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Using Combination of Processing Techniques to Optimize the Extraction of Functional Components in Citrus (Citrus Reticulate) Peels

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Abstract **– Citrus peels (CP) are rich in functional polymethoxyflavones, particularly nobiletin and tangeretin, which are of notable industrial value for functional product development. This study demonstrated using various combinations of operating techniques, including hot-air drying and micronization for preparing varied particle diameters of CP, fermentation and ultrasound assisted extraction. The optimal operating process was determined. The CP from mandarin orange (*Citrus reticulate*) was treated in a hot-air dried oven, grinded and divided by a sieve shaker (30, 325 meshes). The culture medium of *Aspergillus oryzae* with CP30 and CP325 were extracted using a 20 kHz ultrasonic probe (UP20) or a 40 kHz ultrasonic bath (UB40). The analysis of functional components and antioxidant activity of CP extracts were conducted. The results revealed that UP20 micronized large particles and eliminated the physical differences caused by the various processing techniques much more effectively, compared with UB40. The contents of functional components were significantly reduced after fermentation. The antioxidant activities of the fermented extracts were significantly lower than those of the unfermented groups. New metabolites were generated during fermentation. Comparing the extraction yields and antioxidant activities revealed that the unfermented CP325-UP20 group exhibited the most favorable results, followed sequentially by the CP325-UB40, CP30-UP20, and CP30-UB40 groups.**

Keywords **– Citrus Peels, Micronization, Ultrasound Assisted Extraction, Fermentation, Optimization.**

I. INTRODUCTION

In traditional Chinese medicine, *Citrus spp.* are considered to have the properties of "regulating qi-flows for strengthening the spleen, and drying dampness in body to eliminate phlegm." Aside from being a source for essential oils, the citrus peels (CP) of *Citrus reticulate* are rich in functional flavonoids, particularly polymethoxyflavones (e.g. nobiletin and tangeretin) that have been proven to possess pharmacological activities in anti-oxidation, anti-inflammation, preventing cancer and cardiovascular diseases [1-4]. Moreover, recent studies have also reported that extracts from CP can help prevent metabolic diseases, such as obesity and type II diabetes [5-7]. However, CP had always been discarded as waste until very recently when the food industry gradually pays attention to enhance the economic value CP by suitably processing techniques to develop functional food products [8].

The application of nanotechnologies in natural products preparation has received attention in recent years. In particular, micronization makes food ingredients highly soluble, adsorptive, and fluidal because of their increased surface areas and porosity. This technique has been prevalently applied in the manufacturing and development of health-promoting products and pharmaceuticals because it improves the bioavailability of ingredients for readily extracted or absorbed [9]. A specific method for micronization using jet mill to accelerate food particles in high-velocity air flows to create cutting, smashing, and grinding effects by making the particles collide and scrap with each other, and thus breaking them into very fine powders [10]. The present study used an electrical sieve shaker and a jet mill micronizer to prepare various diameters of micronized CP powders to examine the influences of their physicochemical properties on the extraction of functional substances.

Ultrasound assisted extraction (UAE) is an enhanced extraction technique that uses ultrasonic cavitation, mechanical, and thermal effects to enhance the mass transfer of extracts, expand the liquid–solid interface for increased extract solubility, and facilitate diffusing and mix cell materials in a solvent through rupturing the cell wall and reducing the material particle size [11-13]. Sonication devices for UAE can be categorized into probe-type and indirect bath ultrasound. The non-invasive bath system not only reduces the pollution and loss of extracts, but also enables mass extraction operation. However, the bath sonication typically causes an uneven distribution of ultrasonic power. The direct probe-type system is more effective in conducting extraction and degradation because they can concentrate ultrasonic power within a defined area, and thus provide an ultrasonic power that can be 100 times more intense than that of the bath system [14].

Previous published literatures revealed that most of the studies examining the extraction of natural products have focused on extracting health-promoting polyphenols [11, 14, 15]. For example, numerous studies have compared the UAE method of extracting polyphenols with other extraction methods under varied conditions, such as how different ultrasonic conditions (e.g. frequency, power, temperature, and duration) would affect the extraction of hesperidin, phenolic acids, and flavonoid glycoside in various citrus species [16-19]. Because conventional extraction methods require high temperatures and a substantial amount of time, and innovative methods, such as the enzyme extraction, supercritical fluid extraction, and microwave extraction, have notable limitations (e.g. the operational environments are harmful to antioxidant

polyphenols), the method has been recognized as superior in numerous ways [20-22].

