Food Chemistry 153 (2014) 279–284

Contents lists available at ScienceDirect

Food Chemistry

journal homepage: www.elsevier.com/locate/foodchem

Antioxidant-rich phytochemicals in miracle berry (*Synsepalum dulcificum*) and antioxidant activity of its extracts

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ARTICLE INFO

Article history:
Received 17 September 2013
Received in revised form 19 November 2013
Accepted 17 December 2013
Available online 24 December 2013

Keywords:
Miracle berry
Antioxidants
Phytochemicals
Phenolics
Fish oil

ABSTRACT

Miracle berry is known for its unique characteristic of modifying sour flavours to sweet. Twelve phenolics were identified and quantified in the miracle berry flesh at a level from 0.3 for kaempferol to 17.8 mg/100 g FW for epicatechin. Lutein and α-tocopherol were also quantified at a level of 0.4 and 5.8 mg/100 g FW, respectively. The TP and TF contents were 1448.3 GA and 9.9 QR mg Equiv/100 g FW for the flesh, respectively, compared with 306.7 GA and 3.8 mg QR mg Equiv/100 g FW of the seeds. The free radical scavenging and reducing percentage of the flesh extract was 96.3% and 32.5% in DPPH and ABTS assays, respectively. Additionally, the flesh extract had a high FRAP of 22.9 mmol/100 g. It significantly inhibited the oxidation of PUFA in fish oil as well. Thus, miracle berry could also serve as an antioxidant-rich fruit to provide health promoting function.

1. Introduction

Miracle berry (*Synsepalum dulcificum*), also called miracle fruit or red berry, is an indigenous tropical plant growing in West Africa. Although it belongs to the berry family, miracle berry is not as prevalent as other berry fruits such as blueberry, blackberry, cranberry, raspberry and grapes. Generally, miracle berry is about an inch in length with a bright red colour. It has a big seed surrounded by a thin layer of berry flesh with a faint cherry-like flavour (Inglett & Chen, 2011). There has been a growing interest in the potential use of miracle berries in foodstuffs, as it has a unique ability to convert sour tasting foods to sweet. The glycoprotein miraculin in miracle berry was reported to be responsible for this unique function by binding to the sweet receptor cells of the tongue, thus suppressing the response of a sour taste in the central nervous system (Yamamoto et al., 2006). This effect would last until the miraculin was diluted and eliminated by saliva. With the taste modification function, miracle berry has a great potential in food application as an alternative sweetener or taste modifier to mask undesirable sour tastes in food products (Wong & Kern, 2011).

Unlike other common berry fruits which nutrient, phytochemical and antioxidant activity have been extensively studied, the phytochemical profile and antioxidant properties of miracle berry have not been well documented. In fact, miracle berry could also be an abundant source of antioxidant-rich phytochemicals, just like blueberry, blackberry, cherry and grapes. These phytochemical antioxidants have been confirmed to possess health promoting functions in preventing various chronic diseases, such as cardiovascular diseases, obesity, diabetes and certain cancers (Xu, 2012a). In this study, the hydrophilic and lipophilic phytochemicals in miracle berry were identified and quantified. Furthermore, the antioxidant activity of miracle berry flesh seed extracts were evaluated by traditional free radical scavenging methods, ABTS (2,2′-azino-bis(3-ethyl-benzothiazoline-6-sulfonic acid), DPPH (1,1-diphenyl-2-picrylhydrazyl) and FRAP (ferric-reducing/antioxidant power). Also, a fish oil emulsion model was used to measure the antioxidant activity of the extracts in stabilizing lipid oxidation. As the long chain unsaturated fatty acids in fish oil, eicosapentaenoic acid (EPA) and docosahexaenoic acid (DHA) are extremely vulnerable to oxidation, the efficiency of miracle berry in inhibiting the fatty acids oxidation could reflect its antioxidant capability in stabilizing lipid oxidation. The high antioxidant activity of miracle

Abbreviations: ABTS, 2,2′-azino-bis(3-ethyl-benzothiazoline-6-sulfonic acid); DHA, docosahexaenoic acid; DPPH, 1,1-diphenyl-2-picrylhydrazyl; EPA, eicosapentaenoic acid; Equiv, equivalent; FRAP, ferric-reducing antioxidant power; FW, fresh weight; GA, gallic acid; GC, gas chromatography; HPLC, high performance liquid chromatography; MFH, miracle fruit extract at a level of 6 mg/ml of emulsion; MFL, miracle fruit extract at a level of 3 mg/ml of emulsion; PUFA, polyunsaturated fatty acids; QR, quercetin; TF, total flavonoids; TP, total phenolics.

