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ANTIOXIDANT EFFICACY OF UNRIPE BANANA (*MUSA ACUMINATA* COLLA) PEEL EXTRACTS IN SUNFLOWER OIL DURING ACCELERATED STORAGE

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ABSTRACT

Background. Sunflower oil is prone to oxidation during storage time, leading to production of toxic compounds that might affect human health. Synthetic antioxidants are used to prevent lipid oxidation. Spreading interest in the replacement of synthetic food antioxidants by natural ones has fostered research on fruit and vegetables for new antioxidants.

Material and methods. In this study, the efficacy of unripe banana peel extracts (100, 200 and 300 ppm) in stabilizing sunflower oil was tested under accelerated storage (65°C) for a period of 24 days. BHA and α-tocopherol served as comparative standards besides the control. Established parameters such as peroxide value (PV), iodine value (IV), *p*-anisidine value (*p*-AnV), total oxidation value (TOTOX), thiobarbituric acid reactive substances (TBARS) and free fatty acid (FFA) content were used to assess the extent of oil deterioration.

Results. After 24 days storage at 65°C, sunflower oil containing 200 and 300 ppm extract of unripe banana peel showed significantly lower PV and TOTOX compared to BHA and α-tocopherol. TBARS, *p*-AnV and FFA values of sunflower oil containing 200 and 300 ppm of unripe banana peel extract exhibited comparable inhibitory effects with BHA. Unripe banana peel extract at 200 and 300 ppm demonstrated inhibitory effect against both primary and secondary oxidation up to 24 days under accelerated storage conditions.

Conclusions. Unripe banana peel extract may be used as a potential source of natural antioxidants in the application of food industry to suppress lipid oxidation.

Key words: unripe banana peel extract, sunflower oil, accelerated storage, antioxidants, lipid oxidation

INTRODUCTION

Dietary lipids, naturally occurring in raw food materials or added during food processing, play an important role in food nutrition and flavour. Meanwhile, lipid oxidation is a major cause of food quality deterioration, and has been a challenge for manufacturers and food scientists alike (Shahidi and Wanansundara, 2002). Consequently, lipid oxidation will give rise to the development of off-flavours and loss of essential amino acids, fat-soluble vitamins, and other bioactives in food systems. Lipids may undergo autoxidation, photo-oxidation, thermal oxidation, and enzymatic oxidation, most of which involve some type of free

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radical or oxygen species, producing lipid hydroperoxides (Shahidi and Zhong, 2005; Shahidi and Wanansundara, 2002). Lipid hydroperoxides and some of their end products can interact with biological components, such as proteins, cell membranes and enzymes in human body, affecting vital cell functions (Shahidi and Zhong, 2005). Failure to repair damaged biological components will cause the physiological overproduction of free radicals. The scientific community has discovered that fatigue and many human illnesses are associated with free radicals (Halliwell, 2012). Hence, the consumption of antioxidants will help to reduce oxidative damage *in vivo,* thereby reducing the incidence of human diseases (Halliwell, 2012).

To prevent such a quality loss due to lipid oxidation, synthetic antioxidants have been widely utilized to retard lipid oxidation in various food systems. However, the uses of synthetic antioxidants have raised concerns about possible toxicity and side effects (Maqsood et al., 2013). Hence, the usage of natural antioxidants has been increasingly adopted as an effective methodology to prevent rancidity in various edible oils. Many phenolic-rich plant extracts have successfully demonstrated the retarding effect on lipid oxidation of various edible oil due to their good antioxidant capacity (Zhang et al., 2010; Dillard and German, 2000). For example, oyster mushroom, garlic, kenaf seed, pomegranate, olive, tea and *Moringa oleifera* extracts have been demonstrated to delay lipid peroxidation in various oils compared to synthetic antioxidants (Malheiro et al., 2013; Nyam et al., 2013; Yim et al., 2013; Pazos et al., 2005). There are some studies which demonstrated the effectiveness of fruit peel, such as *rambutan* (*Nephelium lappaceum* L.), mangosteen (*Garcinia mangostana* Linn.) and pomegranate peel extracts to prevent the oxidative rancidity of sunflower oil under accelerated conditions (Chong et al., 2015; Winne Sia et al., 2014; Iqbal and Bhanger, 2007). It is well known that the antioxidant capacities of fruit extracts are due to the presence of phenolic compounds (Pedraza-Chaverri et al., 2008). However, there is still lack of studies to determine the efficacy of fruit/fruit peel extracts in preventing oxidative rancidity of vegetable oil (Okonogi et al., 2007).

Banana (*Musa* species) is originated from the family of Musaceae and can be classified into two main species, which are *Musa acuminata* and *Musa balbisiana* (Valmayor et al., 2002). Bananas are an important food crop in the subtropics and tropics being a good source of nutrients and energy. Bananas are often categorized as 'dessert' sweet bananas, which are ripened and eaten raw, but also edible when fully ripe (Perrier et al., 2011). The peel of banana is a typical waste after consumption or disposed by banana crisp industries (Oliveira et al., 2008). Besides its flesh, banana peel contains potent antioxidant compounds. Vitamin A, vitamin C, and carotenoids are the most abundant antioxidants in banana flesh as well as in banana peel (Arora et al., 2008; Pereira and Maraschin, 2015; US Department..., 2014). Banana peels have been shown to demonstrate higher antioxidant capacity (AC) than the pulp (Pereira and Maraschin, 2015; Oliveira et al., 2008; Someya et al., 2002). Moreover, unripe banana peels have been shown to demonstrate higher antioxidant capacity than the ripened banana peel (Pereira and Maraschin, 2015). Hence, banana peel could be one of the natural sources of antioxidant (Pereira and Maraschin, 2015; Oliveira et al., 2008).

