Enhanced yield of phenolic extracts from banana peels (*Musa acuminata* Colla AAA) and cinnamon barks (*Cinnamomum varum*) and their antioxidative potentials in fish oil

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Abstract The bioactive compounds of banana peels and cinnamon barks were extracted by vacuum microwave and ultrasonic-assisted extraction methods at pre-determined temperatures and times. These methods enhance the yield extracts in shorter time. The highest yields of both extracts were obtained from the conditions which employed the highest temperature and the longest time. The extracts' yield from cinnamon bark method was higher by ultrasonic than vacuum microwave method, while vacuum microwave method gave higher extraction yield from banana peel than ultrasonic method. The phenolic contents of cinnamon bark and banana peel extracts were 467 and 35 mg gallic acid equivalent/g extract, respectively. The flavonoid content found in banana peel and cinnamon bark extracts were 196 and 428 mg/g quercetin equivalent, respectively. In addition, it was found that cinnamon bark gave higher 2,2-Diphenyl-1-picrylhydrazyl (DPPH) radical scavenging activity and total antioxidant activity (TAA). The antioxidant activity of the extracts was analyzed by measuring the peroxide and *p*-anisidine values after oxidation of fish oils, stored for a month (30 days) at 25 °C and showed lesser peroxide and *p*-anisidine values in the fish oils containing the sample extracts in comparison to the fish oil without containing any extract. The banana peel and cinnamon extracts had shown the ability as antioxidants to prevent the oxidation of fish oil and might be considered as rich sources of natural antioxidant.

Keywords Banana peel · Cinnamon bark · Phenolic extracts · Fish oil · Microwave · Ultrasonic · Antioxidant

Introduction

The fruit and vegetable wastes (e.g. peels, seeds) are the non-product flows of raw materials whose economic values are less than the cost of collection and recovery for reuse; and therefore discarded as wastes. These wastes could be considered valuable by-products if there were appropriate technical means and if the value of the subsequent products were to exceed the cost of reprocessing (Scheiber et al. 2001). The agro-residues cannot be regarded as the wastes but become an additional valuable resource to augment existing natural materials. Recycling, reprocessing and eventual utilization of food processing residues offer potential of returning these by-products to beneficial uses rather than their discharge to the environment which cause detrimental environmental effects.

Phenolics are found in a plenty of plants and consist of an aromatic ring within the molecular structure (Singh et al. 2011). The phenolic compounds having antioxidant properties can prevent the oxidations of oil (Kaur and Kapoor 2001). Banana fruits contain various antioxidants such as gallic acid and dopamine. Interestingly, banana peel extracts have also been found to contain a high capacity to scavenge 2,2-diphenyl-1-picrylhydrazyl (DPPH•) and 2,2'-azino-bis (3-ethylbenzothiazoline) -6-sulfonic acid (ABTS•+) free radicals (González-Montelongo et al. 2010; Kedare and Singh 2011). Moreover, the extraction of antioxidants from banana peels is a great way for waste management because the main by-product from banana

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processing industry is its peel. Cinnamon barks are used extensively as spices of food or to produce essential oils. The plant leaf and bark have a hot taste and evolves a spicy odor when crushed (Wojdyło et al. 2007). Extraction and solubility of phenolics is governed by their chemical nature in the plant that may vary from simple to very highly polymerized substances. Plant materials may contain varying amounts of phenolic acids, phenylpropanoids flavones, flavonols, anthocyanins, and tannins (Wang and Weller 2006; Kaushik et al. 2010). There is possibility of interaction of phenolics with other plant components such as carbohydrates and proteins. These interactions may lead to the formation of complexes that may enhance insolubility (Arnao 2000). Solubility of phenolics is also affected by the polarity of solvents used during extraction. Solvents, such as methanol, ethanol, propanol, acetone, ethyl acetate, dimethylformaldehyde and their combinations have mostly been used for the extraction of plant phenolics, frequently with different concentrations of water (Kwon et al. 2003). The recovery of polyphenols from fruits and vegetables is also influenced by the extraction time, temperature and the related factors. Conventional extraction is usually performed by maceration. This method is tedious, time consuming and requires relatively large quantities of solvents with low efficiency. Ultrasound and microwave radiation could accelerate the extracting process and this may improve the extraction of bioactive compounds. Extraction using microwave and ultrasonication can result the increased in yield in shorter time at the same temperature using less solvent. Microwave-assisted extraction heats the extracts quickly and accelerates the extraction process for adsorption and desorption of the targeted compounds from matrix. Microwaves have been used for the extraction of few of the bioactive compounds, such as extraction of essential oils from the leaves of rosemary and peppermint (Chen and Spiro 1994) and extraction of glycyrrhizic acid from licorice root (Pan et al. 2000).