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http://dx.doi.org/10.1016/j.foodchem.2013.12.072

berry could provide health promoting function in its food application, in addition to the taste modifying function. In general, the results obtained from this study would be helpful to explore the mechanism of the health promoting functions of miracle berry and provide a potential utilisation of miracle berry extract as a food ingredient with both antioxidant and taste modification functions.

2. Materials and methods

2.1. Chemicals and materials

HPLC grade acetonitrile, acetic acid, methanol and hexane were purchased from Fisher Chemicals (Fair Lawn, NJ, USA). Acetone was purchased from Macron (Charlotte, NC, USA). Ethyl acetate was from EM Science (Gibbstown, NJ, USA). Tween 20, 2,2-diphenyl-L-picrylhydrazyl (DPPH), 2,4,6-tripyridyl-s-triazine (TPTZ), Folin–Ciocalteau reagent, Trolox, menhaden fish oil, heptadecanoic acid (C17:0), EPA, DHA, α-tocopherol, α-tocotrienol, γ-tocopherol, γ-tocotrienol, cyanidin chloride, epicatechin, rutin, myricetin, quercetin, kamepferol, gallic, ellagic, syringic and ferulic acid standards were purchased from Sigma–Aldrich (St. Louis, MO, USA). Fresh miracle berry *(S. dulcificum)* was obtained from the South Subtropical Crops Research Institute in Zhanjiang, China.

2.2. Extraction of phytochemicals, tocopherols and carotenoids in miracle berry

After miracle berry seeds were separated from the flesh, the seeds and flesh were separately ground using a kitchen blender. Twenty grams of the ground flesh or seed sample was homogenously mixed with 50 ml of methanol to extract phytochemicals at 60 °C for 30 min. After 10 min of centrifugation, the methanol layer was transferred to a clean tube. The residue was mixed with 50 ml of methanol to repeat the extraction. The methanol layer was combined with the previously obtained methanol layer. Miracle berry flesh or seed extract was obtained after the methanol was evaporated by a vacuum centrifuge evaporator (Labconco, Kansas City, MO, USA). After the extracts were weighed, each extract was used to prepare a stock solution (100 mg/ml) with its corresponding extraction solvent. For quantifying tocopherols and carotenoids in the flesh, hexane and acetone instead of methanol were used to perform the extraction, respectively, with the same extraction procedure as the phytochemicals extraction.

2.3. Determination of total phenolic and flavonoid contents in the flesh and seed extracts

2.3.1. Total phenolics

The method used to determine the total phenolic content was described by Jang and Xu (2009). Folin–Ciocalteau reagent (0.75 ml) was diluted 10 times and mixed with 0.1 ml of diluted extract solution (1 mg/ml). The reaction was carried out for 5 min in dark. Then, sodium bicarbonate (60 g/l, 0.75 ml) was added. The reaction mixture was incubated at 25 °C for 90 min. The absorbance was measured by a UV–Vis double beam spectrometer (1600 Shimadzu, Kyoto, Japan) at 750 nm. Gallic acid was used to plot the calibration curve for calculation. The total phenolics content of the extract was calculated and expressed as mg gallic acid equivalent (GA Equiv)/100 g FW.

2.3.2. Total flavonoids

Total flavonoid content was determined based on the method described by (Kim, Chun, Kim, Moon, & Lee, 2003). One milliliter of diluted extract solution (1 mg/ml) was mixed with 0.3 ml of 5% $NaNO_2$ and 4 ml of distilled water. An aliquot (0.3 ml) of 10% $AlCl_3$ was added to the mixture followed by adding 2 ml of 1 M NaOH. The solution was immediately diluted to 10 ml using distilled water. The absorbance of the solution was measured at 506 nm. The total flavonoid content was calculated by using a calibration curve obtained from a quercetin standard and expressed as mg quercetin equivalent (QR Equiv)/100 g FW.

