± 0.03^c	36.27 ± 0.25^b	7.59 ± 0.85^b	0.90 ± 0.09^b	3.83 ± 0.41^b	0.95 ± 0.16^b	49.99 ± 0.03^a
Raoping green mume	0.61 ± 0.13^{ab}	38.13 ± 0.76^a	6.63 ± 0.16^c	1.21 ± 0.21^a	3.43 ± 0.81^c	1.34 ± 0.06^a	51.35 ± 1.75^a

Means ± SD (n = 3) within each column followed by the same letter are not significantly different at the 5% level.

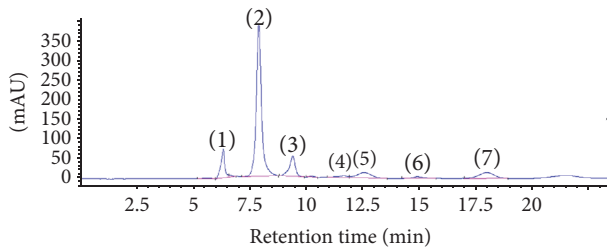

Figure 1: Representative chromatogram of organic acid in umezu (λ = 210 nm). (1) Oxalic acid; (2) citric acid; (3) malic acid; (4) succinic acid; (5) lactic acid; (6) acetic acid; (7) unknown.

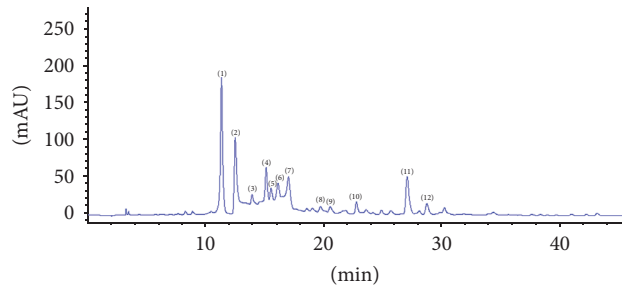

Figure 2: Representative chromatogram of phenolic compounds in umezu (λ = 280 nm). (1) Neochlorogenic acid; (2) unknown; (3) unknown; (4) chlorogenic acid; (5) unknown; (6) unknown; (7) cryptochlorogenic acid; (8) unknown; (9) caffeic acid; (10) unknown; (11) p-coumaric acid; (12) unknown.

with that of *P. mume* fruit reported by others [2, 19]. Citric acid and malic acid are the two most abundant acids, and they account for more than 90% of total acid content in *P. mume* fruit [18] and more than 85% of total acid content in umezu in this study (Table 2). Noteworthily, the differences of these two major organic acids (citric acid and malic acid) among all umezu samples were significant ($p < 0.05$). Except for the different growing environment and species, the fruit maturity might also be a major influence factor [4]. As revealed by Chung et al. [4], malic acid in the fruit is higher than citric acid during the early stage, while citric acid is much more abundant than malic acid during the late maturation stage. Based on Table 2 and Figure 1, it could be concluded that umezu, enriched in organic acids, exhibited potential values in seasoning products development.

3.3. Phenolic Compound Profile. Phenolic compound is another important index for umezu exploitation. Mitani et al. [20] reported that phenolics content in fully matured *P. mume* flesh was as high as 1% on a dry weight basis. Phenolic acids are considered to be anti-inflammatory, anticarcinogenic, and antimicrobial agents, as well as antioxidants [21, 22]. Total and individual phenolic compound contents of umezu samples were shown in Tables 1 and 3, respectively.

Phenolic composition and contents varied in umezu samples. Umezu from Raoping, Guangdong, presented the highest total phenolic content (330.74 mg/L), followed by umezu from Zhaoan, Fujian (300.77 mg/L), while the other three samples had similar total phenolic content. The individual phenolic compounds in umezu samples were assessed by HPLC/DAD and the typical phenolic chromatogram was shown in Figure 2. Five phenolic compounds were identified and quantified, namely, neochlorogenic acid, chlorogenic acid, cryptochlorogenic acid, caffeic acid, and p-coumaric acid. Neochlorogenic acid, chlorogenic acid, and cryptochlorogenic acid accounted for 58.3%–63.6%, 12.4%–18.1%, and 15.4%–20.5% of total quantified phenolic compounds, respectively. In addition, caffeic acid and p-coumaric acid were very low in umezu samples.

Table 3: Phenolic composition of umezu of *Prunus mume* from different factories (mg/L).

Umezu samples	Neochlorogenic acid	Chlorogenic acid	Cryptochlorogenic acid	Caffeic acid	p-Coumaric acid
Fujian green mume	161.45 ± 21.99^{a}	39.66 ± 11.99^{b}	39.87 ± 4.55^{b}	3.57 ± 0.39^{c}	9.25 ± 2.49^{b}
Fujian white mume	152.68 ± 33.75^{b}	39.58 ± 14.60^{b}	37.45 ± 7.32^{b}	3.29 ± 0.86^{c}	9.75 ± 3.05^{b}
Luhe green mume	106.64 ± 36.11^{c}	21.91 ± 8.62^{c}	36.18 ± 9.28^{b}	1.98 ± 0.02^{d}	9.58 ± 2.35^{b}
Puning green mume	147.48 ± 14.09^{b}	39.08 ± 9.18^{b}	37.13 ± 4.38^{b}	7.04 ± 0.88^{a}	9.26 ± 2.74^{b}
Raoping green mume	163.38 ± 48.04^{a}	50.79 ± 14.21^{a}	45.77 ± 12.28^{a}	4.77 ± 1.36^{b}	15.75 ± 3.62^{a}

Means ± SD ($n = 3$) within each column followed by the same letter are not significantly different at the 5% level.