Ultrasound-assisted extraction is faster and more complete in comparison with the conventional method such as maceration/stirring. Benefits of ultrasound are generally attributed to acoustic cavitation phenomenon that is formation, growth and collapse of microbubbles inside a liquid phase submitted to ultrasonic cavitations. These impulsive collapses lead to extreme conditions with the generation of micro-jets and shock waves that imply strong conditions on the solid phase resulting in erosion, fragmentation or disaggregation of the samples (Mason et al. 1996).

The lipid oxidation in foods is associated almost exclusively with the unsaturated fatty acids and is often autocatalytic especially, the fish and some vegetable oils. The fish oil contains abundant amount of polyunsaturated fatty acids

(PUFA) and is very much prone to be oxidized. Due to safety and limitation of synthetic antioxidant usage, natural antioxidants obtained from edible materials, edible byproducts and residual sources have alternately interesting. Plant extracts provide phenolic antioxidants that might exhibit strong antioxidative activity. Cinnamon extracts were able to reduce lipid peroxidation in the β -carotene-linoleic acid system, and the inhibitory effect was comparable to the synthetic one such as butylated hydroxyanisole (BHA) (Mathew and Abraham 2006). Similarly, banana peels extracts have been evaluated for the antioxidative activity, measured as the effect on lipid oxidation, in relation to its gallicocatechin content (Someya et al. 2002). The aim of this research is to improve the techniques using ultrasonic and microwave-assisted techniques for enhancing the yield of extracts in the form of bioactive compounds from fruit and vegetable waste. This research also focuses for evaluating the effects of storage period on the antioxidant activity of banana peel and cinnamon bark extracts in fish oil as model substrate and compares the effects among all samples.

Material and methods

Ripen banana (*Musa acuminata* Colla AAA) and cinnamon bark (*Cinnamomum varum*) were brought from the local market in Bangkok, Thailand. The banana peels and cinnamon barks were cut into small pieces and dried at 50 °C for 48 h using hot air oven. The dried samples were crushed into the powder by using a blender and kept in a vacuum aluminum bag under refrigeration (4 °C) until further use. Purified salmon fish oil (without containing any preservative) was obtained from Ultradog Product Company, Thailand. The analytical grade of chloroform, ethanol, ferric chloride, and all other chemicals used were bought from Sigma Chemical Company Limited (St. Louis, MO, USA). Purified water from an Ultrapure Water System was used for the preparation of all solutions.

Extracts yield from banana peels and cinnamon barks

The powdered samples (5 g) were extracted with 100 ml of 95 % (v/v) ethanol using microwave and ultrasonic extraction at 40, 50 and 60 °C. The extraction times were 10, 15, 20 min and 30, 60, 90 min for microwave (CRS Concave Reflex System, DAOVOO KOR-6327 Model) and ultrasonic (Cole-Palmer Instrument Company Limited, Germany), respectively. The solutions (containing extracts and solvents) were filtered with whatman no. 1 filter paper. The solvents were then evaporated using rotary evaporator (BUCHI R-144V, Germany) at 50 °C under 100 mbar.

The dried extract was accurately weighed and the extract yield was then calculated and expressed as the percentage of the crude extract to the raw materials:

$$\text{Extract yield(\%)} = (\text{g of extract} / \text{g of dried samples}) * 100 \quad (1)$$

Total phenolic contents of extracts from banana peels and cinnamon barks

The total phenolic contents were determined using the Folin–Ciocalteu method as described by Singleton et al. (1999). An aliquot of extract (100 µL) was diluted with 5 ml distilled water followed by addition of freshly prepared 250 µL of Folin–Ciocalteu reagent. After 5 min of incubation at room temperature (21 °C), 1 mL of 10 % (w/v) sodium carbonate in ultrapure water was added and mixed well. After 20 min of standing at room temperature, the absorbance of the mixtures was determined at 760 nm against the blank. The phenolic content was expressed as mg of gallic acid as a standard. All the experiments were done in triplicate.

Flavonoid contents in the extracts from banana peels and cinnamon bark

The flavonoid content was analyzed following the method described by Meda et al. (2005) with slight modifications. An aliquot of extract solution (100 µL) was diluted with 5 ml of ultrapure water, followed by addition of 5 % sodium nitrite (300 µL). After incubation of this mixture for 5 min under ambient temperature, aluminum trichloride (10 %, w/v), solubilised in ethanol (300 µL) was added. The mixture was incubated for 6 min at room temperature, followed by addition of 4 ml of 0.1 M sodium hydroxide and 0.4 mL of ultrapure water just before measuring the absorbance at 415 nm by UV–vis spectrophotometer against a blank sample (the mixture solution without sample extracts). Quercetin was used as a standard in different concentrations (0–0.30 mg/ml) to quantify the flavonoid contents. All the experiments were done in triplicate.