2.4. Identification and quantification of phytochemicals, ascorbic acid and tocols in the extracts

Phytochemicals such as anthocyanins, phenolic acids and ascorbic acid, as well as carotenoids, were determined by a reversed phase HPLC (2690, Waters, Torrance, USA) coupled with C18 column (id 250 × 4.60 mm, 5 μ, Phenomenex, Torrance, USA) and a diode array detector. The conditions of the HPLC methods for phytochemicals and ascorbic acid were developed based on the studies of Yue and Xu (2008) and Xu (2012b). The mobile phase was a mixture of A: 1% acetic acid in water and B: acetonitrile, with the percentage of B ramped from 0% to 100% in 100 min and then changed back to 0% at 101 min for 9 min with a constant flow rate of 0.8 ml/min. The detector was set at 520 nm for monitoring anthocyanins. The wavelength for monitoring each phenolic or ascorbic acid was based on the maximum absorption of its standard. Each anthocyanin was identified by comparison of an elution order with that of the study of Yue and Xu (2008). The concentration of each anthocyanin was calculated by the calibration curve of cyanidin chloride in molar concentration and converted to μg/g of sample based on its molecular weight. The concentrations of other phenolics were calculated by the external calibration curves of their corresponding standards. The HPLC condition for carotenoids analysis was described in the study of Kopec, Cooperstone, Cichon, & Schwartz (2012), with minor modifications. Tocopherols and tocotrienols were determined by a normal phase HPLC (1100 series, Agilent, Santa Clara, CA, USA) with a Supelcosil LC-Si column (id 250 × 4.60 mm, 5 μ, Supelco, Bellefonte, PA, USA). The conditions for the HPLC method were the same as the method described in the study of Jang and Xu (2009).

2.5. Determination of antioxidant activities of the extracts by using DPPH, ABTS and FRAP methods

2.5.1. DPPH

The DPPH assay was performed according to a previous study by Liyana-Pathirana and Shahidi (2005) with minor modifications. One milliliter of 0.135 mM DPPH methanolic solution was mixed with 1 ml of the extract (1000 μg/ml), kamperferol, catechin, gallic acid solution (100 μg/ml), or methanol as a blank. The mixture was then vortexed vigorously and left for 30 min at room temperature in the dark before its absorbance (Abs) was measured at 517 nm. The DPPH free radical scavenging activity was calculated by the equation below and expressed as the percentage of inhibition rate compared with the blank:

$$\text{DPPH free radical scavenging activity}(\%) = (1 - \text{Abs}_{\text{sample}}/\text{Abs}_{\text{blank}}) \times 100$$

where $\text{Abs}_{\text{sample}}$ was the absorbance of the mixture of the test sample and DPPH reagent after reaction; $\text{Abs}_{\text{blank}}$ was the absorbance the mixture of methanol and DPPH reagent after reaction.

2.5.2. ABTS

The ABTS assay was based on the procedure described in the study of Re et al. (1999). The solution consisting of 7 mM of ABTS and 2.4 mM potassium persulfate (1:1 v/v) was reacted in the dark for twelve hours at room temperature. Then, it was mixed with methanol to obtain an absorbance value 0.700 at 734 nm. One

milliliter of the diluted solution was mixed with 1 ml of the extract (1000 μg/ml), kamperferol, catechin, gallic acid solution (100 μg/ml), or methanol as a blank. After a 7 min reaction, the absorbance (Abs) was measured at 734 nm. The free radical scavenging capability was calculated by the equation below and expressed as the percentage of inhibition rate of free radial scavenging compared with the blank.

$$\text{ABTS radical scavenging activity}(\%) = (1 - \text{Abs}_{\text{sample}}/\text{Abs}_{\text{blank}}) \times 100$$

where $\text{Abs}_{\text{blank}}$ and $\text{Abs}_{\text{sample}}$ were the absorbance of the mixtures of blank and test sample, respectively.

2.5.3. FRAP

The FRAP procedure was based on the method developed by Benzie and Strain (1996). The FRAP reagent contained 25 ml of sodium acetate (300 mM in acetic acid, pH 3.6), 2.5 ml of TPTZ solution (10 mM in 40 mM HCl) and 2.5 ml of $FeCl_3 \cdot 6H_2O$ solution (20 mM). Ten microliters of the extract (1000 μg/ml), kamperferol, catechin, gallic acid solution (100 μg/ml), or $FeSO_4$ (1.0 mmol/l) as a reference was mixed with 1 ml distilled water and 1.8 ml of the FRAP solution. Then the mixture was reacted at 37 °C for 10 min. The absorbance of the reaction solution was recorded at 593 nm. The ferric reducing capability was calculated by comparing the absorbance of the reaction solution to the absorbance of the $FeSO_4$ reference and converting to mmol/100 g extract or standard.