Table 4: Antioxidant activities of umezu from different factories.

Umezu samples	ORAC (μmol TE/L)	ABTS (μg/L)	FRAP (μg/L)	DPPH (EC_{50}) (mg/mL)
Fujian green mume	12298.34 ± 145.66^{b}	104.67 ± 3.05^{b}	176.43 ± 3.89^{b}	66.00
Fujian white mume	7994.96 ± 266.62^{d}	94.52 ± 10.25^{c}	162.09 ± 9.16^{d}	43.67
Luhe green mume	10361.99 ± 173.19^{c}	24.96 ± 62.72^{d}	172.95 ± 2.44^{c}	56.83
Puning green mume	7741.43 ± 99.94^{d}	94.96 ± 25.21^{c}	162.09 ± 4.24^{d}	65.96
Raoping green mume	17433.72 ± 145.66^{a}	195.83 ± 26.44^{a}	186.43 ± 3.39^{a}	81.70

Means ± SD ($n = 3$) within each column followed by the same letter are not significantly different at the 5% level.

Mitani et al. [20] identified 5-O-caffeoylquinic acid (neochlorogenic acid) and 3-O-caffeoylquinic acid (chlorogenic acid) from the *P. mume* flesh, indicating that the majority of phenolics in *P. mume* fruit were hydroxycinnamic acid derivatives. Xia et al. [23] identified three chlorogenic acid isomers, namely, 3-O-caffeoylquinic acid, 5-*O*-caffeoylquinic acid, and 4-*O*-caffeoylquinic acid (cryptochlorogenic acid), from *P. mume* seeds and found 3-O-caffeoylquinic acid was of the highest level in these three isomers. In this study, we also detected the chlorogenic acid isomers in *P. mume* seeds but did not find them (data not shown). According to literatures and our analysis, it is likely that chlorogenic acid isomers in umezu were from *P. mume* flesh, not seeds. During pickling, phenolic compounds in *P. mume* flesh could permeate into umezu. Chlorogenic acid isomers are stable under acidic pH; therefore, they kept a high concentration after pickling was finished. Low level of caffeic acid and p-coumaric acid in umezu might be hydrolysate of chlorogenic acid isomers. Narita and Inouye [15] reported that chlorogenic acid could hydrolyze into phenolic acids, such as caffeic acid, cis/trans-p-coumaric acid, and ferulic acid.

3.4. Antioxidant Activities. *P. mume* fruits contain multiple antioxidants, including organic acids, isoflavones, and phenolic acids [24]. Antioxidant activities of umezu samples were compared in terms of ORAC, $ABTS^{+}$, FRAP, and $DPPH^{\bullet}$ scavenging capacity (Table 4).

Umezu obtained from Fujian white mume and Puning green mume had similar antioxidant properties in terms of ORAC and $ABTS^{+}$ scavenging as well as FRAP. In all antioxidant activity assays, umezu acquired from Raoping green mume and Fujian green mume, however, exhibited significantly higher radical scavenging capacity and reducing power than other samples. Particularly, umezu that came from Raoping green mume presented almost twofold antioxidant capacity compared with those from Fujian white mume and Puning green mume in ABTS and ORAC values. This could be explained by their higher level of antioxidant ingredients, of which total phenolic acids (330.74 mg/L) and organic acids (51.53 g/L) ranked almost first among all umezu samples (Tables 1 and 2). Reflecting such wealth of organic acids and phenolic acids, the reducing power reflected by FRAP value for umezu samples showed high values, ranging from 162.9 μg/L to 186.43 μg/L Trolox equivalent, depending upon the species and origin areas. In accordance with our results, Debnath et al. [24] revealed that the extract of *Mume Fructus* exhibited high scavenging activities on DPPH, ABTS, hydroxyl, and superoxide radicals with the IC_{50} of 0.40, 0.36, 1.75, and 1.60 mg/ml, respectively. It was reported that the reducing power properties are generally associated with the presence of reductones, which have been shown to exert an antioxidant action by breaking the free radical chains by donating a hydrogen atom [25].

Noteworthily, in all antioxidant activity assays, only ORAC and FRAP of the umezu followed the same trends as their phenolic acids contents; $ABTS^{+}$ and $DPPH^{\bullet}$ scavenging capacity went different. Luhe green mume and Raoping green mume presented the lowest and strongest scavenging activities of $ABTS^{+}$, with which ABTS values being 24.96 μg/L and 195.83 μg/L, respectively. The $DPPH^{\bullet}$ scavenging capacity of samples exhibited the descending order: Raoping green mume > Fujian green mume > Puning green mume > Luhe green mume > Fujian white mume. Umezu samples obtained from Fujian green mume and Puning green mume displayed almost equivalent scavenging activity on $DPPH^{\bullet}$ radical ($p > 0.05$). The $DPPH^{\bullet}$ scavenging ability of umezu samples obtained from Fujian white mume and Luhe green mume was much lower than other samples, but their EC50 values were still higher than 40 mg/ml, which may represent its medicinal value [26].