DPPH scavenging activity of the extracts from banana peels and cinnamon barks

The antioxidant activity of extracts was measured in terms of hydrogen donating or radical scavenging ability using the stable DPPH method (Blois 1958). In brief, the extract sample solution (2 mL) dissolved in 50 % ethanol (v/v) was mixed with 4 ml of 0.2 mM DPPH dissolved in ethanol. The reaction mixture was incubated for 30 min at room temperature in the dark. When DPPH reacts with an antioxidant compound that can donate hydrogen, it gets reduced and the resulting decrease in absorbance at 517 nm was

recorded at 10 min intervals up to 30 min using a UV–vis Spectrophotometer (Shimadzu UV–vis 2100). The control contained all reagents without the extract sample and was used as blank. The means values were obtained from triplicate experiments. The percentage of DPPH scavenging activity of the sample was calculated as:

$$\text{DPPH(\%)} = (1 - \text{sample absorbance} / \text{control absorbance}) * 100 \quad (2)$$

Determination of total antioxidant activity

The total antioxidant capacities of the extract samples from both banana peels and cinnamon barks were determined according to the method of Prieto et al. (1999) with minor modifications. In brief, the extract solution (100 µl) was mixed with 1 mL of the reagent solution (0.6 M sulphuric acid, 28 mM sodium phosphate and 4 mM ammonium molybdate) in capped tubes. The tubes were then incubated at 95 °C for 90 min. Following the cooling down of the samples to 25 °C, the absorbance was measured at 695 nm against a blank. The blank contained 1 ml of the reagent solution without the extract sample. The total antioxidant activity was represented as the absorbance of the sample. The higher absorbance value indicates the higher antioxidant activity. All the experiments were done in triplicate.

Reductive potential

The reductive potential of the extracts from banana peels and cinnamon barks was determined following the method described by Oyaizu (1986) with slight modification. The different concentrations of extracts and the standards (125–1,000 µg/mL) were dissolved in one ml of purified water and then mixed with equal volume of 0.2 M phosphate buffer (2.5 mL, pH 6.5) and 1 % (w/v) potassium ferricyanide (2.5 mL). The mixture was then incubated at 50 °C for 20 min. A portion (2.5 mL) of trichloroacetic acid (10 % w/v) was added to the mixture, which was then followed by centrifugation for 10 min at 1,000 g. The supernatant (upper layer) of the solution (2.5 mL) was mixed with purified water (2.5 mL) and 0.5 ml of ferric chloride (0.1 % w/v). The absorbance was measured at 700 nm by UV–vis Spectrophotometer. Higher absorbance of the reaction mixture indicated the greater reductive potential. All the experiments were done in triplicate.

Application of banana peel and cinnamon bark extracts to fish oil

Fish oil was used as substrate to study the antioxidant activity of the extract samples. The tests were conducted at ambient temperature. The oil samples (100 mL) were placed in capped amber glass bottles. The pre-determined amounts

of sample extracts (800, 1,600 and 2,400 mg/kg) were added into the oil samples. The prepared oil samples were kept at 25 °C 30 days-storage. The control samples were the oils without the sample extracts and kept at the same condition. The oil sample of each treatment was measured for the peroxide and p-anisidine values after 3, 7, 15 and 30 days of storage to analyze the antioxidant activity of plant samples extract.

Determination of Peroxide Value (POV)

Peroxide value was analyzed according to the method of Pearson (1973) with slight modification. The pre-determined weight of fish oil (300 mg) was dissolved in 9.9 ml of chloroform: methanol (7:3 v/v) before adding 50 µL of 10 mM xylenol orange and 50 µl of ferric chloride solution. The mixture solution was incubated at room temperature for 5 min at 5 °C. The supernatant was used to measure of an absorbance at 560 nm with UV-visible spectrophotometer. All the experiments were done in triplicate. The peroxide value was expressed as meq active oxygen per Kg using the following formula:

$$POV(\text{meq/kg of oil}) = [(A_s - A_b) * M_i] / (W * 55.84 * 2) \quad (3)$$

Where,

POV	Peroxide value (meq/kg of oil)
A _s	Absorbance of sample
A _b	Absorbance of blank
M _i	Inverse of the slope obtained by standard curve with ferric chloride standard solution
W	Weight of the sample and 55.84 was the atomic weight of iron per µmol.