2.6. Determination of antioxidant activity of the berry flesh extract in stabilizing fish oil

An emulsion consisted of 1% menhaden fish oil and 1% Tween 20 in phosphate buffer (pH 7.0). The emulsion was homogenised by a microfluidizer materials processor (M-110P, Microfluidics, Newton, MA, USA). Then, 3.0 or 6.0 mg/ml of the berry flesh hydrophilic extract in the emulsion was prepared in the same way as the treatments. Based on the results obtained from the determination of the total phenolic content, the total phenolic content of 3.0 mg of the extract was equivalent to 0.5 mg of gallic acid. Thus, 0.5 mg/ml of gallic acid in the emulsion was also prepared as a reference. The emulsion without the extract or gallic acid was used as a blank. Each treatment or blank emulsion (20 ml) was added to a 40 ml test vial. The vials were incubated at 37 °C with continuous agitation by a multiple magnet stirrer (Multistirrer, VELP Company, Italy) until the experiment was completed. The levels of EPA and DHA in each fish oil emulsion were determined at 0, 24, 48 and 72 h. The analysis method of EPA and DHA was described in the study of Zhang, Shen, Prinyawiwatkul, King, and Xu (2013) with modifications. One milliliter of each emulsion sample was extracted with 2 ml of hexane which contained C17:0 as the internal standard (100 μg/ml). The hexane layer was separated and evaporated to obtain dry oil extracts in a clean test tube. Then, 2 ml of BCl_3 was added to the dried oil to perform esterfication at 60 °C in a water bath for 30 min. Then, the reaction solution was mixed and vortexed with 1 ml hexane and 1 ml water. The upper hexane layer was dehydrated by anhydrous sodium sulfate and transferred to a GC vial. The GC condition was the same as that in the study of Zhang et al. (2013). The retained EPA and DHA in the emulsion were calculated with the following formula:

$$\text{Retained rate}(\%) = (C_t/C_0) \times 100$$

where C_0 was the concentration of EPA or DHA at 0 h; C_t was the concentration of EPA or DHA at 0, 24, 48 or 72 h in the same emulsion.

2.7. Data analysis

Each determination was repeated in triplicate. The results of the total phenolic and flavonoid content, DPPH, ABTS and FRAP assays, and identified components in the extracts were expressed as means ± standard deviation. The significant differences among treatments were conducted by one-way ANOVA at $P < 0.05$ (SAS, 9.1.3, Cary, NY, US).

3. Results and discussions

3.1. Extraction yields, total phenolic and flavonoid contents and phytochemicals in miracle berry flesh and seeds

The yields of the hydrophilic extracts from the berry flesh and seeds were 8.6 and 0.6%, respectively (Table 1). The total phenolic content of the berry flesh was 1448.3 mg GA Equiv/100 g FW, which was approximately five times higher than that of the seeds (306.7 ± 44.1 mg GA Equiv/100 g FW) (Table 1). Also, compared with the total phenolics in other reported berry fruits, such as blackberry (435.0 mg GA Equiv/100 g FW), blueberry (348.0 mg GA Equiv/100 g FW), Corema album berry (121.4 mg GA Equiv/100 g FW) or strawberry (83.9 mg GA Equiv/100 g FW), the miracle berry in this study had a much higher total phenolic content (Heinonen, Lehtonen, & Hopia, 1998; León-González et al. 2013). For the total flavonoid content, the berry flesh contained 9.9 ± 0.5 mg of QR Equiv/100 g FW, which was approximately three times higher than in the berry seeds (3.8 ± 0.4 mg of QR Equiv/100 g FW) (Table 1). The total phenolic and flavonoid contents are two general indices widely used to represent the overall antioxidant capability in a sample (Xu, 2012b). Based on these results, the overall antioxidant capability of miracle berry flesh was much higher than that of the berry seeds. In other words, more antioxidant-rich phytochemicals in miracle berry are located in the flesh rather than the seeds.