In addition to physical and chemical processes, fermentation has become a popular method of improving the extraction yield of polyphenols. It primarily relies on the enzymes produced during fermentation to dissolve cell walls and facilitate the release of free and cell wall-bound phenolic compounds (e.g., cellulose, hemicellulose, lignin, pectin, and protein) [23]. Additionally, various microbial bioconversion pathways that generate new metabolites, such as glycosylation, deglycosylation, methylation, glucuronidation, and sulfate conjugation, can potentially facilitate the production of extracts with high added values from plant-based substrates [23]. For example, Yamane *et al*. isolated cellulose-degrading enzymes from a solid-state culture of *Aspergillus oryzae* to improve the utilization of rice endosperm cells in sake mash fermentation [24]. Mamma *et al*. also determined that citrus peels, which are a common by-product of the citrus processing industry, can be used in solid-state fermentation to produce fungal cellulolytic and xylanolytic enzymes [25].

Because citrus peels contain valuable polymethylflavones, this study try to establish an optimal processing procedure by comparing various combinations of processing techniques. CP was first dried in hot air at varied temperatures (100 °C and 150 °C) before been ground into powders that were further treated by either an automatic sieve shaker or a jet mill to obtain CP powders with different diameters. Meanwhile, carboxymethyl cellulose (CMC) was used as the sole carbon source in the culture medium to grow *A. oryzae* for the fermentation of the CP powders at varied diameters (CP30 & 325 mesh). Finally, a 20 kHz ultrasonic probe (UP20) and a 40 kHz ultrasonic bath (UB40) were used to extract the CP powders. The contents of polyphenols, antioxidant activities and major functional components in CP extracts were compared to determine the optimal processing procedure.

II. Materials and Methods

A. Materials and Chemicals

The mandarin oranges (*Citrus reticulata Blanco*) used in the study were acquired from the Farmers' Association of the Shek Kong District, Taichung, Taiwan. Reagent-grade chemicals were used for extractions and analytical assays. The reagents for antioxidant assays, including *Folin–Ciocalteu* reagent, 2, 2 – di (4–*tert*-octylphenyl) – 1 - picrylhydrazyl (DPPH), and 2, 2′ - azino – bis (3 - ethylbenzo thiazoline-6-sulfonic acid), carboxymethyl cellulose (CMC) and culture medium for fermentation were all purchased from Sigma-Aldrich Chemical Co. (St. Louis, MO, USA).

B. Preparation of Different CP Extracts

The peels of the mandarin oranges (CP) were placed in a hot-air drying oven for 10 h at 100 °C and 150 °C. Afterwards, the CP were grinded into powder, which was subsequently divided by a sieve shaker (#30, #30–325, and #325 meshes) into three diameter ranges (CP-1 to CP-3). A portion of the CP powder that passed through the #30 mesh was micronized in a spiral jet mill (OM2 Micronizer; Sturtevant, Int., Hanover, MA, USA) with the following settings: feeding rates at 10 or 15 g/min, grinding pressure of 105 PSIG, and feeding pressure of 70 PSIG. The powder particles were thus reduced to two diameter ranges (CP-4 & CP-5), and then sent into a static light scattering particle size distribution analyzer (Beckman Coulter LS13 320; Beckman Coulter, Inc., Fullerton, CA, USA) with the Tornado DPS module to analyze their particle sizes. The particle diameters of the powder samples were subsequently calculated by analyzing the scattering signals after applying background corrections and standard operating procedures. The abbreviations of each prepared CP powders were as follows:

CP100 (150) °C-1: hot air dried at 100 (150) °C and sieved though #30 mesh.

CP100 (150) °C-2: hot air dried at 100 (150) °C and sieved though #30-325 mesh.

CP100 (150) °C-3: hot air dried at 100 (150) °C and sieved though #325 mesh

CP100 (150) °C-4: hot air dried at 100 (150) °C and sieved though #30 mesh (15g/min).

CP100 (150) °C-5: hot air dried at 100 (150) °C and sieved though #30 mesh (10g/min).