Sunflower oil contains about 59% of polyunsaturated fatty acids (PUFA; linoleic acid) and 30% of monounsaturated fatty acids (MUFA; oleic acid; Normand et al., 2001). Due to its high PUFA content, sunflower oil is highly susceptible to lipid oxidation (Aladedunye and Przybylski, 2009; Normand et al., 2001). Hence, sunflower oil has been used as a model to investigate the ability of various plant extracts in preventing its peroxidation, as previously reported (Winnie Sia et al., 2014; Nyam et al., 2013). Ripe banana peel water-extract have been shown to suppress the auto-oxidation of linoleic acid by 65–70% after 5-day incubation in an emulsion system by determining its peroxide value and thiobarbituric acid reactivity (Kanazawa and Sakakibara, 2000). However, no study has ever been conducted on the efficacy of unripe banana peel in preventing lipid peroxidation under accelerated storage conditions. Hence, this study aims to determine the effectiveness of unripe banana peel extract in preventing sunflower oil rancidity under 24 days of accelerated storage conditions. Crude unripe banana peel extract was standardized to the levels of 100, 200, 300 ppm and added to each sunflower oil sample. Sunflower oil samples that are stabilised with α-tocopherol (200 ppm) and BHA (200 ppm) were

used as standard, whereas, the control sunflower oil sample was without additives. Oxidative changes were monitored by the peroxide value, *p*-anisidine value, iodine value, 2-thiobarbituric acid reactive substances and free fatty acid value, as well as the calculated total oxidation value.

METHODOLOGY

Materials and reagents

Refined, bleached and deodorized (RBD) sunflower oil without additives and antioxidants was purchased from MOI Foods Malaysia Pte. Ltd., Selangor, Malaysia. The unripe tropical fruit, banana (*Musa acuminata* Colla) was purchased from a local wet market in Kuala Lumpur, Malaysia. The fruits were cleaned and inspected to remove damaged or diseased fruits. Following this, the peel was separated from its edible pulp. The unripe banana peel was then washed, air dried followed by drying in the oven at 40°C for 24 h and ground to powder using a food processor after filter through a 1.0 mm sieve using a miller. The unripe banana peel powder was vacuum packaged into a sterile sample bag by using a vacuum packaging machine (DZQ 400/500, Zhejiang, China) prior to analysis. All chemicals and reagents used were of analytical grade from Merck (Merck KGaA, Darmstadt, Germany). BHA and α-tocopherol were purchased from Sigma-Aldrich (St. Louis, MO, USA). Water used throughout the study was of ELGA Lab Water (Micromeg, UK) quality.

Extraction

Oven-dried unripe banana peel powder (50 g) was dissolved in 500 ml ethanol-distilled water (9:1, v/v) and subjected to agitation at 150 rpm at room temperature on a shaker for 120 min (Green Seriker, Korea). Subsequently, the mixture was centrifuged at 4000 rpm for 10 min (Universal 320R, Hettich Zentrifugen, MA, USA) and supernatant was filtered through a Whatman No. 1 filter paper. The residue was re-extracted twice where both extracts were combined and concentrated in a rotary evaporator (Rotavapor R-200, Buchi, Switzerland) at 35°C. Crude extracts obtained where then freeze-dried (ALPHA 1–4LD Plus, Christ, Germany), vacuum packaged and stored under refrigeration until further analyses.

Preparation of sample extract for determination of oxidative stability

Sunflower oil was stored under accelerated conditions at 65°C over a period of 24 days according to method described (Iqbal and Bhanger, 2007) with slight modifications according to Chong et al. (2015). The accelerated storage condition involves placing a known volume of oil sample in a forced-draft oven at 65°C, where each day under such oven storage test at 65°C is equivalent to one month of the storage at ambient temperature (Chong et al., 2015; Yim et al., 2013). First, crude extracts of unripe banana peel were dissolved with 150 µL absolute ethanol and added into 250 mL pre-heated sunflower oil (at 50°C for 3 h) at concentrations of 100 and 200 ppm. All the samples were prepared separately for different day of storage time. Samples were not drawn from the sample bottle. They were kept in the oven until till day of analysis. After that, the oil samples were placed in a water bath sonicator for 1 h at 60°C to ensure homogenous dispersion. Synthetic antioxidant (BHA) at its legal limit of 200 ppm and α-tocopherol (200 ppm) were used as the comparative standards. All samples (250 ml) were placed in closed amber bottles and stored in oven at a fixed temperature of 65°C. Control sample of sunflower oil was placed under identical condition without the addition of sample extract. Oxidative changes were monitored by the peroxide value, iodine value, *p*-anisidine value, 2-thiobarbituric acid reactive substances and free fatty acid value at 0 day and at regular intervals of 6 days for 24 days.

Analysis of Peroxide Value

Peroxide Value (PV) of all samples was measured according to the AOAC method 965.33 with slight modification (O'Keefe and Pike, 2010; Zhang et al., 2010). Sunflower oil samples (2.00 g) were dissolved in 30 mL of acetic acid-chloroform (3:2; v/v) solution. Then, 1 mL saturated solution of potassium iodide (KI) was added. The mixture was shaken by hand for 1 min and was kept in the dark for 5 min. After addition of 75 mL distilled water, the mixture was titrated against 0.022 M sodium thiosulphate ($Na_2S_2O_3$) until the yellow colour was disappeared. Subsequently, 0.5 mL of starch indicator (1%) was added where titration was continued until the blue color of the mixture disappeared. The blank was analysed under similar

conditions. Determination of PV of each sample was carried out in triplicates and PV (mEq/kg) was calculated according to the equation:

$$PV = [C \times (V - V_k) \times 12.69 \times 78.8] / \text{sample weight (g)}$$

where:

C – concentration of $Na_2S_2O_3$, mEq/kg,
V – the titer value of $Na_2S_2O_3$ for sample, mL,
V_k – the titer value of $Na_2S_2O_3$ solution for blank, mL.

Measurement of *p*-Anisidine Value

The *p*-anisidine value (*p*-AnV) was determined according to AOCS method Cd 18–90 (O'Keefe and Pike, 2010). To begin, sunflower oil samples (2 g) were first dissolved in 25 mL isooctane. Then, 5 mL aliquot of this mixture was mixed with 1 mL 0.25% *p*-anisidine in acetic acid (w/v). The mixture was shaken vigorously and kept in the dark for 10 min, and absorbance was measured at 350 nm using a spectrophotometer (XTD 5, Secomam, Alés Gard, France). The blank which consists of 5 mL isooctane was measured under similar condition. The *p*-AnV was calculated according to the equation:

$$p\text{-AnV} = 25 \times [(1.2\ A_s - A_b)] / \text{sample weight (g)}$$

where:

A_s – the absorbance of fat solution after reaction with the *p*-Anisidine reagent,
A_b – the absorbance of blank.