The level of ascorbic acid in the miracle berry flesh was 28.9 mg/100 g FW (Table 2) and higher than blueberry or blackberry which was reported in a range of 4–19 mg/100 g FW (Ruiz et al., 2013). Epicatechin, rutin, quercetin, myricetin, kaempferol, ellagic, gallic, ferulic, syringic acid and three anthocyanins were identified in the flesh (Fig. 1). Epicatechin was the dominant phenolic in the miracle fruit flesh at 17.8 mg/100 g FW and attributed to 41.4% of the total phenolics identified (Table 2). Although ellagic and syringic acids are not commonly found in most berry fruits, they were present in the miracle berry flesh at levels of 0.4 and 3.3 mg/100 g FW, respectively (Table 2). Also, the levels of gallic and ferulic acids in the miracle berry flesh were 10.7 and 5.8 mg/100 g FW, respectively (Table 2), compared with 0.1–6.2 mg/100 g FW for gallic acid and 0.2–1.9 mg/100 g FW for ferulic acid in the antioxidant-rich berries, such as cranberry, blueberry, red raspberry and strawberry (Häkkinen et al., 1999). The level of quercetin in the miracle berry flesh was 1.1 mg/100 g FW and higher than that of cherry or red raspberry, which was reported at below 0.5 mg/100 g FW in a study by Jakobek, Seruga, Novak, and Medvidovic-Kosanovic (2007). Also, the miracle berry flesh contained a higher level of myricetin (0.8 mg/100 g FW) than red

Table 1
Extraction yield and total phenolic and flavonoid contents of miracle berry flesh and seed extracts.

(Unit:/100 g FW)	Flesh	Seed
Extraction yield (g)	8.6 ± 0.1	0.6 ± 0.1
Total phenolic content (mg GC Equiv)	1448.3 ± 96.1	306.7 ± 44.1
Total flavonoid content (mg QR Equiv)	9.9 ± 0.5	3.8 ± 0.4

Table 2
The levels and maximum absorption wavelengths of ascorbic acid, phenolics, anthocyanins, carotenoids and tocopherols in miracle berry flesh.

Name	Concentration (mg/100 g FW)	Wavelength (nm)
Ascorbic acid	28.9 ± 0.9	243
Epicatechin	17.8 ± 0.3	280
Gallic acid	10.7 ± 0.2	272
Ferulic acid	5.8 ± 0.1	324
Syringic acid	3.3 ± 0.2	276
Rutin	2.8 ± 0.1	326
Quercetin	1.1 ± 0.1	370
Myricetin	0.8 ± 0.1	370
Ellagic acid	0.4 ± 0.0	250
Kaempferol	0.3 ± 0.0	364
Delphinidin glucoside	0.8 ± 0.1	520
Cyanidin galactoside	2.6 ± 0.1	520
Malvidin galactoside	10.1 ± 0.7	520
Lutein	0.4 ± 0.0	447
α-Tocopherol	5.8 ± 0.3	290
α-Tocotrienol	0.6 ± 0.1	290
γ-Tocopherol	1.0 ± 0.1	290

current and sweet cherry, which were in a range of 0.02–0.2 mg/100 g FW (Jakobek et al., 2007). Anthocyanins in the berry flesh were at a total level of 13.5 mg/100 g FW, and may be responsible for the red colour of miracle berry. This was similar to the level (14.5 mg/100 g FW) obtained by a spectrophotometric method reported in the study of Buckmire and Francis (1976). However, with the HPLC method, delphinidin-glucoside, cyanidin-galactoside and malvidin-galactoside were identified as the three primary anthocyanins in the miracle berry flesh at an approximate ratio of 12:3:1 (Table 2).

For lipophilic antioxidants, the level of α-tocopherol was much higher than that of α-tocotrienol and γ-tocopherol (Table 2). The level of α-tocopherol (5.8 mg/100 g FW) in the miracle berry flesh was superior to that in other berry varieties, which are reported in a range of 0.7–2.1 mg/100 g FW (Carvalho, Fraser, & Martens, 2013). Also, lutein was the only carotenoid detected in the miracle berry flesh at a level of 0.4 mg/100 g FW (Table 2). Compared with other antioxidant-rich berries, the miracle berry had higher levels of important hydrophilic phenolics and lipophilic tocols and carotenoid, which may contribute to the antioxidant capability and health promoting functions of miracle berry.