Determination of para-anisidine value

p-Anisidine value of oil was analyzed according to the method of AOCS Recommended Practice (AOCS 1990). The weight of fish oil (100 mg) was dissolved in 25 mL of isooctane and measured at 350 nm using UV-visible spectrophotometer. This solution (2.5 mL) was mixed with 0.5 ml of 0.5 % (w/v) para-anisidine (p-anisidine) in acetic acid for 10 min. The absorbance was recorded at 350 nm. The p-anisidine value was calculated by the following formula:

$$p - \text{anisidine value}(\text{absorbance unit/g}) = 25 * [(1.2 * A_2 - A_1) / W] \quad (4)$$

Where;

A1	Absorbance before adding p-anisidine
A2	Absorbance at 350 nm after adding p-anisidine
W	Weight of Sample (g)

Statistical analysis

Data were expressed as means ± standard deviation (SD) of three replicate determinations and then analyzed by SPSS V.13 (SPSS Inc. Chicago, USA). One Way Analysis of Variance (ANOVA) test was used to determine the differences among the means. *P* values <0.05 were regarded to be significant.

Results and discussion

The vacuum microwave and ultrasonic were used to extract the bioactive compounds from the dried banana peels and cinnamon barks. The phenolic content was used as a parameter to analyze for the best and most suitable method and conditions for enhancing the yield of extracts. The extract samples were analyzed for total polyphenolic contents, total flavonoid contents and DPPH radical scavenging activity. Moreover, antioxidant activity of each sample extracts was analyzed by measuring the oxidation of the oil after adding the extracts to the oil.

Effects of extraction conditions on extraction yields

The vacuum microwave and ultrasonic extraction were found as few of the convenient methods because the variables with temperatures can easily be manipulated. As a result, these methods need less solvent and are much faster than other conventional methods, such as soxhlet and maceration method. Figure 1 illustrates the effects of extraction conditions for the extraction yield by microwave and ultrasonic extraction. The percentage of yield increased as the temperature and time increased. At higher temperature, the antioxidant and soluble compounds readily dissolve into the solvent, thus increase the extraction yield. In both microwave and ultrasonication methods of extraction, the extraction yield increased with the extraction temperatures. The highest extraction yields of both methods were obtained at 60 °C. There were significant differences (*P*<0.05) in the yields among the different time at 60 °C, while there was no significant difference between the variations in periods at 40 °C for both the microwave and ultrasonic methods. There is significant increase (*P*<0.05) in yield when the temperature was raised for both methods. The temperature and time of extraction showed the significant effects on the extraction yield which agree with the previous research (Xiao et al. 2009).

For the extraction of banana peel using vacuum microwave method, the highest yield (13.03 % (w/w)) was obtained with the condition at 60 °C and 20 min, while the lowest yield (6.94 % (w/w)) was obtained at 40 °C and 10 min. For the extraction of cinnamon bark using vacuum

Fig. 1 Vacuum microwave and ultrasonic extraction yields of bioactive compounds at different temperatures incubated at different pre-determined times ($n=3$, $P<0.05$)

microwave method, the highest (18.36 %, w/w) and the lowest (13.95 %, w/w) yields of cinnamon bark extract were obtained with the conditions at 60 °C and 40 °C, respectively. The highest yield of banana peels and cinnamon bark extracts were 11.26 and 20.83 % (w/w), respectively, which were obtained with the condition at 60 °C and 90 min by ultrasonic method. The lowest yield of both extracts was obtained at 40 °C. There is no significant difference ($p<0.05$) in the cinnamon bark extract yield between the times at the 40 °C and 50 °C.

Effects of extraction conditions on total phenolic contents

Most of the antioxidants in fruits are derived from phenolic and polyphenolic compounds, which can be measured using the Folin–Ciocalteu method (Singleton et al. 1999). The results of triplicate analysis are expressed as mg of gallic acid/g of sample extract. The total phenolic contents in banana peels and cinnamon barks by using different extraction method and conditions are shown in Table 1.

Table 1 Total phenolic contents in banana peel and cinnamon bark extracts, determined by Folin–Ciocalteu reagent in the extracts. The values are compared between two raw materials and the extraction methods (vacuum microwave and ultrasonication)

Methods	Temperature (°C)	Time (min)	Phenolic content(mg/g)	
			Banana peel	Cinnamon bark
Vacuum microwave	40	10	18.2±3.61 ^h	370.1±11.44 ^{defg}
		15	18.4±0.68 ^h	358.3±12.69 ^{fg}
		20	18.9±1.07 ^h	340.8±13.71 ^g
	50	10	19.0±0.57 ^h	383.6±34.91 ^{cdef}
		15	22.5±2.26 ^{fg}	426.6±11.20 ^b
		20	20.1±3.03 ^{gh}	392.7±39.76 ^{bcd}
	60	10	30.6±3.55 ^{bc}	394.0±15.27 ^{bcd}
		15	26.2±3.20 ^{de}	400.1±46.11 ^{bcd}
		20	20.0±3.30 ^{gh}	385.3±55.04 ^{cdef}
Ultrasonic	40	30	24.9±1.98 ^{ef}	403.9±24.08 ^{bcd}
		60	27.8±2.05 ^{cd}	466.8±31.62 ^a
		90	28.3±1.96 ^{cd}	412.9±6.20 ^{bc}
	50	30	29.3±0.61 ^c	378.6±24.91 ^{cdef}
		60	33.2±1.08 ^{ab}	387.1±15.77 ^{cdef}
		90	32.7±1.95 ^{ab}	368.1±24.69 ^{defg}
	60	30	35.1±1.15 ^a	385.6±15.24 ^{cdef}
		60	28.2±1.11 ^{cd}	366.1±23.77 ^{efg}
		90	29.6±1.19 ^c	358.0±26.23 ^{fg}