Determination of Total Oxidation (TOTOX) value

Total oxidation (TOTOX) values of the oil samples were determined based on the obtained PV and AV values as described by (Nyam et al., 2013) using the following equation:

$$TOTOX = 2PV + AV$$

Analysis of Iodine Value

The iodine value (IV) in the oil samples was determined by Wijs method, as describe in the AOAC Official Method 993.20 (O'Keefe and Pike, 2010). First, sunflower oil samples (0.2 g) was dissolved in 15 mL of cyclohexane-acetic acid (1.1; v/v) solvent. The mixture was added with 25 mL Wijs solution [Iodine chloride (ICl) in acetic acid] and kept in the dark for 1 h. Then, the mixture was added with 20 mL 15% KI solution and 150 mL of distilled water. The mixture was gradually titrated against 0.1 M $Na_2S_2O_3$ solution with continuous vigorously shaking until dark brown colour disappeared. The blank was analyzed under similar conditions. The IV was expressed as the gram of iodine absorbed per 100 g sample (g I_2/100 g) and was calculated using the equation:

$$IV = [(B - S) \times M \times 12.69] / \text{sample weight (g)}$$

where:

B – the titration of blank, mL,
S – the titration of test solution, mL,
M – the molarity of $Na_2S_2O_3$ solution, mol/L.

Thiobarbituric Acid Reactive Substances (TBARS) Assay

Lipid oxidation of all samples was determined by 2-thiobarbituric acid (TBA) method according to the methods of Okuda et al. (2005) with slight modifications. First, 1 mL of sunflower oil was mixed with 2 ml of TBA-TCA solution containing 15% TCA and 20 mM TBA (w/v) in distilled water. The mixture was then vortexed and incubated in water bath (95°C) for 15 min until pink colour was developed. Subsequently, samples were cooled under running tap water for 15 min followed by centrifuge for 15 min at 4500 rpm. Blank solution was prepared with 1 mL distilled water under similar conditions. The absorbance of the supernatant was measured at 532 nm using a spectrophotometer against blank solution. A standard curve of malondialdehyde (MDA) was prepared using 1,1,3,3-tetraethoxypropane (TEP) where TBARS value were expressed as mg of MDA equivalents per kg sample.

Measurement of Free Fatty Acids (FFA)

Free fatty acids, as oleic acid percentages in oil samples, were determined using an alkali titration method according to AOAC official method 28.032 (O'Keefe and Pike, 2010). First, oil samples (7 g) were dissolved in 50 mL neutralized ethanol. Then, the mixture was titrated against sodium hydroxide (0.25 M) using phenolphthalein solution as an indicator while shaking the content mixture. The mixture was titrated until permanent faint pink colour developed and persisted for

more than 1 min. Percentage of free fatty acids was expressed as:

$$\% \text{ FFA (oleic acid)} = \text{volume (mL) of 0.25 M NaOH used}$$

Statistical analysis

All independent analyses were carried out in triplicates ($n = 3$) for which the results were expressed as mean ±standard deviation. Data collected were analyzed using SPSS analytical software version 21.0 (SPSS Inc., Illinois, USA). Data were subjected to one-way-analysis of variance (ANOVA) followed by Bonferroni's test for comparison of means as a post-hoc test. Significant levels were based on the confidence level of 95% ($p < 0.05$).

RESULTS AND DISCUSSIONS

The resistance of lipids to oxidation is known as oxidative stability. The determination of oxidative stability using actual shelf life of oil at ambient conditions of storage requires months or years. Therefore, accelerated tests are used to evaluate the oxidative stability of oils and fats. A protocol recommended by the American Oil Chemists' Society (AOCS) states that placing a fat or oil of known volume in a forced-draft oven above ambient temperature but less than 80°C, with 60°C being recommended. Such temperatures are desirable accelerated storage temperatures since the mechanism of oxidation from 60°-65°C is the same as oxidation at room temperature (O'Keefe and Pike, 2010). Khan and Shahidi (2001) and Iqbal and Bhanger (2007) have demonstrated that one day of accelerated storage in Schaal oven test at 65°C, is equivalent to one month of storage at ambient temperature. In this present research, analyses were carried out every 6 days interval. Every 6 days under accelerated storage represents half year storage at ambient temperature. A combination of methods to assess both primary and secondary oxidative changes of experimental sunflower oils during accelerated storage was carried out in this study. The quality indices of sunflower oil were quantitatively determined as well, including formation of primary and secondary oxidation products, losses of unsaturated fatty acids and hydrolysis of fatty acids. Besides, the protective effects of those supplemented antioxidants were compared with those supplemented with unripe banana peel in preventing lipid peroxidation of sunflower oil (SFO). The abbreviations of SFO supplemented with 100, 200 and 300 ppm of unripe banana peel extracts used for the following sections are SFO-100, SFO-200 and SFO-300, respectively.

Peroxide Value

The degree of primary oxidation of SFO was determined by measuring PV in the presence and absence of antioxidants at 65°C for 24 days. As shown in Figure 1, the PV of all the treated samples increased with time. Peroxide value (PV) is a measurement of the concentration of hydroperoxides and peroxides that are produced in the initial stages of lipid oxidation, represents primary reaction products. It is determined based by their ability to liberate iodine from potassium iodide (Nor et al., 2008). The PVs of the samples were in the range of 41.12–69.00 mEq/kg, while it was 41.12 mEq/kg and 41.92 mEq/kg for BHA and α-tocopherol stabilized samples, respectively, after accelerated storage up to 24 days. Highest PV was observed for SFO-100 followed by control, SFO-200, SFO-300, SFO supplemented with α-tocopherol (SFO-TOCO) and SFO supplemented with BHA (SFO-BHA), respectively, at all stages (Fig. 1). PVs of SFO-200 and SFO-300 were significantly lower ($p < 0.05$) than the control on 12th and 18th day, respectively, and almost as low as of SFO-BHA and SFO-TOCO on the 24th day. However, there was significant difference ($p < 0.05$) between the PVs of SFO-BHA and SFO-TOCO with SFO-200 and SFO-300 on 24th day (Fig. 1). Hence, it can be said that the unripe banana peel extracts added to sunflower oil can work as effective as the added BHA and α-tocopherol. However, as compared to the control and SFO-100, the PVs of SFO-200 and SFO-300 were significantly lower ($p < 0.05$), which may indicate good antioxidant capacity due to higher concentrations of the unripe banana peel extract (Fig. 1).