3.2. The antioxidant capabilities of miracle berry flesh and seed extract in DPPH, ABTS, and FRAP assays

Three antioxidant activity assays, DPPH, ABTS and FRAP were applied to assess the antioxidant capabilities of both miracle berry flesh and seed extracts. The antioxidant activity determined by DPPH assay is the activity of quenching free radicals or H-donor capability of the antioxidant (Sasikumar, Patharaj, Adithya, Christabel, & Shamna, 2012). The DPPH free radicals reacted with a hydrogen donated from the antioxidant and form their corresponding hydrazine (Sanchez-Moreno, 2002). The results of this study indicated that the free radical scavenging percentage of miracle berry flesh extract was 96.3%, and not significantly different from that of catechin (94.4%) or gallic acid (92.9%) at 100 μg/ml (Fig. 2). However, it was significantly higher than that of the seed extract (54.3%) or kaempferol (84.4%). For the ABTS method, activity is based on the intensity of an antioxidant in scavenging the radical cation generated by ABTS. The free radical scavenging percentage of the flesh extract was 32.5%, which was twice that of the seed extract (18.0%), similar to kaempferol (30.7%), but lower than catechin (46.7%) or gallic acid (91.3%) used in the assay (Fig. 2). FRAP assay is a SET (singlet electron transformer) method which is based on the reduction capability of ferrous ion (Fe^{3+}) to ferric ion (Fe^{2+}) of a test sample (Ou, Huang, Hampsch-Woodill, Flanagan, & Deemer, 2002). It was found that the reduction capability of the berry flesh extract (22.9 mmol/100 g) was significantly higher than the seed extract (5.2 mmol/100 g), kaempferol (4.0 mmol/100 g), catechin (12.0 mmol/100 g), or gallic acid (7.5 mmol/100 g) (Fig. 2). The higher reduction capability of the berry flesh extract could be attributed to the higher levels of phenolics and ascorbic acid, which were considered to be stronger reductants in donating electrons. They could convert free radicals into stable products and terminate the free radical chain reaction (Sasikumar et al., 2012).

The results obtained from the traditional antioxidant activity assays indicate that the miracle berry flesh extract had significantly higher antioxidant capability than the seed extract (Fig. 2).

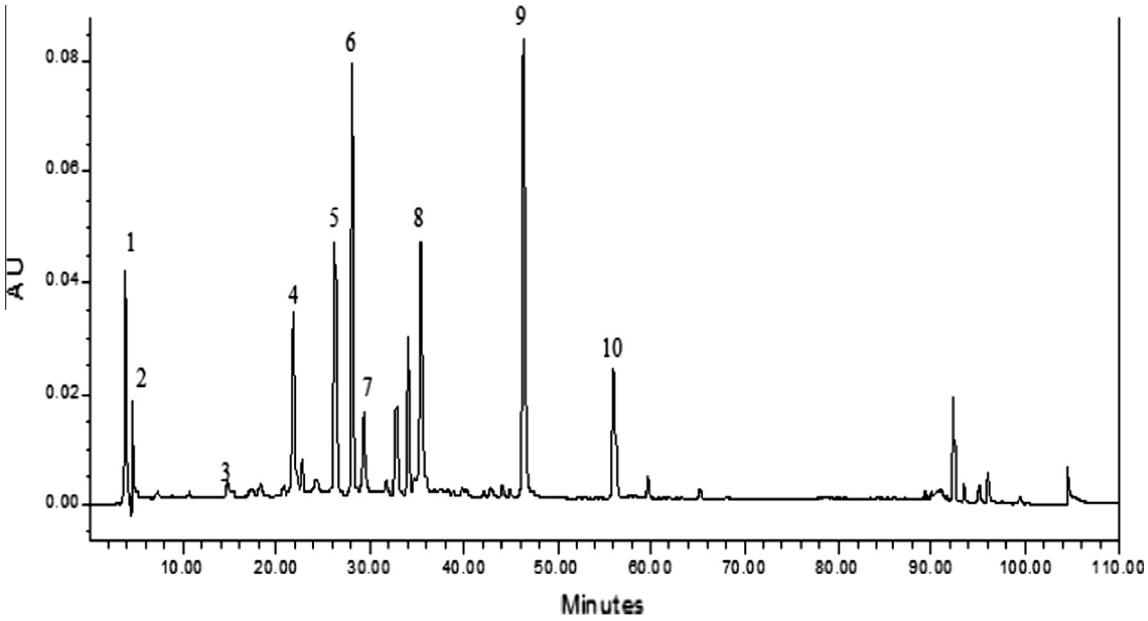

Fig. 1. Chromatogram of ascorbic acid and phenolics in miracle berry flesh at wavelength 370 nm, 1 ascorbic acid; 2 gallic acid; 3 epicatechin; 4 syringic acid; 5 ellagic acid; 6 rutin; 7 ferulic acid; 8 myricetin; 9 quercetin; 10 kaempferol. *Peaks without numbers were unidentified compounds.