4. Conclusions

Chemical composition and antioxidant activities of umezu from five *P. mume* pickling factories in China were investigated. These samples contained 33.00–34.50° Brix solid matter in which NaCl was 20.36–22.01%. Total titratable acid content was 4.55–5.44% which makes it with very low pH (1.85–1.90), while reducing sugar content was only 5.18–8.56%. Umezu was high in organic acids (>43 g/L) in which six organic acids were identified and the dominant ones were citric acid and malic acid. It also contained abundant phenolic compounds (>250 mg/L) in which the predominant phenolic acids were neochlorogenic acid, chlorogenic acid, and cryptochlorogenic acid. Umezu obtained from different factories also exhibited strong radical scavenging activities and ferrous reduction ability. Total phenolics were partially responsible for the antioxidant activity assessed by ORAC, ABTS, DPPH, and FRAP assays. Considering the results obtained, it might be anticipated that the umezu could be used as sauce or a natural preservative in food and/or pharmaceutical industry with suitable desalting technology.

Conflicts of Interest

The authors declare that they have no conflicts of interest.

Acknowledgments

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Research Article

Inhibition of Corn Oil Peroxidation by Extracts from Defatted Seeds of *Camellia oleifera* Abel

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The defatted seed of *Camellia oleifera* Abel is an underexploited byproduct. In the present study, 3 solvents (isopropanol, hexane, and diethyl ether) were used to extract active compounds from defatted seeds of *Camellia oleifera* Abel. The qualitative and quantitative characterization of the main phenolic compounds were performed using the HPLC-DAD. In addition, the antioxidant efficacy of defatted seed extracts in the stabilization of the corn oil was estimated by monitoring its primary and secondary oxidation products. The results showed that isopropanolic extract (IE) exhibited the highest yield of total phenolic compounds (9.23 ± 0.33 mg/g) compared with other solvents. The predominant phenolic compounds identified by HPLC-DAD in defatted seed extracts were epicatechin, naringenin, and catechin. In addition, IE exhibited the best inhibition effect on both primary and secondary oxidation products. The results reveal that IE of defatted seed of *Camellia oleifera* Abel is a useful antioxidant for the stabilization of corn oil.

1. Introduction

Vegetable oils, which are currently the preferred cooking medium, are beneficial and popular due to their cholesterol-lowering effects. However, they are more susceptible to oxidation than animal fats, which predominantly contain saturated fatty acids and thus do not react readily with other chemicals, especially oxygen [1]. The oxidation of lipids limits the shelf life of many products; moreover, some oxidation products are potentially toxic [2, 3].

Many methods have been employed to overcome lipid oxidation. These include the use of low-temperature storage to slow down the oxidation process, advanced packaging technologies to exclude oxygen and light, and antioxidants [4]. Addition of synthetic antioxidants, such as butylated hydroxyanisole (BHA), butylated hydroxytoluene (BHT), and tert-butylhydroquinone (TBHQ), is one of the most effective and popular methods to prevent oxidation and the development of off-flavors (Halliwell and Gutteridge, 1985). However, recent reports have revealed that these compounds may be associated with certain health risks, including carcinogenesis (Hou, 2003; Prior, 2004). Therefore, the use of TBHQ for food application is banned in Japan, Canada, and Europe. Similarly, BHA has been removed from the generally recognized as safe (GRAS) list of compounds (Farag, Badei, and El Baroty, 1989).

It should be noted that oxidation is prevented in living organisms due to a number of endogenous factors, including enzymes, natural antioxidants, and chelators of metal ions [5]. Thus, there has been an increasing trend among food scientists to replace synthetic antioxidants with natural ones, which can be obtained from a wide variety of plant sources, including mustard flour [6], canola meal [7], garlic [8], old tea leaves [9], rice bran [10], wheat bran [11], and peanut hulls [12].

Camellia oleifera Abel is an evergreen shrub or small tree in the *Camellia* family. China is the world's largest *Camellia oleifera* Abel producer with 4 million hectares of *Camellia oleifera* Abel forest [13]. The seeds of *Camellia oleifera* constitute an important oil source that is extensively used to produce a specific type of cooking oil (tea oil), whose beneficial unsaturated fatty acids are comparable to those of olive oil. Four times more seed cake will be produced than tea oil. The defatted seed of *Camellia oleifera* is a substantial byproduct

that is normally used as animal feed, organic fertilizer, or detergent due to its rich concentrations of polyphenols, saponins, proteins, and polysaccharides [14]. Although some studies have examined the antioxidant properties of *Camellia* oil, there are few reports on the efficiency of seed cake extract of *Camellia oleifera* in inhibiting corn oil peroxidation.

The objectives of this study were to extract active compounds from defatted seed of *Camellia oleifera* Abel using different solvents (isopropanol, hexane, and diethyl ether) and examine the antioxidant potential of the extracts using Folin-Ciocalteu assay. In addition, the predominant phenolic compounds of seed cake extracts were identified using HPLC-DAD. The primary and secondary oxidation products of corn oil were monitored by using the peroxide value (PV), anisidine value (AV), conjugated dienes (CD), and thiobarbituric acid reactive substances (TBARS) values.

2. Methods

2.1. Materials and Chemicals. Butylated hydroxyanisole (BHT), Folin-Ciocalteau reagent, 2,4,6-tris(2-pyridyl)-1,3,5-triazine (TPTZ), and *p*-anisidine and thiobarbituric acid (TBA) were purchased from Sigma-Aldrich Chemical Co. (Milwaukee, WI, USA). Phenolic acids, gallic acid, coumaric acid, catechin, epicatechin, chlorogenic acid, epicatechin, rutin, ferulic acid, naringenin, and kaempferol acid were obtained from Merck Darmstadt, Germany. Defatted seed was obtained from Dongguan Chengfu Trade Co., Ltd. (Dongguan, Guangdong, China). The refined edible corn oil without any synthetic antioxidants was obtained from a local store in Hainan. All other chemicals were of analytical grade unless otherwise mentioned.