p-Anisidine Value

The *p*-Anisidine Value (*p*-AnV) is the measure of the secondary lipid oxidation product produced when the hydroperoxide decomposes to carbonyl, aldehydes, carboxylic acids and ketones. This is the stage that leads to the rancid flavor of the oil (O'Keefe and

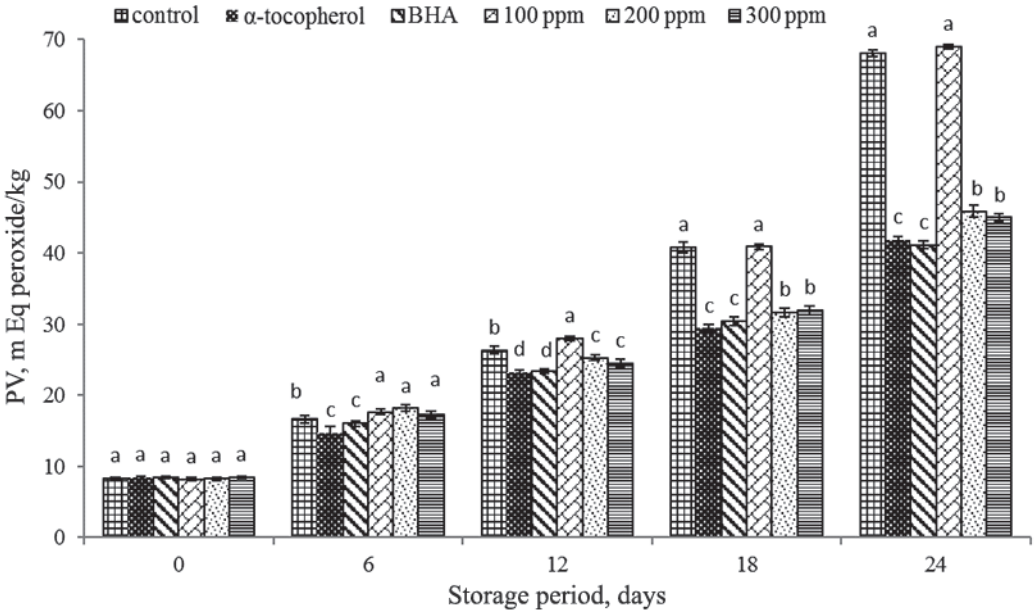

Fig. 1. Peroxide value (PV) of sunflower oil supplemented with unripe banana peel extracts, BHA and α-tocopherol under accelerated storage at 65°C for 24 days. Different letters within each storage period denote that they are significantly different ($p < 0.05$)

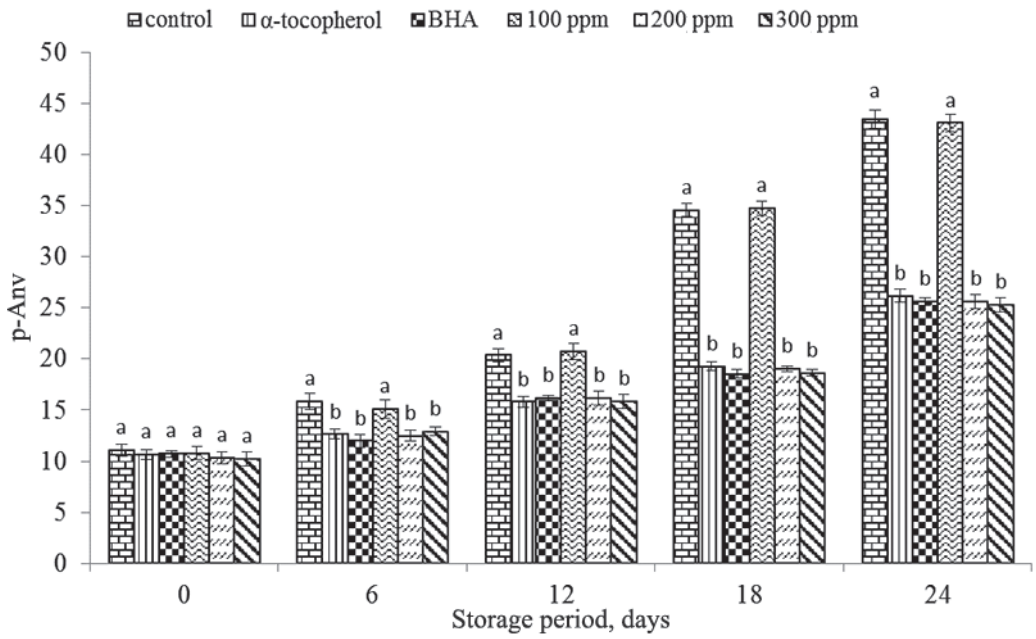

Fig. 2. *p*-Anisidine value (*p*-AnV) of sunflower oil supplemented with unripe banana peel extracts, BHA and α-tocopherol under accelerated storage at 65°C for 24 days. Different letters within each storage period denote that they are significantly different ($p < 0.05$)

Pike, 2010). The *p*-AnV value can be determined by evaluating the absorbance of solution produced from the mixture of oil in isooctane solution with *p*-anisidine (0.25% in glacial acetic acid). The added *p*-anisidine reacts with the aldehyde and produces a yellow colored solution. A lower *p*-AnV value indicates a better quality of oil (O'Keefe and Pike, 2010). Figure 2 demonstrated that there were increase of *p*-AnV for all the samples with irregular patterns throughout the storage period. From 6th day onwards, SFO-200, SFO-300, SFO-BHA and SFO-TOCO demonstrated a similar inhibitory effect, of which their *p*-AnV values were 12.49 ±0.54, 12.89 ±0.42, 11.99 ±0.60 and 12.63 ±0.48, respectively (Fig. 2). Under accelerated storage for 24 days, the total increase of *p*-AnV values were in the following order of SFO (control) > SFO-100 > SFO-TOCO/SFO-BHA = SFO-200/SFO-300 with the maximum values of 43.42 ±0.87, 43.06 ±0.86, 26.17 ±0.63, 25.62 ±0.41, 25.60 ±0.68 and 25.28 ±0.71, respectively, at 24th day. This indicates that the unripe banana peel extracts is effective in retarding the formation of secondary oxidation products in sunflower oil within 2 years of storage (24 days of accelerated storage condition) at ambient temperature.