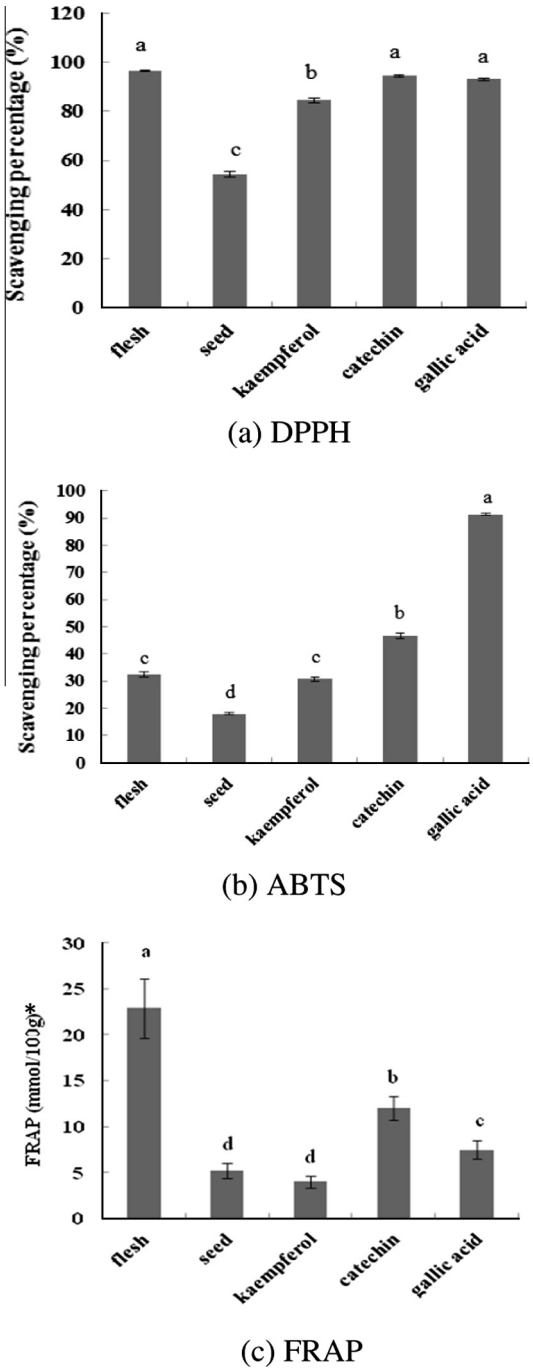

Fig. 2. The free radical scavenging percentages of the berry flesh and seed extracts, kaempferol, catechin and gallic acid in DPPH (a) and ABTS (b) assays and their ferric-reducing antioxidant powers in FRAP (c) assay.

This capability was also equivalent to common phenolic acid and flavonoid antioxidants at the same concentration. In a previous study, the DPPH free radical scavenging activity was in a range of 8.3–23.3% in raspberry, below 16% in blackcurrant and 70% in strawberry (Balogh, Hegedűs, & Stefanovits-Bányai, 2010). However, it was as high as 96.3% in the miracle berry flesh extract, even at a relatively lower concentration than the samples in that study. Although the percentage of scavenging ABTS radical ranged from 63.3% to 95.3% among different cultivars of grapes in the study of Du et al. (2012), the grape extract in their study was diluted at a ratio of 1:10 (w/v), which was hundred fold higher than the miracle berry extract solution used in this study. Thus, miracle berry exhibited significant higher ABTS scavenging activity than grapes. In the FRAP assay, the miracle berry flesh extract maintained stronger ferric-reducing power (22.9 mmol/100 g) than that of myrtle berry extract (0.7–8.4 mmol/100 g) (Tuberoso et al., 2010). Therefore, the antioxidant capability of miracle berry was superior to many other recognised antioxidant-rich fruits in scavenging free radicals.

3.3. The antioxidant capability of miracle berry flesh in stabilizing fish oil

Fish oil is abundant in EPA (C20:5) and DHA (C22:6) fatty acids, which are two long chain unsaturated omega-3 fatty acids and have been recommended for promoting cardiovascular health (Sirot et al., 2012). However, due to the high degree of unsaturation, the incorporation of EPA and DHA in food could increase the tendency for lipid oxidation. In order to retain the omega-3 fatty acids and stabilizing fish oil against oxidation during food processing and storage, usually a synthetic antioxidant is added in the food. However, the safety of long term consumption of synthetic antioxidants is a concern, as the antioxidants could accumulate in the liver or even cause carcinogenesis (Iqbal & Bhanger, 2007). In this study, the capability of stabilizing EPA and DHA against oxidation was evaluated. Also, the fish oil was homogenised with tween in phosphorous buffers (pH = 7.2) and incubated at 37 °C to simulate the environment of vulnerable lipids in the human serum. The retention of EPA or DHA in the emulsion reflected the status of lipid oxidation in fish oil. The results of retained EPA and DHA in different treatments and blank are shown in Fig. 3. After 24 h oxidation, EPA and DHA dropped dramatically in the blank, followed by the GA group. However, only 14% of EPA and 17% of DHA were oxidised in the MFL (0.3 mg/ml) group. After 48 h, the retained EPA and DHA were only 17% and 13% and 34% and 24% in the blank and GA group, respectively, while they were 59% and 48% in the MFL group. The retained EPA and DHA in MFL