Mean values ($n=3$) within a column with different letters are significantly different at $p<0.05$

Banana peels contain phenolics (catecholamines, flavanones, flavonols, tocopherols etc.) (Someya et al. 2002) and non-phenolic antioxidants (ascorbic acid, carotenes, cyanidin) (Seymour 1993). Sterols (stigmasterol, β -sitosterol and campesterol) and tripenic alcohols (cyacloartenol, cycloeucalenol, 2,4-methylene cyacartanol) are the lipids without phenolic ring and without antioxidant activity, present in banana peels (Subagio et al. 1996). The phenolic contents in banana peel ranged from 18.21 to 35.06 mg gallic acid/g extract and the highest value was obtained by using ultrasonic method at 60 °C and 30 min. These amounts were much higher than those described in previous research (Someya et al. 2002). At 60 °C with the extended period of extraction time (20 min), the phenolic contents in the extracts were observed lesser in comparison the extracts at 10 and 15 min of duration. At the higher temperature and longer extraction time, some of the phenolic compounds are likely to get oxidized (González-Montelongo et al. 2010). The increased amount of phenolic contents was found with the extraction time at 40 °C while at 50 °C, the phenolic content increased only after certain period of time (in this case is 30 min) and slightly decreased when extracted for longer extraction time. Higher extraction temperature can be related to shorter extraction time, which is beneficial for

extraction and leads to higher phenolic contents (Prasad et al. 2009). The results obtained from ultrasonic and vacuum microwave showed the same trend, however, the ultrasonic gave significantly ($P<0.05$) higher phenolic content than microwave in all tested temperature. At 40 °C of microwave extraction, there was no significant difference ($P<0.05$) in phenolics among all the extraction times tested.

The phenolic contents in cinnamon bark range from 358 to 467 mg gallic acid/g extract by using vacuum microwave and ultrasonic method. These values were much higher than the previous reported research (Provan et al. 1994). The highest value was obtained by using ultrasonic method at 40 °C and 60 min. The extraction of cinnamon by two methods produced greatly different results as illustrated in Table 2. For ultrasonic extraction of cinnamon bark, the phenolic content at 40 °C was highest and significantly different from 50 °C to 60 °C. For vacuum microwave extraction, there was no significant difference among three extraction temperatures at the same time (10 min). At 50 °C, the phenolic contents of vacuum microwave were similar to ultrasonic, which increased with time and decreased when extracted for longer time. The phenolic content at 40 °C, on the other hand, gradually decreased with the time. The highest phenolic content (450 mg/g of gallic acid) was observed at the 50 °C by vacuum microwave method.

The cinnamon bark extracts had much higher phenolic content than banana peel extract. For the extraction of both samples, ultrasonic method provided significantly higher phenolic content ($P<0.05$) in compare to vacuum microwave. The high frequency causes the destruction of chemical entity of antioxidant. Vacuum microwave irradiation effects on phenolic content by hydrolyzing the β -ether bound to the phenolic compounds in the cell walls (Proestos et al. 2006). In addition, high dielectric solvents (e.g. ethanol) can absorb more vacuum microwave energy so the polarity of the solvent is very important in vacuum microwave extraction. Polar solvents are usually believed to be better in efficiency than non-polar solvent (Wang and Weller 2006). However, the vacuum microwave method does have some benefits. For example, vacuum microwave can increase the yield in shorter time at the same temperature. Also, the vacuum microwave energy results in more effective heating, faster heat transfer, reduced thermal gradient and faster response to process heating control (Provan et al. 1994).

Table 2 The flavonoid content, DPPH radical scavenging activity and total antioxidant activity of banana peel and cinnamon bark extracts ($n=3$)

Sample extracts	Flavonoid (mg/g)	%DPPH	TAA (Abs.)
Banana peel	196.1 $\pm$ 6.70	84.5 $\pm$ 6.48	0.61 $\pm$ 0.06
Cinnamon bark	427.9 $\pm$ 13.34	93.4 $\pm$ 0.21	0.93 $\pm$ 0.15

DPPH 1,1-Diphenyl-2-picryl hydrazyl, TAA Total antioxidant activity

Flavonoid content, DPPH radical scavenging activity and total antioxidant activity (TAA) of extracts

Table 2 illustrates the flavonoid contents, percentage DPPH radical scavenging activity and total antioxidant activity (TAA) of banana peel and cinnamon bark extracts. Flavonoid is also considered as a class of in phenolic compounds. The DPPH scavenging activities indicate the antioxidant activity. Total antioxidant capacity (TAA) was evaluated by the phosphomolybdenum method based on the reduction of Mo^{6+} to Mo^{5+} by the antioxidant compounds and the formation of a green Mo^{5+} complex at a low pH with a maximal absorbance at 695 nm. A higher absorbance value indicates that the extract has higher antioxidant activity.