Next, 1 g of each prepared CP powder was added separately to 10 mL of ethanol. Each of the solutions was then divided into two batches: extraction by UB for 2 h or by UP for 10 min. The supernatant of each extract was then filtered and stored in the refrigerator for analylsis.

C. Fermentation of CP Powders with A. oryzae and Preparation of CP Extracts

The influence of using CMC as the sole carbon source in inducing cellulose-degrading enzymes in the culture medium of *A. oryzae* (acquired from the Bioresource Collection and Research Center, Food Industry Research and Development Institute, Taiwan) was tested by liquid-state fermentation with two different particle sizes of CP powders (CP30 & 325 mesh), which was then extracted using UB40 and UP20 to compare the extaction yield of each CP extract. *A. oryzae* was cultured for 2–3 days at 25°C. Approximately 3–5 activated single colonies were then loaded into 10 mL of sterile distilled water and vortex mixed for use as a liquid inoculum. Subsequently, 1 mL of the liquid inoculum was added to 100 mL of culture media (containing 1 g of CP powder) in an Erlenmeyer flask, and shaken (150 rpm) at 25°C for 3–5 days. During the period, 4 mL of the culture media was taken from the flask each day and mixed with 8 mL of ethanol for UAE. The resulting mixture was centrifuged at 6,000 rpm for 20 min and the supernatant was concentrated by reduced-pressure evaporator. Finally, 2 mL of methanol was added into the concentrate, which was then filtered through a 0.45 □m membrane and diluted for analysis.

D. Analysis of the Contents of Major Flavones in CP Extracts

The CP extracts prepared as previously described were diluted with methanol, filtered through a 0.45 μm membrane and then diluted to suitable concentrations before being analyzed by high-performance liquid chromatography (HPLC). The running condition for the

HPLC analysis was as follows: (1) Sample (20 µL) injected into the HPLC column (Cosmosil $5C_{18}$-AR-II, 4.6 I.D. × 25 cm; Nacalai Tesque, Japan); (2) mobile phase composition: 0.1% H_3PO_4 [A] and acetonitrile [B]; (3) gradient: 0–5 min [from 90% A, 10% B to 70% A, 30% B], 5–20 min [from 70% A, 30% B to 60% A, 40% B], 20–40 min [from 60% A, 40% B to 10% A, 90% B], and 40–45 min [from 10% A, 90% B to 5% A, 95% B]; and (4) flow = 1.0 mL/min, and detect wave length = 254 nm. The concentrations of major flavonoids in the CP extracts (hesperidine, tangeretin and nobiletin) were calculated through interpolation based on the standard curves plotted using standard samples.

E. Analysis of Antioxidant Activities of Various CP Extracts

(a) Total Phenolics

Total phenolics was determined by the *Folin–Ciocalteu* method using gallic acid as the standard. The regression curve equation of the standard was obtained through the following procedures: First, 1 mL solution of gallic acid in varied concentrations evenly mixed with the same volume of *Folin–Ciocalteu* reagent were prepared and left to stand for 5 min. Next, 2 mL of 20% Na_2CO_3 was added to the solution, which was again left to stand for 5 min and then centrifuged for 5 min. Subsequently, a UV/Vis spectrophotometer was used to measure the absorbance of the supernatant at 730 nm wavelength and the regression curve equation of the standard was determined. The total phenolics of CP extracts were tested by replacing the standard solution with suitable concentrations of CP extracts. The gallic acid equivalent (GAE) per mL of extract was derived by substituting the absorbance of each sample into the aforementioned equation.

(b) DPPH Radical Scavenging Activity

0.1 ml of CP extract diluted with ethanol was mixed with a freshly prepared 10 mM of DPPH ethanolic solution (0.9 mL) and the solution was left in the dark to stand for 30 min. Next, the solution was examined by the spectrophotometer for their absorbance at 517 nm. Notably, a lower absorbance value would represent stronger DPPH radical scavenging activity for CP exact.