2.2. Preparation of Seed Cake Extracts. The seed cake extracts (SCE) were prepared according to the method of Iqbal and Bhanger [8] with some modifications. The defatted seed of *Camellia oleifera* Abel was dried in an oven at 55°C until the moisture content was less than 10% (w/w). The dried defatted seed was ground until it could pass through a 1 mm sieve. Then, 50 g (dry basis) of seed powder was extracted into 250 mL solvent (isopropanol, hexane, or diethyl ether). The slurry was incubated at room temperature in a vibration cultivating box at 180 rpm for 2 h, and the extracts were sonicated at 60°C for 2 h. The extracts were filtered using filter paper, and the residue was again extracted with 200 mL of each solvent. The procedure was repeated thrice to ensure that the phenolic compounds were completely extracted. Next, the solvent of the filtrate was removed using a rotary evaporator at 40°C under reduced pressure. The products were weighed to calculate the yield and were stored under nitrogen prior to further analyses.

2.3. Determination of Total Phenolic Compounds. The total phenolic content of the samples was determined using the Folin-Ciocalteau method [15]. Briefly, each individual extract (0.1 mL) was diluted to 1 mL with distilled water, and 0.5 mL of Folin-Ciocalteu reagent and 2 mL of Na_2CO_3 solution (7.5%, w/w) were added. The solutions were mixed thoroughly and incubated at 40°C for 30 min. The absorbance was detected at 765 nm on a Perkin-Elmer Lambda-2 Spectrophotometer with a 1 cm cell. Gallic acid was used as a standard, and the results were expressed as gallic acid in terms of milligram gallic acid equivalents in every gram of dried SCE. The reaction was conducted in triplicate, and the results were averaged.

2.4. Identification of Phenolic Compounds Using HPLC-DAD. The phenolic compounds of defatted seed extracts were identified using the HPLC-DAD method as reported by Chirinos et al. [16]. HPLC analysis was performed using Shimadzu LC-20A pumps with a diode array detector (DAD), and chromatographic separations were performed on a Phenomenex C18 column (250 mm × 4.6 mm, particle size 5 mm). The composition of solvents and gradient elution conditions were as follows. The mobile phase was composed of solvent (A) water : acetic acid (97 : 3, v/v, pH 2.27) and solvent (B) methanol. The solvent gradient was as follows: 10–30% B for 0–6 min, 30–70% B for 6–10 min, 70–90% B for 10–15 min, 90–100% B for 15–20 min, 100–20% B for 20–25 min, and holding 20% B for 25–30 min. A flow rate of 1.0 mL/min was used, and 10 μL samples were injected. All samples and mobile phases were filtered through a 0.22 μm Millipore filter before injection. Each fraction was analyzed in duplicate.

2.5. Ferric Reducing Antioxidant Power (FRAP) Assay. The FRAP assay was conducted according to Benzie and Strain (1996) with some modifications. The working FRAP reagent was freshly prepared by mixing 25 mL acetate buffer (300 mM, pH 3.6), 2.5 mL TPTZ solution (10 mM), and 2.5 mL $FeCl_3 \cdot 6H_2O$ solution (20 mM). The mixture was kept at 37°C before use, and a reagent blank reading was taken at 593 nm. SCE (100 μL) were allowed to react with 2.85 mL FRAP solution for 30 min in the dark, and the absorbance was read at 593 nm. The Trolox was used as the standard, and the standard curve was linear between 25 and 800 μM Trolox. The results were expressed in mM Trolox equivalent antioxidant capacity (TEAC)/g dried SCE. Additional dilutions were needed if the measured FRAP value was over the linear range of the standard curve.

2.6. Measurement of Peroxide Value (PV), Conjugated Dienes (CD), Anisidine Value (AV), and Thiobarbituric Acid Reactive Substances (TBARS). The antioxidant activity of the collected SCE extracts (IE, NE, and DE) on corn oil was evaluated using the PV, CD, AV, and TBARS values. PV was determined according to the iodine colorimetry reported by AOAC. The extracts of SCE and the synthetic antioxidant were dissolved in a minimum amount of absolute methanol and added to 100 g corn oil after mixing for 10 min to achieve different concentration of 0.25% and 0.5% (w/v) for SCE and 0.02% (w/v) for BHT. After being stored for different times, samples (5 g) were dissolved in 30 mL of acetic acid/chloroform (3 : 2, v : v) solution, with the addition of 0.5 mL of a saturated solution of potassium iodide and a 1% starch solution as an indicator. The mixture was allowed to stand for 1 min and then 30 mL of distilled water and 0.5 mL of 1% starch were added. The liberated iodine was titrated with a sodium

Table 1: Total extractable compounds (TEC), total phenolic compounds (TPC), and the proportion of TPC in TEC[A].

Extraction	TEC (mg/g dried seed cake)	TPC (mg/g dried seed cake)	TPC/TEC (mg/g dried SCE)
IE[B]	180.1 ± 1.78^{c}	9.23 ± 0.33^{c}	51.25
NE	123.3 ± 2.35^{b}	6.45 ± 0.61^{b}	52.31
DE	95.7 ± 1.39^{a}	3.86 ± 0.48^{a}	40.33

[A]Assays were performed in triplicate. Mean ± SD values in the same column with different superscript letter are significantly different ($p < 0.05$); [B]IE, isopropanol extract; NE, normal hexane extract; DE, diethyl ether extract; SCE, seed cake extract.

thiosulfate solution (0.1 mol L^{-1}) until the blue color disappeared. A blank test was also conducted under the same conditions as described with the samples. The peroxide value was calculated as follows:

$$\text{Peroxide value} = \frac{(B - A) \times N \times f \times 100}{m}, \quad (1)$$

where A is the volume (mL) of the standard volumetric sodium thiosulfate solution used for the blank test, B is the volume (mL) of the standard volumetric sodium thiosulfate solution used for the sample titration, N is the normal concentration of the standard volumetric sodium thiosulfate solution used, f is the correction factor, and m is the mass of the sample (kg).