The results reveal that the cinnamon bark extract had higher flavonoid content, %DPPH and TAA than banana peel extract. Cinnamon bark contains higher phenolic contents. The flavonoid content in cinnamon bark extract (427.91 mg/g) was much higher than banana peel (196.05 mg/g) and also higher than previous research (Prasad et al. 2009). The percentage of DPPH of banana peel and cinnamon bark extracts were 84.45 and 93.39 %, respectively. The TAA of banana peel and cinnamon bark extracts were 0.61 and 0.93, respectively. Even though, the phenolic and flavonoid contents of both samples were greatly different, the antioxidant activity parameters (% DPPH and TAA) have not shown significant ($p<0.05$) different.

Reducing power

The reducing power of the extracts from cinnamon barks and banana peels and the reference compound, ascorbic acid increased steadily with the increasing concentrations as shown in Fig. 2. The reducing powers (correlated by measuring the absorbance at 700 nm) of extract from cinnamon barks, extract from banana peels and ascorbic acid were 3.418, 2.918 and 3.542 respectively at a dose of 1 mg showing that the extracts

Fig. 2 Reducing power of ascorbic acid, banana peel extract and cinnamon bark extract ($n=3$, $P<0.05$)

from cinnamon barks and banana peels can act as electron donors and can react with free radicals to convert them to more stable products and thereby terminate radical chain reactions.

Effects of extracts on oxidative stability of commercial fish oil

Increasing primary and secondary lipid peroxidation products of fish oil during storage was measured by peroxide and *p*-anisidine values for the antioxidative effects of extracts from banana peels and cinnamon barks. The antioxidant activity was analyzed by measuring the oil oxidation after applying of sample extracts into the fish oil. The oxidative process of oils and fats is one of the main causes of the deterioration of the principal organoleptic and nutritional characteristics of foodstuffs. Primarily, oxidation products, in which the fatty acids react with oxygen and determine odorless compounds, normally measured with Peroxide Value (PV) test. During the second phase of complex oxidation reactions, the peroxide degrades into many substances as volatile aldehydes, responsible for rancid odor and flavor. The *p*-anisidine value represents the level of non-volatile aldehydes, primarily 2-alkenals present in the fat.

The increase in peroxide and *p*-anisidine values of fish oil containing 800, 1,600 and 2,400 mg/kg of sample extracts are illustrated in Table 3. The initial peroxide and *p*-Anisidine values for fish oil were 3.6 ± 0.16 meq O₂/kg oil and 1.20 ± 0.13 absorbance unit/g oil respectively. These initial lipid

peroxidation products were not significantly different ($p > 0.05$) among groups of fish oil and fish oil containing extracts. Peroxide and *p*-anisidine values of fish oil without sample extracts were significantly higher ($p < 0.05$) than those of the fish oil containing the sample extracts during storage at 25 °C. Peroxide value of fish oil without the sample extracts was rapidly increased ($p < 0.05$) to 71.17 meq O₂/kg oil after 30-days of storage and it was also significantly different ($p < 0.05$) to the other groups. Peroxide values of fish oil containing 1,600 and 2,400 mg/kg sample extracts (both from banana peels and cinnamon barks) was significantly lower ($p < 0.05$) than the fish oil added only 800 mg/kg of the sample extracts after 15-days of storage. There was not significant different ($p > 0.05$) in the peroxide value in the fish oil containing 1,600 and 2,400 mg/kg of the sample extracts during the storage. The fish oil containing cinnamon bark extract showed lower peroxide value (18.3 meq O₂/kg oil) than oil containing the banana peel extract (25.2 meq O₂/kg oil) after 15 days of storage. Similarly, the lower peroxide value was also observed in the oil containing cinnamon bark extract than the oil containing banana peel extracts after 30-days of storage.