DPPH radical scavenging activity（%） = [(A_{517} nm of control) – (A_{517} nm of sample) /(A_{517} nm of control] × 100

(c) Trolox Equivalent Antioxidant Capacity (TEAC) Assay

Miller *et al*. established the trolox equivalent antioxidant capacity (TEAC) assay to measure radical scavenging capacities of substances [26]. The removal of 2, 2'-azino-bis (3-ethylbenzothiazoline-6-sulphonic acid) (ABTS) radical was examined as the assessment index, and trolox was adopted to plot the standard curve. More recently, the method of Erkan *et al*. was adapted to prepare phosphate buffered saline (pH 7.4) by mixing ABTS with $K_2S_2O_8$, which was to be left to stand for 12–16 h in the dark [27]. The mixture turned into a blue-green stock solution with ABTS radicals, with ABTS and $K_2S_2O_8$ concentrations of 7 mM and 2.45 mM, respectively. Erkan et al. also noted that 300 µL of the solution should be diluted to 20 mL and left to stand in the dark for 1 h before use, and that its absorbance at 730 nm should be 0.788 ± 0.02.

In the present experiment, 990 µL of the diluted stock solution was evenly mixed with 10 µL of the CP extract at a suitable diluted concentration, and was then left to stand for 10 min before its absorbance at 734 nm was measured. Trolox in varied concentrations (0.2–2 mM) was used as the standard, and the calibration curve was plotted accordingly (R^2 = 0.9997). Furthermore, the trolox-equivalent concentration of the CP extract was calculated through interpolation, and the experiment was repeated three times.

F. Statistical Analysis

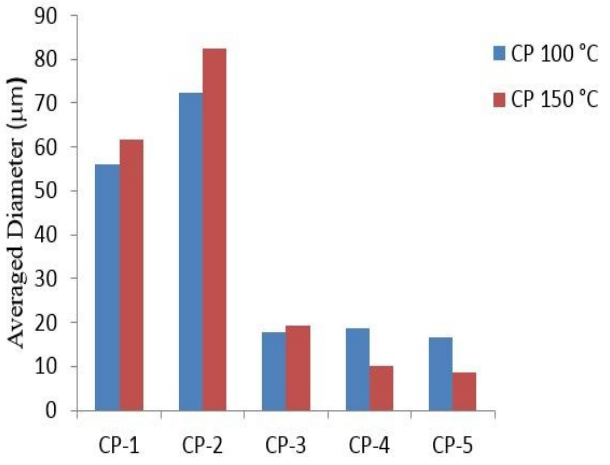
The present study used the SAS software suite to examine the experimental data through analysis of variance, followed by Duncan's multiple range test for post-hoc comparison ($p > 0.05$).

III. Results and Discussion

A. Preparation of CP Powders

Due to the increasing demand on converting food wastes or by-products to functional food ingredients, citrus flavones becomes valuable candidates because of their multiple health-promoting bioactivities [1-7]. In the present study, the CP powders were prepared first by hot-drying at either 100 °C or 150 °C (CP100 °C & CP150 °C), which were further grinded into powders of various diameters (CP-1 to CP-5) through a sieve shaker and a spiral jet mill. The diameters of the CP powders were shown in Figure 1(a). CP-1, CP-2 and CP-3 represented the average diameters of CP powders passing through various mesh sizes, 30, 30-325 and 325 meshes respectively, in the sieve shaker, and the distribution curves of these samples are illustrated in Figure 1(B); by contrast, CP-4 and CP-5 represented the particles generated by the spiral jet mill under different input settings. Comparing the average diameters showed that CP treated with hot-air drying at different temperatures had little effect on particle diameters, as indicated in Figure 1(a); the average diameters in CP100 °C-3 and CP150 °C -3 were 17.87 and 19.16 µm, which were far smaller than those in CP-1 and CP-2. In addition, the minimum diameter among the CP powders was prepared by the spiral jet mill (CP-4 & CP-5), especially in CP150°C -5 that was as small as less than 10 µm.