The p-AV of the samples was measured using a spectrophotometer [3]. PV and AV, which were used as indicators for the primary and secondary oxidation of the corn oil, were determined at 4, 8, 16, 24, 32, 48, and 72 h, and all treatments were carried out three times.

CD value was determined according to the method of Shahidi et al. [2]. The absorbance of the samples was determined. Each sample (10 mL) was thoroughly mixed with 10 mL isooctane. The absorbance was read at 234 nm after samples had been stored for 0, 1, 2, and 3 days. Pure isooctane was used as a reference. The CD value, expressed as percentage of conjugated dienoic acid, was calculated according to the equation below:

$$\text{CD value} = \frac{A}{C \times 1}, \quad (2)$$

where CD is conjugated diene, A is absorbance at 234 nm, C is concentration (g/100 ml), and l is path length (cm).

The TBARS values of the samples were tested and analyzed over 0, 1, 2, and 3 d periods as described by Shahidi et al. [2] with some modification. Briefly, each sample (200 mg) was weighed accurately and transferred into a centrifuge tube to which 5 mL of 1-butanol TCA was added and vortexed at a high speed for 2 min. A quantity of 5 mL aqueous solution of TBA (0.02 M) was then added to each centrifuge tube, which was then vortexed for an additional 30 s. The samples were then centrifuged at 3,000 ×g for 10 min, and the supernatants were filtered through Whatman #3 filter paper. The tubes were placed in a water bath at 95°C for 120 min and cooled to room temperature. The absorbance value of solutions was read at 532 nm. The TBARS value was calculated according to the equation below:

$$\text{TBARS value} = \frac{A \times 0.145}{m}, \quad (3)$$

where A is absorbance at 532 nm and m is the mass of the sample.

3. Results and Discussion

3.1. Extraction of Phenolic Compounds. Total extractable compounds (TEC), total phenolic compounds (TPC) using different solvents, and the proportion of TPC in TEC are shown in Table 1. It is clear that the extraction abilities of isopropanol, normal hexane, and diethyl ether used for the extraction of the COC were significantly different ($p < 0.05$). In general, the TEC of IE, NE, and DE were 180.1 ± 1.78, 123.3 ± 2.35, and 95.7 ± 1.39 mg/g, respectively. The result suggested that TEC depended on the extraction medium, which is consistent with results reported by Shahidi et al. [2].

The TPC contents of IE, NE, and DE from COC had the same trend as did the experimental results of TEC. The results showed that IE contained the highest amount of TPC (9.23 ± 0.33 mg/g), followed by NE (6.45 ± 0.61 mg/g) and DE (3.86 ± 0.48 mg/g). The relationship between the total extractable compounds and their total phenolic compound content was represented in percentages of TPC/TEC. The total phenolic content found in the total extractable compounds ranged from 40.33 to 52.31 mg/g. The amount of TPC observed in camellia oil cake extracts was greater than that observed in corn tassels (0.16%), banana peels (0.91%), and rice bran (3.86%) [17–19].

3.2. Identification of Phenolic Compounds Using HPLC-DAD. The phenolic compounds in defatted seed extract were identified and quantified by HPLC-DAD method [20]. All samples were monitored at 280 nm, and their spectra are shown in Figure 1. As shown in Figure 1(a), peaks 1~9 reflected nine types of phenolic acid standards: gallic acid, coumaric acid, catechin, chlorogenic acid, epicatechin, rutin, ferulic acid, naringenin, and kaempferol acid, respectively. The spectra of phenolic compounds of IE, NE, and DE are shown in Figures 1(b), 1(c), and 1(d), respectively. The species and content of the phenolic compounds of IE, NE, and DE are shown in Table 2. As shown in Figure 1, there were three main phenolic compounds (epicatechin, naringenin, and catechin) in seed extracts. Epicatechin was one of the most predominant phenolic compounds, contributing approximately 8.64 ± 0.03 mg/g in IE and 9.32 ± 0.12 mg/g in NE. However, no epicatechin or naringenin was detected in DE or NE (Table 2). Naringenin and catechin in IE contributed approximately 2.71 ± 0.05 and 4.00 ± 0.04 mg/100 g dried SCE, respectively.