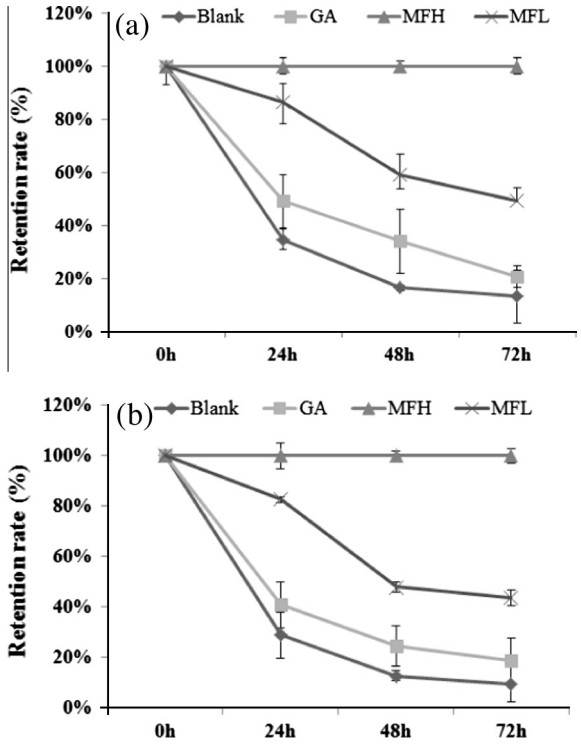

Fig. 3. The retention rates of EPA (a) and DHA (b) in blank, GA-gallic acid (0.5 mg/ml), MFL-miracle flesh extract (3.0 mg/ml) and MFH-miracle flesh extract (6.0 mg/ml) treatments of the fish oil emulsion model.

were still several folds higher than the blank or GA group after 72 h oxidation. In MFH (0.6 mg/ml) group, the retention of the two fatty acids maintained 100% after 72 h. Although the scavenging free radicals capability of gallic acid was significantly higher than the miracle berry flesh extract in the ABTS assay, the inhibition of lipid oxidation of the miracle berry extract was more effective than gallic acid. It also indicated that the traditional antioxidant activity assays could not directly reflect the capability of an antioxidant in preventing lipid oxidation in an oil-water emulsion. Actually, lipid oxidation or the related oxidised products in the human body is the initiator of tissue cell inflammatory and could result in the risk of developing various chronic diseases (Singh et al., 2009). The effectiveness of an antioxidant in preventing lipid oxidation indicates the higher capability in reducing toxic lipid oxidation production and potentially preventing the risk of tissue inflammatory and chronic diseases. Thus, the miracle berry extract could be a food antioxidant to effectively stabilize lipids in food products and prolong their shelf-life. It could also provide effective health promoting functions due to its greater potential in inhibiting lipid oxidation and toxic oxidised products in human serum.

4. Conclusion

Epicatechin, rutin, quercetin, myricetin, kaempferol, gallic, ferulic, syringic acid, three anthocyanins (delphinidin glucoside, cyanidin galactoside and malvidin galactoside), three tocopherols (α-tocotrienol, α- and γ-tocopherol) and lutein were identified and quantified in the miracle berry flesh. Some of the important antioxidant-rich phenolics and ascorbic acid in the miracle berry had much higher levels than those in well recognised antioxidant-rich berries, such as blueberry and blackberry. In the ABTS and DPPH assays, the free radical scavenging activities of the flesh extract was similar to other antioxidant standards. However, in the FRAP assay, the activity of the flesh extract was significant higher than other antioxidant standards. Furthermore, the miracle berry extract exhibited greater capability in preventing lipid oxidation in the fish oil emulsion than gallic acid in this study. Thus, it could potentially be used as a food ingredient not only to replace synthetic food antioxidants, but also to provide health promoting functions in reducing the risk of chronic diseases associated with lipid metabolism problem.

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