(a)



(b)

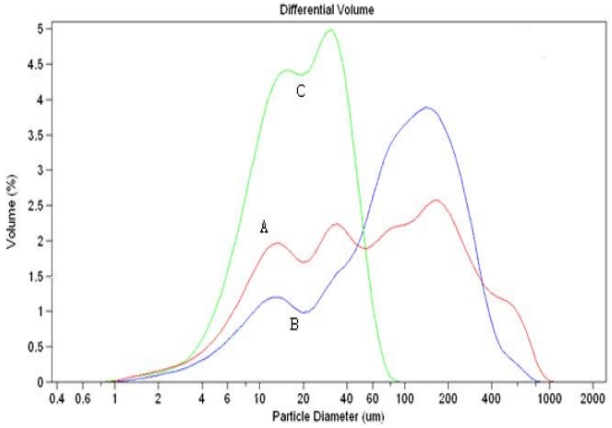


Fig. 1 (a) Average particle diameters of CP powders (b) The particle diameter distribution of three different CP powders (CP1-CP-3) prepared by sieve shaker. A: CP-1 B: CP-2 C: CP-3

B. Comparison of Polyphenol Contents in CP Extracts under Different Treatments

The CP powders prepared under varied conditions described previously were extracted using either a 20kHz probe-type ultrasound (UP20) or a 40 kHz ultrasonic bath (UB40), and the optimal processing procedure was determined by comparing the contents of polyphenols and other functional substances of CP extracts, as well as their antioxidant activities. As shown in Table 1, the CP powders were divided into two groups (CP100 °C & CP150 °C) according to the temperatures of their drying treatment, and each group was further divided into five subgroups by different micronization methods and diameter ranges. As indicated in Table 1, by comparing among the five CP extracts treated with UB40 in each of CP100 °C and CP150 °C groups, significantly different contents of polyphenols among the CP extracts in both group was observed because of their distinct particle sizes. Interestingly, the polyphenol content was not linearly correlated with particle diameter as expected, instead, CP100 °C -3 and CP150 °C -5 contained the highest amounts of polyphenols of 1.617 ± 0.027 mg/mL GAE and 1.619 ± 0.027 mg/mL GAE, correspondently. The powders of CP-4 and CP-5, despite having the smallest diameters after prepared by the spiral jet mill, were found to yield variable concentrations of polyphenols under UB40 treatment, which were less than that of CP-3.

Table 1. The contents of polyphenols in CP extracts after various treatments

GAE mg/mL	UB40	UP20
CP100 °C-1	1.112 ± 0.039^{a}	1.064 ± 0.124^{a}
CP100 °C-2	1.203 ± 0.114^{ab}	1.476 ± 0.151^{b}
CP100 °C-3	1.617 ± 0.027^{c}	1.562 ± 0.042^{b}
CP100 °C-4	1.470 ± 0.05^{bc}	$1.707 \pm 0.017^{b*}$
CP100 °C-5	1.403 ± 0.028^{bc}	$1.634 \pm 0.015^{b*}$
CP150 °C-1	1.330 ± 0.091^{b}	1.462 ± 0.017^{ab}
CP150 °C-2	1.046 ± 0.026^{a}	$1.397 \pm 0.010^{a*}$
CP150 °C-3	1.340 ± 0.061^{b}	1.570 ± 0.022^{c}
CP150 °C-4	1.197 ± 0.056^{ab}	$1.519 \pm 0.016^{bc*}$
CP150 °C-5	1.619 ± 0.027^{c}	1.589 ± 0.017^{c}

UB40: 40 kHz ultrasonic bath
UP20: 20 kHz ultrasonic probe
Data are expressed as mean ± standard deviation (n = 4).
Means within a column with different superscripts are significantly different ($p < 0.05$).
*Data from UP20 group are significantly different from those from UB40 group.

However, in another set of experiment of UP treatment, as noted in Table 1, the statistical analysis of the CP extracts ($p < 0.05$) revealed that among the CP100 °C groups (aside from CP100°C -1, which yielded relatively less polyphenols), the yields of CP100 °C -2, -3, -4, and -5 were not significantly different. The CP150 °C group exhibited similar result, which showed that the within-group leveling effect was prominent among CP150 °C -3, 4 and 5. Overall, the results suggested that the UP setting may have eliminated the interparticle electrostatic forces in the CP4 and CP5 subgroups prepared by the spiral jet mill. Additionally, the UP20 was evidently more capable of micronizing larger powder particles than the UB40.