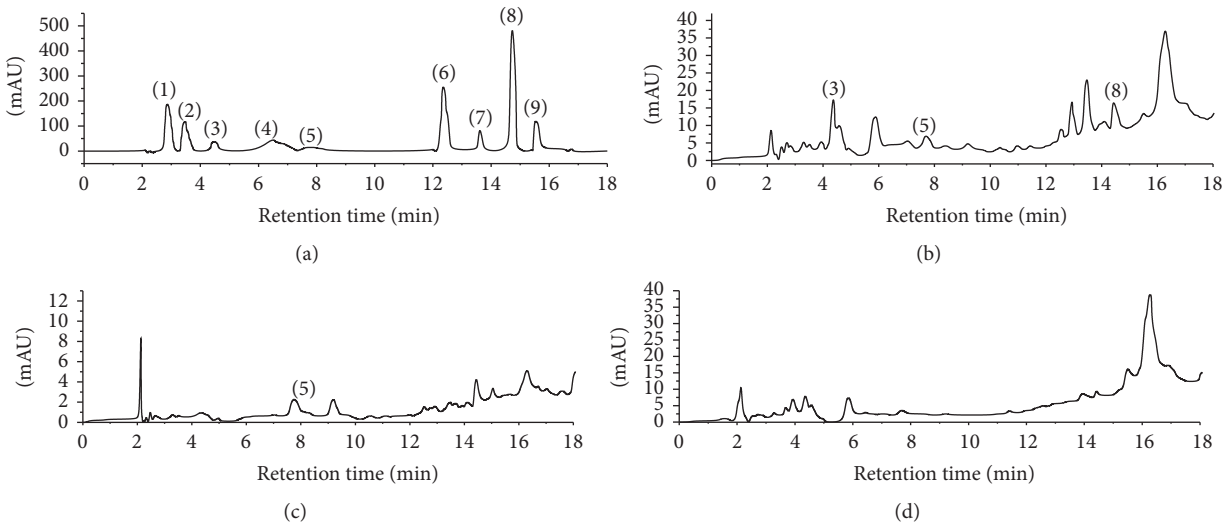

Figure 1: HPLC/DAD chromatogram of phenolic compounds in camellia oil cake. Detection was at 280 nm. (a) Standards of phenolic acids. Peak: (1) gallic acid; (2) coumaric acid; (3) catechin; (4) chlorogenic acid; (5) epicatechin; (6) rutin; (7) ferulic acid; (8) naringenin; (9) kaempferol acid. (b) Isopropanol extract (IE). Peak: (3) catechin; (5) epicatechin; (8) naringenin. (c) Normal hexane extract (NE). Peak: (5) epicatechin. (d) Diethyl ether extract (DE).

Table 2: Phenolic compound content (mg/g dried SCE).

Compounds	IE (mg/g)[A]	NE (mg/g)	DE (mg/g)
Epicatechin	8.64 ± 0.03^{a}	9.32 ± 0.12^{a}	ND[B]
Naringenin	2.71 ± 0.05^{c}	ND	0.38 ± 0.03^{b}
Catechin	4.00 ± 0.04^{b}	0.60 ± 0.03^{b}	0.73 ± 0.04^{a}

[A]Assays were performed in triplicate. Mean ± SD values in the same column with different superscript letter are significantly different ($p < 0.05$); [B]ND: not detected.

3.3. Total Antioxidant Power. The ferric reducing antioxidant power (FRAP) assay is often used to determine the antioxidant power on the basis of their electron-donating ability [21]. The principle of the FRAP assay is that the electrons donated from antioxidants (defatted seed extracts) are capable of reducing ferric ions (Fe^{3+}) to ferrous ions (Fe^{2+}). Through the standard curve of the ferric reducing antioxidant power, the total antioxidant powers of BHT and the three extracts are shown in Figure 2. BHT is one of the most effective and popular antioxidants used in the food system. As reported, BHT is not the most suitable reference in FRAP evaluation when compared with that of other synthetic antioxidants. However, BHT was usually used as the positive control in FRAP evaluation and showed good FRAP values [22–25] (Halliwell and Gutteridge, 1985). Data presented in Figure 2 showed that the FRAP values of the three defatted seed extracts were significantly ($p > 0.05$) different. IE has the highest FRAP values (3.29 ± 0.12 mM TEAC/g dried SCE) compared with NE (2.26 ± 0.19 mM TEAC/g dried SCE) and DE (0.89 ± 0.15 mM TEAC/g dried SCE). According to the result of the HPLC-DAD measurement (Table 2), three main phenolic compounds were epicatechin, naringenin, and catechin. In addition, no significant ($p > 0.05$) difference between the FRAP values of IE and BHT (3.63 ± 0.21 mM TEAC/g dried SCE) was observed, which suggested that IE could be potentially employed as a substitute for synthetic antioxidants in the food system.

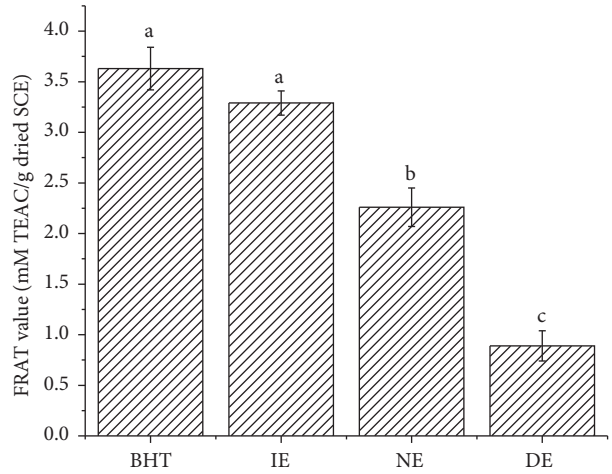

Figure 2: The FRAP values of camellia oil cake phenolic extracts and BHT on corn oil (BHT, butylated hydroxytoluene; IE, isopropanol extract; NE, normal hexane extract; DE, diethyl ether extract; TEAC, Trolox equivalent antioxidant capacity). The data are averages and standard deviations of triplicate measurements. Values in each bar with different superscript letters (a, b, c) are significantly different ($p < 0.05$).