Meanwhile after 24 days of storage, by comparing the increase of *p*-AnV in SFO-100 with that of SFO-200 and SFO-300, SFO-100 demonstrated a higher increase compared to that of SFO-200 and SFO-300 (Fig. 2). This suggests that 200 and 300 ppm of unripe banana peel extracts is sufficient enough to exhibit a protective effect in retarding secondary oxidation of oil. According to the Malaysian Food Act (1983), the maximum safety limit of BHA in food system is 200 ppm (equiv. 1100 μM). Therefore, unripe banana peel could be considered as a good source of natural antioxidant in a food system when compared to the maximum safety limit of BHA. After 24 days of accelerated storage, the control and SFO-100 demonstrated the highest *p*-AnV, demonstrating the higher rate of carbonyl formation compared with SFO-200, SFO-300, BHA and α-tocopherol stabilized samples. In another study, Nor et al. (2008) reported that *Pandanus amaryllifolius* leaf extract (0.1%) was demonstrated to be capable of retarding oxidation in palm olein as effectively as the synthetic antioxidant in *p*-AnV test.

Determination of Total Oxidation (TOTOX Value)

The total oxidation of the oil sample can be determined based on the calculated PV and *p*-AnV values. These values are reported as TOTOX value. TOTOX value measures primary and secondary oxidation products, reflecting the initial and later stages of the oil oxidation. Therefore, it provides a better estimation of the progressive oxidative deterioration of the oil (O'Keefe and Pike, 2010). Lower TOTOX value indicates a greater stability of oil sample against oxidative rancidity (Shahidi and Wanasundara, 2002). The TOTOX value of all the samples including the synthetic antioxidants were in the order of SFO-100 > control > SFO-200/SFO-300 = SFO-TOCO/SFO-BHA with maximum values of 180.88 ±1.51, 179.52 ±1.25, 117.36 ±1.64, 115.28 ±1.21, 110.00 ±0.78 and 107.85 ±1.13, respectively (Fig. 3). All the supplemented samples demonstrated positive effects in inhibiting oxidative rancidity except SFO-100. As compared to the control, all the supplemented samples had significantly ($p < 0.05$) lower TOTOX values except SFO-100 (Fig. 3). This could be due to the fact that SFO-100 was not effective in inhibiting oxidative rancidity of sunflower oil. SFO-300 reached a maximum TOTOX value which was higher than the TOTOX value of SFO-BHA. This might due to the fact that unripe banana peel extracts added to sunflower oil did not work as effectively as expected. After 24 days of storage, SFO-100 ppm was observed to be the least effective where it had significantly ($p < 0.05$) higher TOTOX values than α-tocopherol and BHA (Fig. 3). The low antioxidant activity of SFO-100 was mainly because of its low efficacy in delaying hydroperoxides formation due to the low concentration of unripe banana peel extract.

Iodine Value

Sunflower oil contains more than 70% polyunsaturated fatty acids (PUFAs). These PUFAs are highly prone to lipid oxidation (Zhang et al., 2010). During storage, the double bonds of these PUFAs are attacked by free radicals, which results in the formation of conjugated bonds (Kanner et al., 2012). Hence, measuring the amount of unsaturated fatty acids present in sunflower oil can be used as a reference to determine the freshness of the oil (Winne Sia et al., 2014). The freshness of sunflower oil can be determined quantitatively

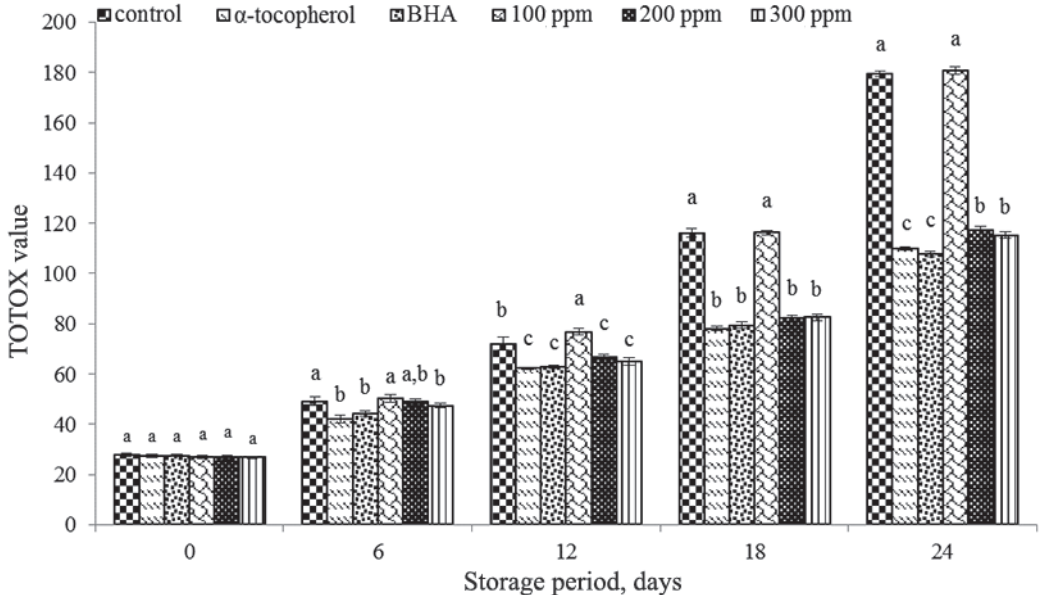

Fig. 3. TOTOX value of sunflower oil supplemented with unripe banana peel extracts, BHA and α-tocopherol under accelerated storage at 65°C for 24 days. Different letters within each storage period denote that they are significantly different ($p < 0.05$)

by adding iodine monochloride to the oil samples. The unsaturated fatty acids react with iodine monochloride to release free iodine. The free iodine can then react with sodium thiosulphate for the determination of iodine value (IV) (O'Keefe and Pike, 2010).