Similarly, secondary lipid peroxidation product of fish oil was determined by examining *p*-Anisidine values. Determination of *p*-anisidine value was based on color intensity of the reaction between *p*-anisidine and aldehydes. The evolution of *p*-anisidine values of fish oil without containing sample extracts were significantly higher ($p < 0.05$) than those of the fish oils containing samples extracts after 7-days of storage. There was

Table 3 Effect of banana peel and cinnamon bark extract on the oxidative stability of fish oil stored for a month (30 days) at 25 °C. The three different concentrations (800, 1,600, and 2,400 mg/kg) of extracts were used while the control was without the addition of any extracts

Extracts	Conc. (mg/kg of fish oil)	Storage time (days) at 25 °C				
		0	3	7	15	30
Peroxide value (meq/kg of fish oil)						
Control	0	3.7±0.16 ^a	6.5±0.57 ^{ab}	15.3±0.73 ^b	56.5±0.29 ^{ab}	71.2±0.04 ^c
Banana peel	800	2.5±0.09 ^a	3.5±0.54 ^a	4.7±0.08 ^a	34.4±0.07 ^a	44.2±0.56 ^b
	1600	2.7±0.21 ^a	3.6±0.36 ^a	4.4±1.07 ^a	25.4±1.10 ^b	35.4±0.49 ^b
	2400	2.8±0.36 ^a	3.6±0.29 ^a	4.9±0.14 ^a	22.2±0.98 ^b	33.2±1.18 ^b
Cinnamon bark	800	2.0±0.07 ^a	2.9±0.43 ^a	3.2±0.51 ^c	25.7±0.11 ^b	35.4±0.36 ^b
	1600	2.2±0.18 ^a	3.4±0.07 ^a	3.9±0.94 ^c	18.4±0.82 ^c	26.2±0.04 ^d
	2400	2.2±0.18 ^a	2.8±0.36 ^a	4.5±0.51 ^c	18.3±0.94 ^c	25.2±0.45 ^d
p-Anisidine value (absorbance unit/g of fish oil)						
Control	0	1.2±0.13 ^a	4.6±0.30 ^b	7.1±0.47 ^{ab}	20.3±0.59 ^{cd}	55.5±0.48 ^c
Banana peel	800	1.2±0.12 ^a	2.3±0.61 ^a	3.8±0.13 ^b	11.9±0.26 ^b	22.1±0.59 ^{ab}
	1600	1.5±0.15 ^a	2.4±0.18 ^a	3.3±0.06 ^b	7.5±1.38 ^{ab}	14.6±0.57 ^{bc}
	2400	1.5±0.17 ^a	2.6±0.41 ^a	2.4±0.30 ^c	6.8±2.14 ^{ab}	13.4±2.52 ^b
Cinnamon bark	800	1.2±0.05 ^a	2.8±0.25 ^a	2.9±1.30 ^b	9.9±0.37 ^b	12.4±0.12 ^b
	1600	1.9±0.52 ^a	2.2±0.67 ^a	2.7±0.83 ^b	4.1±1.02 ^{ab}	8.7±3.01 ^a
	2400	1.8±0.24 ^a	2.1±0.40 ^a	2.4±0.23 ^b	3.9±0.29 ^{ab}	8.3±2.39 ^a

Mean values ($n=3$) within a column with different letters are significantly different at $p < 0.05$

no significant difference ($p>0.05$) of *p*-anisidine values between fish oils containing 1,600 and 2,400 mg/kg of both (banana peels and cinnamon barks) extracts after a week storage as shown in Table 3. The *p*-anisidine values are slightly lower in the oil containing cinnamon bark extracts (4.04) in comparison to the oil containing banana peel extracts (6.85) after 15-days of storage. Similarly, the *p*-anisidine values were slightly lesser in the oil containing cinnamon bark extract than containing banana peel extracts after 30-days of storage.

Conclusion

The agro-residues could be regarded as the valuable resources after recycling and reprocessing. This study showed the extraction method, temperature and time had significant effects on the extraction yields and phenolic contents. Higher extraction temperature relates to shorter extraction time, which is beneficial for extraction and provides high phenolic content. The ultrasonic extraction gave significantly higher phenolic contents than vacuum microwave extraction. Moreover, this study showed cinnamon bark had about ten times higher phenolics and flavonoid contents than banana peels. In addition, the banana peel and cinnamon extracts had the ability as antioxidants to prevent the oxidation of fish oil. The concentrations of sample extracts and the storage time affected the antioxidant activity. At the optimum concentration of both extracts in fish oil, cinnamon gave lower peroxide value than banana peel extract. This might be attributed to the higher phenolic compounds acting as antioxidant present in cinnamon. More studies are being continued with the extraction of phenolic and other bioactive compounds and their molecular identifications from similar vegetable and fruit wastes and their applications in our lab.