3.4. Peroxide Value (PV). During the storage of corn oil, a series of free radical chain reactions occurred, which resulted in a great number of oxidation compounds. The antioxidant effects of phenolic compounds of SCE were assessed using the peroxide value (PV) to determine the primary oxidation products [26]. Hydroperoxides are the primary oxidation

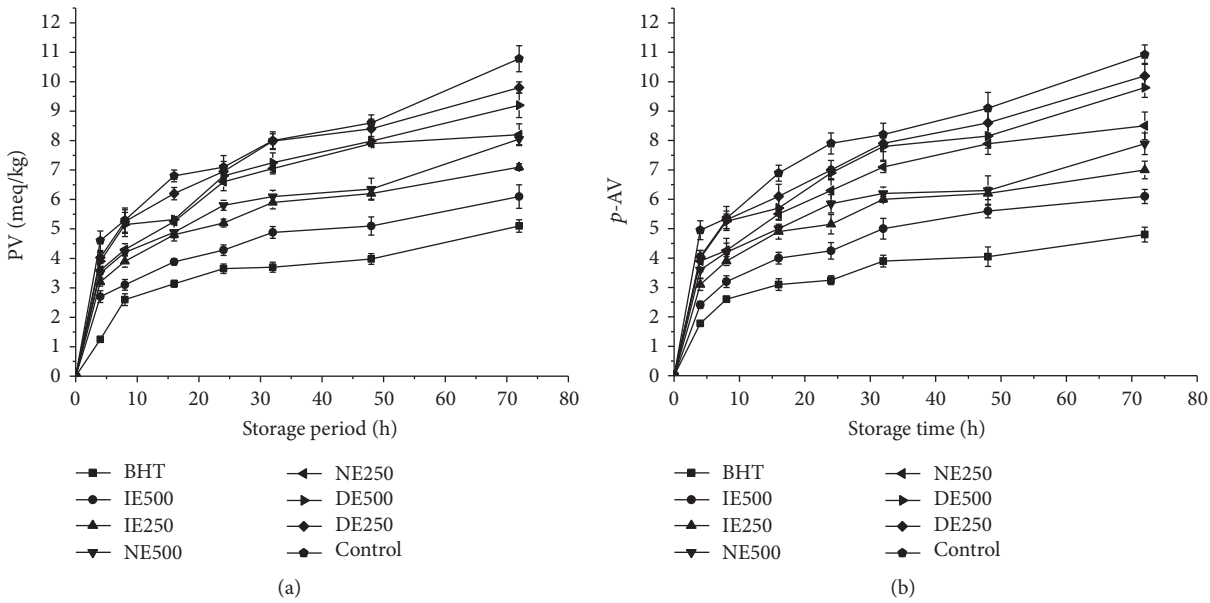

Figure 3: The changes of PV (a) and p-AV (b) during different storage time (h) (BHT, butylated hydroxytoluene; IE, isopropanol extract; NE, normal hexane extract; DE, diethyl ether extract).

products of lipid oxidation, and their content is often used as an indicator for the initial stages of oxidation. The effect of SCE and BHT on the development of PV of corn oil during storage at 50°C is shown in Figure 3. PV in all samples increased as the storage time was extended. This result may be attributed to the formation of hydroperoxides during primary oxidation processes. At all stages, highest PV was observed for the control sample, followed by DE-250, DE-500, NE-250, NE-500, IE-250, IE-500, and BHT. The result suggested that, to some extent, PV depends on the category rather than the concentrations of antioxidants. As shown in Figure 3, PV of the control increased to 10.67 ± 0.13 meq/kg after 72 h of storage. Significant differences ($p < 0.05$) were found between the control and other oil samples to which IE, NE, DE, or BHT was added, thus revealing the good antioxidant efficacy of the antioxidants in stabilization of corn oil. The synthetic antioxidant BHT showed a lower PV (5.17 ± 0.08 meq/kg) relative to the other tested samples. IE and NE extracts were found to be more effective (with less PVs) than DE that had higher PVs.

For concentration, lower PVs were obtained when the extract concentrations were high (NE, DE, and IE). IE500 showed the best effect in lowering peroxide formation compared with the other samples. IE500 gave a PV of 6.28 ± 0.12 meq/kg at 72 h, whereas the corresponding values of the control sample were 10.67 ± 0.13 meq/kg. DE250 demonstrated the worst effect and gave a PV of 9.76 ± 0.31 meq/kg at 72 h. From the analysis above, we may conclude that extracts from defatted seed at 0.25% and 0.5% (w/v) concentrations were effective in stabilization of corn oil during storage at 50°C and that defatted seed extracts at higher concentrations were better at inhibiting corn oil oxidation in the initial stage. IE displayed the best antioxidant activity among the three solvent extracts, which is consistent with the results of FRAP.

3.5. Conjugated Dienes (CD) Values. CD value is also a good indicator of oxidative deterioration of oils in the initial stages, and it may thus also be a good indicator of the effectiveness of antioxidants [27]. Table 3 indicates that CD of corn oil with defatted seed extracts and BHT varies after storage for 0, 24, 48, and 72 h at 50°C. The CD values of all samples increased with storage time, which may reflect the lipid oxidation process. The control sample exhibited the highest CD value in all storage stages and showed a sixfold increase after 72 h (12.10± 0.64%), relative to the initial period (2.15 ± 0.49%). High values of CD may be related to the presence of higher contents of polyunsaturated fatty acids in the oil samples [8] (Liu and White, 1992). Compared to the control, the CD values of the corn oil samples containing defatted seed extracts or BHT showed lower increase during the same storage period. After storage for 72 h, the lowest CD was observed for BHT sample (5.58 ± 0.30%) followed by IE (7.05 ± 0.33%), NE (8.71 ± 0.39%), and DE (10.83 ± 0.40%). The CD results were consistent with the measures of PV.