Abdulkarim et al. (2007) reported that the IV indicates the degree of unsaturation of oils. Anwar et al. (2007) and Nor et al. (2008) demonstrated that the IV values greatly reduced with the increase of storage time. The IV values of the stabilized unripe banana peel extract, BHA, α-tocopherol and control sunflower oil, over an accelerated storage period of 24 days at 65°C, was shown in Figure 4. It was observed that the IV value decreases significantly ($p < 0.05$) with the increase time of storage for all samples. The rate of reduction in control was higher than that in sunflower oil containing both synthetic and natural antioxidants. A decreasing IV value of oils is generally attributed to the destruction of the fatty acid double bonds caused by oxidation process. Throughout the 24 days storage period, the total reduction in IV among the samples were in the following order: SFO (control) > SFO-100 > SFO-200 >SFO-300 > SFO-TOCO > SFO-BHA (Fig. 4). The untreated sunflower oil sample (control) decreased noticeably throughout 24 days until a minimum value of 91.43 I_2/100 g. On 0th and 24th day, SFO-100 demonstrated higher reduction compared to SFO-200 and SFO-300, whereby the IV value of SFO-100 were significantly different ($p < 0.05$) compared to that of the IV values obtained for SFO-200 and SFO-300. On the 12th day, SFO-300, α-tocopherol, and BHA demonstrated stronger inhibitory effects against lipid oxidation, while on the 24th day, no significant differences ($p < 0.05$) were noticed between the crude extract at 300 ppm, BHA, and α-tocopherol (Fig. 4). As shown in Figure 4, it was clear that both BHA and α-tocopherol inhibited oxidation of sunflower oil significantly ($p < 0.05$) as compared to SFO-100 and SFO-200 throughout the storage period. The highest IV of SFO-BHA and SFO-TOCO compared to SFO-100, SFO-200 and SFO-300 at every interval demonstrated the higher efficacies of BHA and α-tocopherol to protect the unsaturated bonds of fatty acids in sunflower oil being oxidized by free radicals. From this, it can be deduced that unripe banana peel antioxidant is less effective as the synthetic antioxidants in preventing lipid oxidation.

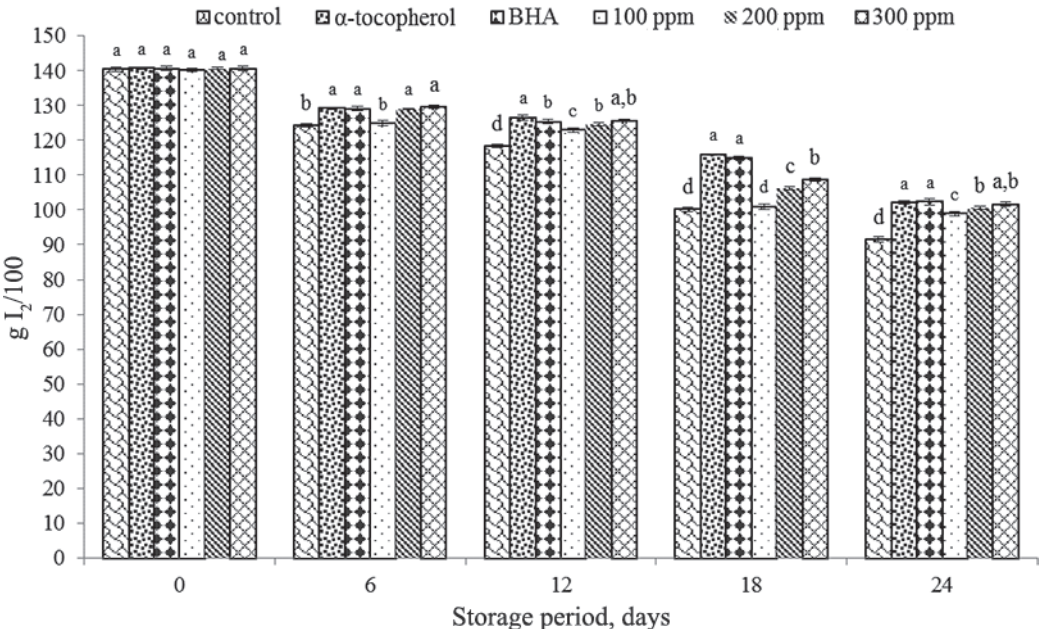

Fig. 4. Iodine value (IV) of sunflower oil supplemented with unripe banana peel extracts, BHA and α-tocopherol under accelerated storage at 65°C for 24 days. Different letters within each storage period denote that they are significantly different ($p < 0.05$)

Thiobarbituric Acid Reactive Substances (TBARS)

Thiobarbituric Acid (TBA) is defined as the quantity of malondialdehyde (MDA; mg) presented in 1 kg of sample (Zhang et al., 2010). MDA is the standard biomarker for measurement of lipid peroxidation of cell membrane despite other biomarkers such as 4-hydroxynonenal and isoprostanes. MDA are generated from the peroxidation of fatty acids with three double bonds and more where it is the most abundant active carbonyl generated from lipid peroxidation in foods containing high amounts of unsaturated fats (Kanner et al., 2012). Thus, MDA is a robust biomarker in terms of measuring lipid peroxidation *in vivo*. The primary oxidation product, lipid hydroperoxides react with oxygen to form MDA, which contributes to the off-flavour of the oil (Zhang et al., 2010). The MDA levels in an oil sample can be determined through its reaction with thiobarbituric acid (TBA). In this test, MDA reacts with TBA to form TBA-MDA complex which is pink in colour (O'Keefe and Pike, 2010).

Figure 5 depicts the effects of 100, 200 and 300 ppm unripe banana peel extract, BHA, α-tocopherol and control on TBARS values of sunflower oil at 65°C for 24 days accelerated storage. As shown in Figure 5, regular increase in TBARS value as a function of storage time was observed at all intervals. As the sample concentration increases, the amounts of secondary products detected were lower. This result was in accordance with the obtained *p*-AnV value. This result was also in accordance with the result obtained in the study of garlic extract and pomegranate peel added to sunflower oil, which reported to have stronger protective effects at higher concentrations (Iqbal and Bhanger, 2007). The TBARS values of control in the absence of antioxidant and crude unripe banana peel extract at 100 ppm increase significantly throughout the storage period especially after the 6th day, reaching the maximum value of 0.14 mg MDA/mL on 24th day. It was also apparent that the TBARS values of sunflower oil samples stabilized with unripe banana peel extracts and synthetic antioxidants were found to be significantly ($p < 0.05$) lower than the control and SFO-100.