C. Comparison of Antioxidant Activities of CP Extracts under Various Treatments

Next, we also compared the antioxidant activities of the CP100 °C and CP150 °C groups. First, the DPPH radical scavenging activities of CP extracts were measured as shown in Tables 2. Statistically, the most prominent significant difference was that the antioxidant activities of the UP20-treated extracts were significantly higher than those of the UB40-treated extracts (CP-UP20 > CP-UB40). Similar to previous result for polyphenol contents, the UP20-treated CP extracts exhibited less significant difference of leveling effect in antioxidant activities than the UB40-treated ones, especially within the CP 100 °C group, where the percentage of DPPH inhibition with range from 29.351 % to 38.488 % under UB40 treatment, was reduced to the range from 36.972 % to 42.839 % under UP20 treatment. This demonstrated that ultrasonic probe can effectively eliminate the physical differences in CP powders prepared by diverse processing procedures, including the differences in particle sizes and inter-particle electrostatic forces.

We also unexpectedly observed that when the TEAC assay was used to compare the CP extracts prepared through various processing procedures (whether different drying temperatures or ultrasonic frequencies), the within-group comparison of CP-1 to 5 would all exhibit little significant difference, especially even under UB treatment, TEAC values within the CP 100 °C group exhibited not statistical difference, with range from 10.438 ± 0.025 mM/mL(CP 100 °C-1) to 12.876 ± 0.023 mM/mL (CP 100 °C-3) (Table 2). This finding is probably explained by the different reaction environments in the two antioxidant assays (one was in alcoholic phase, whereas the other was aqueous phase). The major antioxidant polyphenols in CP extracts, which consist primarily of nobiletin and tangeretin, were lypophilic and alcohol-soluble compounds.

D. Comparison of Functional Components in CP Extracts under Various Treatments

The major functional components in the CP 100 °C and CP 150 °C groups of CP powders are listed in Table 3. The

results indicated that greater deviations in the analysis of the concentration of nobiletin and tangeretin among the CP extracts within CP 100 °C or CP 150 °C group was observed and the leveling effect after ultrasonic probe treatment was not prominent. The obvious deviation might be explained by the different process for preparing CP powders, resulting in the differentiated distribution of peel tissues in CP powders. In our previous study, the CP-3 contained less amount of cellulose fibers than the other CP powders, which might result in higher concentration of functional components in the CP-3 extracts**.** It is notable that the concentrations of nobiletin and tangeretin in the extracts were in the order of CP-3 > CP-1, CP -4, CP-5 > CP-2, and the content in the UP20 extracts was significantly higher than that in the UB extracts (i.e., CP-UP20 > CP-UB40). The highest concentrations of nobiletin in each group were 1.792 ± 0.114 mg/mL (CP 100 °C-3-UB40), 1.315 ± 0.199 mg/mL (CP 150 °C-3-UB40), 1.797 ± 0.039 mg/mL (CP 100 °C-3-UP20) and 1.739 ± 0.112 mg/mL (CP 150 °C-3-UP20). In contrast, the lowest concentrations of nobiletin in each group were 1.012 ± 0.307 mg/mL (CP 100 °C-2-UB40), 0.471 ± 0.087 mg/mL (CP 150 °C-2-UB40), 1.367 ± 0.081 mg/mL (CP 100 °C-2-UP20) and 0.931 ± 0.133 mg/mL (CP 150 °C-2-UP20). The remarkable reduced amount of nobiletin in CP 150 °C group was observed, in comparison with CP 100 °C under either UB40 or UP20 treatment. It is reasonable that high hot-air drying temperature over 100 °C might destroy functional flavones. The similar trend was also found in the analysis of tangeretin.

Due to the economic value of citrus peels, a number of studies have been reported to utilize UAE for extracting citrus polyphenols and optimize the extraction conditions, such as frequency, power, temperature, and duration [15-19]. A recent study focused on the effect of ultrasonic irradiation surface on yield of hesperidin from citrus peels by response surface methodology [18]. In addition, another recent study by M'hiria *et al*., compared four innovative extraction methods for orange peels, including ultrasound assisted extraction, microwave assisted extraction, supercritical CO_2 extraction and high pressure extraction, however, each method has its own notable limitation to reach the optimal extraction result [22]. In this study, we tried to combine a series of processing techniques to optimize the extraction yields of functional components in citrus peels, which has not been reported in other literatures.