3.6. Secondary Oxidation Products Determination. In this study, secondary oxidation products were assessed using thiobarbituric acid reactive substances (TBARS) and p-anisidine value (p-AV). TBARS is the most widely used method for measuring secondary oxidation products, which may contribute to the off-flavor of oxidized oil. As shown in Table 3, TBARS for all samples increased gradually as the storage period was extended (0~72 h). As observed for the primary oxidation product (PV and CD values), the control showed the highest level of TBARS at all storage times, and the values for the samples treated with extracts and antioxidant were significantly lower than those of the control. The TBARS of IE stabilized corn oil increased from 0.30±0.12 to 1.08 ± 0.46 μmol/g oil. This was lower than that of NE and

Table 3: Conjugated diene (CD) and TBARS values of corn oil stabilized by camellia oil cake phenolic extracts (0.5%) and BHT (0.02%) during different storage time at 50°C[A].

Time (h)	BHT[B]	Control	NE	IE	DE
		CD values (% of conjugated dienoic acid)			
0	2.15 ± 0.49^{a}	2.15 ± 0.49^{a}	2.30 ± 0.22^{a}	2.03 ± 0.23^{a}	2.25 ± 0.18^{a}
24	3.20 ± 0.22^{e}	4.67 ± 0.20^{a}	3.83 ± 0.21^{c}	3.60 ± 0.18^{d}	4.28 ± 0.25^{b}
48	5.08 ± 0.38^{e}	8.05 ± 0.78^{a}	6.88 ± 0.48^{c}	6.48 ± 0.15^{d}	7.33 ± 0.28^{b}
72	5.58 ± 0.30^{e}	12.10 ± 0.64^{a}	8.71 ± 0.39^{c}	7.05 ± 0.33^{d}	10.83 ± 0.40^{b}
		TBARS value (μmol/g oil)			
0	0.29 ± 0.10^{a}	0.34 ± 0.16^{a}	0.27 ± 0.13^{a}	0.30 ± 0.12^{a}	0.30 ± 0.14^{a}
24	0.39 ± 0.17^{e}	0.85 ± 0.24^{a}	0.67 ± 0.23^{c}	0.58 ± 0.24^{d}	0.79 ± 0.26^{b}
48	0.55 ± 0.29^{e}	1.19 ± 0.38^{a}	0.73 ± 0.41^{c}	0.62 ± 0.24^{d}	1.08 ± 0.43^{b}
72	0.77 ± 0.22^{e}	1.92 ± 0.47^{a}	1.48 ± 0.47^{c}	1.08 ± 0.46^{d}	1.67 ± 0.28^{b}

[A]Assays were performed in triplicate. Mean ± SD values in the same column with different superscript letter are significantly different ($p < 0.05$); [B]BHT, butylated hydroxytoluene; IE, isopropanol extract; NE, normal hexane extract; DE, diethyl ether extract.

DE samples (1.48 ± 0.47 and 1.67 ± 0.28 μmol/g oil, resp.) but higher than that of BHT sample (0.77 ± 0.22 μmol/g oil) after 72 h. These results suggested that the antioxidant activity of the defatted seed extracts and synthetic antioxidant was in the following order: BHT > IE > NE > DE.

The *p*-AV is also useful for assessing oil at the secondary stage of oxidation. The *p*-AV of corn oil samples stabilized by defatted seed extracts and BHT during different storage times is shown in Figure 3(b). As shown in Figure 3(b), across all storage times, the highest *p*-AV was observed for controls, followed by DE, NE, and IE, indicating that IE and NE were more effective than DE. Relative to BHT, the phenolic extracts obtained from defatted seed extracts seemed to be less effective in inhibiting secondary products. In addition, *p*-AV decreased as the concentration of extracts increased. These results suggested that defatted seed extracts with certain concentration ranges could effectively improve the stabilization of corn oil.

4. Conclusion

Three solvents (isopropanol, hexane, and diethyl ether) were used to extract active compounds from defatted seeds of *Camellia oleifera* Abel. The results showed that isopropanolic extract (IE) exhibited the highest yield of total phenolic compounds (9.23±0.33 mg/g), followed by normal hexane extract (6.45±0.61 mg/g) and diethyl ether extract (3.86±0.48 mg/g). Three predominant phenolic compounds (epicatechin, naringenin, and catechin) from the defatted seed extracts were identified using the HPLC-DAD method. In addition, the antioxidant efficacy of IE, NE, and DE has been estimated in stabilization of corn oil by monitoring the primary and secondary oxidation products of oil. The peroxide value (PV), anisidine value (AV), conjugated dienes (CD), and thiobarbituric acid reactive substances (TBARS) each indicated a similar trend in the antioxidant activity of the three extracts: IE > NE > DE. The results revealed that IE of defatted seed of *Camellia oleifera* Abel is a useful antioxidant for the stabilization of corn oil.

Additional Points

Practical Applications. The seeds of *Camellia oleifera* constitute an important oil material that is extensively used to produce a specific type of cooking oil (tea oil), whose beneficial unsaturated fatty acids are comparable to those of olive oil. Tea oil is a good raw material for industrial use and is used to manufacture soap, margarine, hair oil, lubricants, and paint and in the syntheses of other high-molecular weight compounds.

Disclosure

The authors alone are responsible for the content and writing of the paper.

Conflicts of Interest

The authors report no conflicts of interest.

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