After 24 days of accelerated storage, BHA and α-tocopherol managed to show inhibition ability in retarding lipid oxidation with the TBARS value of 0.12 ±0.00 mg MDA/mL. This indicates that both BHA and

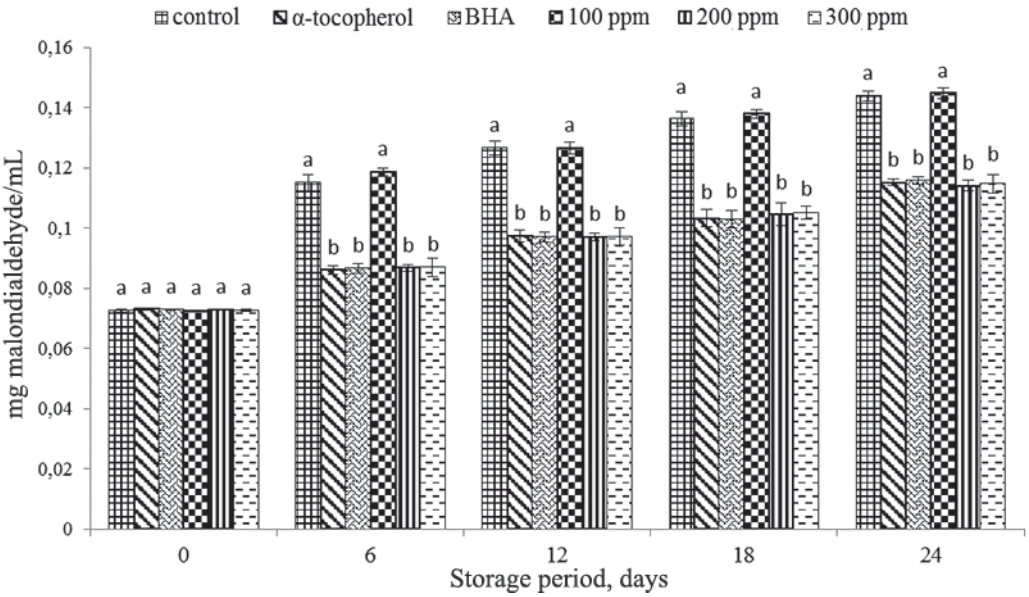

Fig. 5. TBARS value of sunflower oil supplemented with unripe banana peel extracts, BHA and α-tocopherol under accelerated storage at 65°C for 24 days. Different letters within each storage period denote that they are significantly different ($p < 0.05$)

α-tocopherol were effective in retarding lipid oxidation. SFO-200 and SFO-300 demonstrated the lowest TBARS values, which was 0.11 mg MDA/mL on the 24th day. This shows that both SFO-200 and SFO-300 demonstrated a higher antioxidant activity and hence greater protective effect than SFO-100. SFO-200, SFO-300, SFO-BHA and SFO-TOCO exhibited comparable inhibitory effects against lipid oxidation as observed on 24th day of the storage duration. This implies that both SFO-200 and SFO-300 are able to prolong the shelf-life of sunflower oil for 2 years at ambient storage by having the antioxidant potency as effective as the allowed BHA concentration of 200 ppm as a food additive. In another study, Monfared et al. (2011) have shown that water and ether extracts of *Urtica dioica* (stinging nettle) are capable of inhibiting both primary and secondary oxidation of sunflower oil during storage compared to the control, which is in consistent with the results of this study.

Free Fatty Acids (FFA)

Free fatty acid value is used as an indicator of fat hydrolysis, specifically, the presence of free fatty acids. FFAs are formed due to the hydrolysis of triglycerides and can be promoted by moisture content (O'Keefe and Pike, 2010). The FFA content of SFO (control), SFO-100, SFO-200, SFO-300, SFO-BHA and SFO-TOCO during 24 days of accelerated storage were shown in Figure 6. The FFA contents in the sunflower oil demonstrated a gradual increase throughout the storage period. As a comparison among the samples, the total increase of FFA values during the storage period were in the order of SFO (control) > SFO-100 > SFO SFO-200/SFO-300 = SFO-BHA/SFO-TOCO. The antioxidant capacities of SFO-200 and SFO-300 are comparable with α-tocopherol and BHA. After 0 day of storage, the FFA values of both control and SFO-100 increased significantly and gradually throughout the storage period until 24th day. Based on the result obtained, it was clear that SFO-200, SFO-300, SFO-BHA and SFO-TOCO are able to retard the hydrolysis of triglycerides, thus, preserving sunflower oils for 2 years at room temperature (Fig. 6).

After 24 days of storage time, the untreated sunflower oil sample (control) demonstrated the highest FFA content (0.38 ±0.02%). Meanwhile, crude extract at 300 ppm demonstrated the lowest FFA content (0.23 ±0.04%) as shown in Figure 6. This shows that