E. Effects of Fermentation of CP Powders on Antioxidant Activities and Functional Components in CP Extracts

Finally, we further tried to optimize the processing by utilizing fermentation of CP powders (CP30 & 325 mesh) with *A. oryzae* cultivated in a culture medium that used CMC as the sole carbon source to induce cellulose-degrading enzymes. After fermentation, the ultrasonic bath and probe were used for extraction. The functional components in CP extracts and their antioxidant activities were then compared to determine the optimal processing technique. The results in Table 4 showed that by comparing the functional components (nobiletin, tangeretin) before and after fermentation revealed that significantly smaller concentrations in CP30-UP20, CP30-UB40 and CP325-UP20 groups after fermentation were observed. The results of the DPPH antioxidant assay (Table 4) also demonstrated that the antioxidant activity in the extracts following fermentation was significantly lower than that occurred before fermentation, indicating that *A. oryzae* did not facilitate the extraction of functional components in from the CP powders. In our previous study, the evaluation of the functionality of both fermented and unfermented citrus peels treated under hot air drying process indicated the unfermented citrus peel extract showed better antioxidant activity, containing more total polyphenols and functional flavonoid components, including nobiletin and tangeretin, among all of the CP extracts [28]. We also proposed that the biochemical transformations occurred in the fermentation process with microbials, resulting in the the aglycone converting into other unknown metabolites. A previous study indicated biotransformation of nobiletin by *Aspergillus niger* led to 4'-hydroxy-5, 6, 7, 8, 3'-pentamethoxyflavone [29]. In this study, the amounts of nobiletin and tangeretin gradually decreased after two days of fermentation by *A. oryzae*; additionally, new metabolites were generated from fermentation as indicated in HPLC analysis (data no shown). The mass spectrometry of one of the metabolites obtained through column separation determined its molecular weight to be 402. Finally, comparing the extraction yields and antioxidant activities signified that the unfermented CP325-UP20 (421.98 ± 19.44 ppm for nobiletin; 341.93 ± 13.12 ppm for tangeretin) attained the most favorable results, followed sequentially by the CP325-UB40, CP30-UP20, and CP30-UB40 groups.

IV. Conclusions

In this study, we tried to combine a series of processing techniques to optimize the extraction yields of functional components in citrus peels. The result indicated that UP20 effectively micronized large particles and eliminated the physical differences caused by the different processing techniques. Additionally, the results showed that the contents of functional components (nobiletin, tangeretin) were significantly reduced after fermentation, indicating that *A. oryzae* does not facilitate the extraction of functional components in CP. The study might provide information for extraction of valuable functional polymethoxyflavones from citrus (*Citrus reticulate*) peels.

Table 2. DPPH radical scavenging activities and TEAC antioxidant capacities in CP extracts after various treatments

DPPH % of Inhibition	UB40	UP20
CP100 °C-1	29.712 ± 0.017^{b}	$36.972 \pm 0.087^{b*}$
CP100 °C-2	29.351 ± 0.118^{b}	41.211 ± 0.179^{a}
CP100 °C-3	38.488 ± 0.073^{a}	41.944 ± 0.063^{a}
CP100 °C-4	35.132 ± 0.107^{ab}	42.839 ± 0.005^{a}
CP100 °C-5	33.976 ± 0.050^{ab}	$41.982 \pm 0.050^{a*}$
CP150 °C-1	30.482 ± 0.209^{b}	37.531 ± 0.025^{bc}
CP150 °C-2	27.101 ± 0.053^{b}	$36.363 \pm 0.048^{c*}$
CP150 °C-3	33.727 ± 0.073^{ab}	$39.943 \pm 0.024^{ab*}$
CP150 °C-4	28.866 ± 0.104^{b}	$39.135 \pm 0.029^{ab*}$
CP150 °C-5	39.818 ± 0.085^{a}	40.453 ± 0.037^{a}
TEAC TE mM/mL	UB40	UP20

DPPH % of Inhibition	UB40	UP20
CP100 °C-1	10.438 ± 0.025[a]	13.197 ± 0.013[a*]
CP100 °C-2	10.500 ± 0.050[a]	14.255 ± 0.034[a]
CP100 °C-3	12.876 ± 0.023[a]	14.807 ± 0.009[a]
CP100 °C-4	12.722 ± 0.035[a]	14.884 ± 0.006[a]
CP100 °C-5	11.941 ± 0.012[a]	14.500 ± 0.012[a*]
CP150 °C-1	12.416 ± 0.037[ab]	15.589 ± 0.017[a]
CP150 °C-2	9.135 ± 0.006[c]	14.056 ± 0.022[a*]
CP150 °C-3	12.155 ± 0.024[ab]	15.650 ± 0.018[a*]
CP150 °C-4	10.822 ± 0.029[ab]	15.282 ± 0.023[a*]
CP150 °C-5	14.929 ± 0.043[a]	15.604 ± 0.038[a]

UB40: 40 kHz ultrasonic bath
UP20: 20 kHz ultrasonic probe

Data are expressed as mean ± standard deviation (n = 4).