References

- AOCS (1990) *p*-Anisidine value (Ti la-64). In: Firestone D (ed) Official methods and recommended practices of American Oil Chemists' Society. AOCS Press, Campaign
- Arnao MB (2000) Some methodological problems in the determination of antioxidant activity using chromogen radicals: a practical case. *Trends Food Sci Technol* 11:419–421
- Blois MS (1958) Antioxidant determinations by the use of a stable free radical. *Nature* 181:1199–1200
- Chen SS, Spiro M (1994) Study of microwave extraction of essential oil constituents from plant materials. *J Microw Power Electromagn Energy* 29:231–241
- González-Montelongo R, Gloria Lobo M, González M (2010) Antioxidant activity in banana peel extracts: testing extraction conditions and related bioactive compounds. *Food Chem* 119:1030–1039
- Kaur C, Kapoor HC (2001) Antioxidants in fruits and vegetables—the millennium's health. *Int J Food Sci Technol* 36:603–725
- Kaushik R, Pradeep N, Vamshi V, Geetha M, Usha A (2010) Nutrient composition of cultivated stevia leaves influence of polyphenols and plant pigments on sensory and antioxidant properties of leaf extracts. *J Food Sci Technol* 47:27–33
- Kedare SB, Singh RP (2011) Genesis and development of DPPH method of antioxidant assay. *J Food Sci Technol* 48:412–422
- Kwon JH, Belanger JMR, Jocelyn P, Yaylayan VA (2003) Application of microwave-assisted process to the fast extraction of *Ginseng saponins*. *Food Res Int* 36:491–498
- Mason TJ, Paniwnyk L, Lorimer JP (1996) The uses of ultrasound in food technology. *Ultrason Sonochem* 3:S253–S260
- Mathew S, Abraham E (2006) Studies on the antioxidant activities of cinnamon (*Cinnamomum verum*) bark extracts through various in vitro models. *Food Chem* 94:520–528
- Meda A, Lamien CE, Romito M, Millogo J, Nacoulma OG (2005) Determination of total phenolics, flavonoid and proline contents in Burkian Fasan honey as well as their radical scavenging activity. *Food Chem* 91:571–577
- Oyaizu M (1986) Studies on products of browning reactions prepared from glucosamine. *Jpn J Nutr* 44:307–314
- Pan X, Niu G, Liu JH, Shu YY (2000) Microwave-assisted extraction of glycyrrhizic acid from licorice root. *J Biochem Eng* 5:173–177
- Pearson D (1973) Laboratory techniques in food analysis. Butterworth & Co. (Publishers) Ltd, Kingsway, pp 157–161
- Prasad KN, Yang B, Dong X, Jiang G, Zhang H, Xie H, Jiang Y (2009) Flavonoid contents and antioxidant activities from *Cinnamomum* species. *Innov Food Sci Emerg Technol* 10:627–632
- Prieto P, Pineda M, Aguilar M (1999) Spectrophotometric quantification of anti-oxidant capacity through the formation of a phosphomolybdenum complex: specific application to the determination of vitamin E. *Anal Biochem* 269:337–341
- Proestos C, Bozaris IS, Nychias JG-E, Komaitis M (2006) Analysis of flavonoids and phenolic acids in Greek aromatic plants: investigation of their antioxidant capacity and antimicrobial activity. *Food Chem* 95:664–671
- Provan J, Scobbie L, Chesson A (1994) Determination of phenolic acids in plant cell walls by microwave digestion. *J Sci Food Agri* 64:63–65
- Scheiber A, Stintzing FC, Carla R (2001) By-products of plants food processing as a source of functional compounds—recent development. *Trends Food Sci Technol* 12:401–413
- Seymour GB (1993) Banana. In: Semour G, Taylor J, Tucker G (eds) *Biochemistry of fruit ripening*. Chapman & Hall, London, pp 95–98
- Singh B, Sharma HK, Sarkar BC (2011) Optimization of extraction of antioxidants from wheat bran (*Triticum spp.*) using response surface methodology. *J Food Sci Technol*. doi:10.1007/s13197-011-0276-5
- Singleton VL, Orthofer R, Lamuela-Raven-tos RM (1999) Analysis of total phenols and other oxidation substrates and antioxidants by means of Folin–Ciocalteu reagent. *Methods Enzymo* 299:152–178
- Someya S, Yoshiki Y, Okubo K (2002) Antioxidant compounds from bananas (*Musa Cavendish*). *Food Chem* 79:351–354
- Subagio A, Morita N, Sawada S (1996) Carotenoids and their fatty-acid esters in banana peel. *J Nutr Sci Vitaminol* 42:553–566
- Wang L, Weller CL (2006) Recent advanced in extraction of nutraceuticals from plants. *Trends Food Sci Technol* 17:300–312
- Wojdyło A, Oszmianski J, Czemerys R (2007) Antioxidant activity and phenolic compounds in 32 selected herbs. *Food Chem* 105:940–949
- Xiao X, Wang J, Wang G, Li G (2009) Evaluation of vacuum microwave-assisted extraction technique for the extraction of antioxidants from plant samples. *J Chromatogr A* 1216:8867–8873