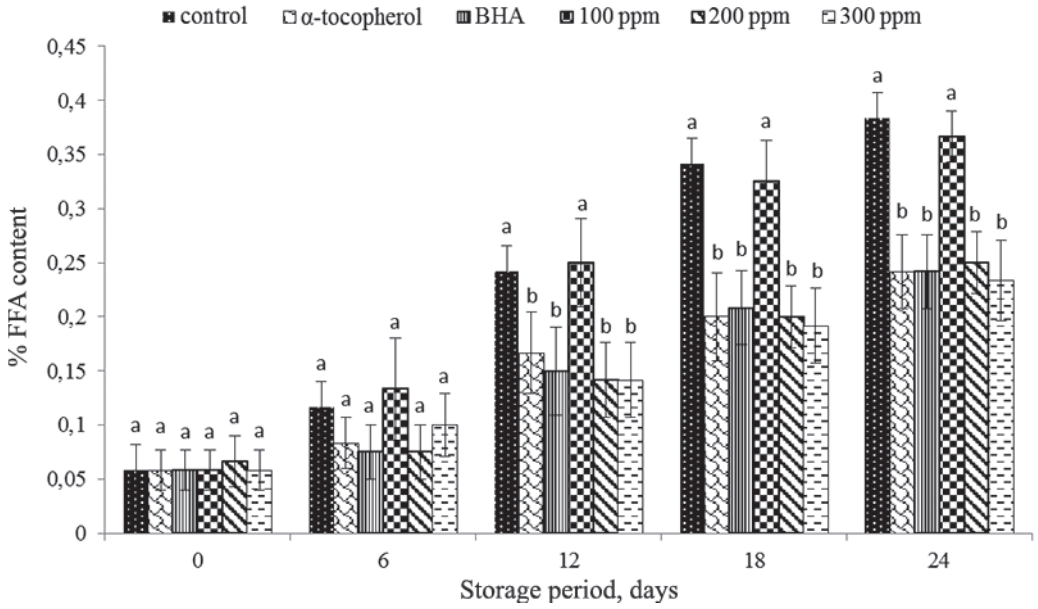

Fig. 6. FFA value of sunflower oil supplemented with unripe banana peel extracts, BHA and α-tocopherol under accelerated storage at 65°C for 24 days. Different letters within each storage period denote that they are significantly different ($p < 0.05$)

all SFO-200, SFO-300, SFO-BHA and SFO-TOCO have the similar inhibitory effect on the lipid oxidation of sunflower oil and are capable of hindering the hydrolysis of triglycerides by extending its shelf-life up to 2 years. Similarly, Iqbal and Bhanger (2007) reported that garlic extract at 1000 ppm is more effective than BHA and BHT at 200 ppm in preventing hydrolysis of triglycerides over longer storage period. Thus, higher concentration of unripe banana peel extracts may be able to give a better protective activity. Future research should be carried out to investigate the effectiveness of unripe banana peel extracts at higher concentrations.

Discussions

Although it is difficult to stabilize sunflower oil because of its high linoleic acid content, unripe banana peel extract at the concentration of 200 and 300 ppm has been shown to have stabilizing efficacy comparable to synthetic antioxidants such as BHA at its legal limit. Unripe banana peel extract has a strong antioxidative effect during initial and final stages of oxidation under accelerated storage conditions at 65°C for 24 days. It improves the resistance of sunflower oil against thermal deteriorative changes. Moreover, polyunsaturated fatty acid content has been saved appreciably by inducing resistance of sunflower oil against oxidative rancidity.

Some studies have demonstrated the effectiveness of other plant extracts in preventing sunflower oil oxidation. For example, carnosic acid from rosemary dried leaves demonstrated strong antioxidant efficacy by protecting sunflower oil from being oxidized (Zhang et al., 2010). Iqbal and Bhanger (2007) investigated the efficacy of garlic extracts and synthetic antioxidants added to sunflower oil. Garlic extract at 1000 ppm demonstrated greater antioxidant efficacy to prevent lipid oxidation compared with synthetic antioxidants. The present study gives an impact for expanding the use of natural antioxidants from fruit wastes, such as banana peel in food industry.

Bananas have a characteristic array of phytochemicals. The phytochemicals that have been reported most in both banana pulp and peel are carotenoids, phenolic compounds and biogenic amines (Oliveira et al., 2008; Pereira and Maraschin, 2015; Tsamo et al., 2015). These phytochemicals have been shown to correlate well with its good antioxidant capacities

(Bennett et al., 2010; Lim et al., 1997; Sulaiman et al., 2011). However, literature on the phytochemical profiles of the peel and pulp of bananas is still scarce and inconsistent.

Someya et al. (2002) demonstrated that total phenolic content (TPC) was high in banana peels, accounting for 907 mg/100 g of dry weight of sample, where the phenolic compounds identified were mainly epigallocatechin, catechin, and epicatechin. Biogenic amines, such as dopamine have also been identified in banana peel, where the concentration of dopamine in the banana peel was higher than the pulp (Teeranud et al., 2005). There was another study demonstrating that banana peel contains large amounts of catecholamines, dopamine and L-dopamine with significant antioxidant activity (Rafaela et al., 2010). Dopamine have been shown to demonstrate higher antioxidant capacity *in vitro* [determined using (2,2-diphenyl-1-picrylhydrazyl) DPPH assay] compared to synthetic antioxidants, such as ascorbic acid, reduced glutathione, and some phenolic compounds, such as epigallocatechin gallate (Kanazawa and Sakakibara, 2000).

Kanazawa and Sakakibara (2000) reported that banana peel contain flavonoids, such as naringenin and rutin. Meanwhile, saponin (24 mg/g) has also been found in *Musa sapientum* peels (Anhwange, 2008). Unripe banana peel has been shown to contain leucocyanidin, a flavonoid that helps in accelerating the healing of skin wounds as well as surgery wounds (Atzingen et al., 2013). The antioxidative efficacy of unripe banana peel has been shown effective in the prevention and treatment of peptic ulcers in rat model. It is interesting to know that the active agent in unripe bananas is water soluble and becomes inactive in ripe banana peel (Best et al., 1984). More research should be carried out to determine the complete profiles of phytochemicals, such as, phenolic acids, flavonoids, phytoestrogens and carotenoids of bananas (pulp and peel) in relation to their antioxidant capacities or other bioactivities. The combination of these bioactive compounds (phenolic compounds and biogenic amines) might provide synergistic effects to the antioxidant capacity that further enhanced the oxidative stability of oils using unripe banana peel as shown in this study. Considering the potent antioxidant efficacy of unripe banana peel extract, it could be used as food additives for increasing the shelf life of food by preventing lipid peroxidation.

CONCLUSION

From the present study, it can be concluded that unripe banana peel extract at 200 and 300 ppm has been shown to have strong protective effects against lipid peroxidation of sunflower oil during accelerated storage conditions and demonstrated stabilizing efficacy comparable to commonly used synthetic antioxidants, BHA (200 ppm) at its legal limit. Unripe banana peel extract prolongs the shelf-life of sunflower oil against heat deteriorative changes. Unripe banana peel could be one of the most important sources of natural antioxidants. Therefore, unripe banana peel extract can be recommended as a potent source of antioxidants for the stabilization of food systems, especially unsaturated vegetable oils.

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