Means within a column with different superscripts are significantly different ($p < 0.05$).

*Data from U20 group are significantly different from those from U40 group

Table 3. The contents of functional components in CP extracts after various treatments

UB40	Nobiletin (mg/mL)	Tangeretin (mg/mL)
CP100 °C-1	1.314 ± 0.082	0.745 ± 0.095
CP100 °C-2	1.012 ± 0.307	0.548 ± 0.164
CP100 °C-3	1.792 ± 0.114	1.098 ± 0.162
CP100 °C-4	1.203 ± 0.081	0.661 ± 0.131
CP100 °C-5	1.171 ± 0.081	0.671 ± 0.087
CP150 °C-1	1.433 ± 0.249	0.516 ± 0.199
CP150 °C-2	0.471 ± 0.087	0.292 ± 0.087
CP150 °C-3	1.315 ± 0.199	0.801 ± 0.066
CP150 °C-4	0.899 ± 0.121	0.579 ± 0.098
CP150 °C-5	1.157 ± 0.134	0.650 ± 0.060
UP20	**Nobiletin (mg/mL)**	**Tangeretin (mg/mL)**
CP100 °C-1	1.453 ± 0.021[a]	0.855 ± 0.090[ab]
CP100 °C-2	1.367 ± 0.081[a]	0.666 ± 0.104
CP100 °C-3	1.797 ± 0.039	0.916 ± 0.068
CP100 °C-4	1.496 ± 0.212	0.759 ± 0.060
CP100 °C-5	1.661 ± 0.239	0.775 ± 0.081[b]
CP150 °C-1	1.346 ± 0.032	0.700 ± 0.080
CP150 °C-2	0.931 ± 0.133[b]	0.520 ± 0.067[b]
CP150 °C-3	1.739 ± 0.112	0.971 ± 0.086[b]
CP150 °C-4	1.297 ± 0.083[b]	0.673 ± 0.097
CP150 °C-5	1.350 ± 0.039	0.694 ± 0.060[b]

[a]Data from CP100 °C group are significantly different from those from CP150 °C group.
[b]Data from UP20 group are significantly different from those from UB40 group.

Table 4. Comparison of DPPH radical scavenging activities and the contents of functional components in CP extracts before/after fermentation

	DPPH % of inhibition	Nobiletin ppm	Tangeretin ppm
C-CP30-UP20	37.32 ± 4.50	335.88 ± 29.79	236.89 ± 33.71
F-CP30-UP20	18.47 ± 4.44[a]	265.78 ± 14.96[ac]	181.69 ± 27.79[a]
C-CP30-UB40	35.06 ± 7.54	278.85 ± 92.68	176.96 ± 98.68
F-CP30-UB40	17.55 ± 2.83[a]	193.70 ± 11.49	107.85 ± 6.68
C-CP325-UP20	43.76 ± 6.48	421.98 ± 19.44	341.93 ± 13.12 [c]
F-CP325-UP20	36.00 ± 2.33	350.87 ± 12.87[ab]	285.45 ± 8.42[ab]
C-CP325-UB40	36.30 ± 2.39	352.21.11 ± 36.50	233.75 ± 26.06
F-CP325-UB40	27.02 ± 2.60[ab]	382.09±30.98[b]	242.98 ± 8.85[b]

C: unfermented control group; F: fermented (3 days) group
CP30: sieved though #30 mesh; CP325: sieved though #325

Data are expressed as mean ± standard deviation (n = 4).
[a]Data from F group are significantly different from those from C group.
[b]Data from CP 325 group are significantly different from those from CP 30 group.
[c]Data from UP 20 group are significantly different from those from UB 40 group.

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