Oxidative behaviour of four Malaysian edible plant extracts in model and food oil systems

Irwandi Jaswir¹*, Fitri Octavianti², Ridar Hendri³, Elmi⁴ and Alfi Khatib⁵

¹Department of Biotechnology Engineering, Faculty of Engineering, International Islamic University Malaysia, Jalan Gombak 53100 Kuala Lumpur, Malaysia.
²Dental Faculty, MAHSA University College, Pusat Bandar Damansara, Kuala Lumpur, Malaysia.
³Faculty of Fisheries, Riau University, Pekanbaru, Riau, Indonesia.
⁴Department of Paediatrics, State General Hospital, Pekanbaru, Riau, Indonesia.
⁵Department of Food Science, Faculty of Food Science and Technology, Universiti Putra Malaysia, 43400 Serdang, Selangor DE, Malaysia.

Accepted 27 December, 2011

The present study was conducted to investigate the antioxidative behaviour of four local plant extracts in Malaysia-- red spinatch (Amarantus gangeticus), “kucai” (Allium odorum), Chinese cabbage (Brassica chinensis), and “kesom” (Polygonum minus)-- in palm olein system and in a linoleic acid model system. The antioxidative capacities of these local plants were then compared to the activity of rosemary and sage, two types of antioxidant commonly found in the market. From the analysis using Oxygen Consumption Method, it was found that among the samples evaluated, red spinatch had the longest time to reach the 50% oxygen in the chamber, with 110 min, meaning that this sample had the highest level of antioxidative activity. This was followed by the extracts of Chinese cabbage (80 min), “kucai” (60 min), and “kesom” (40 min). The antioxidative activities of these plants, however, were lower than those of rosemary and sage. These commercial antioxidants were found to have 150 and 130 min time, respectively, to reach the 50% oxygen in the chamber. Results from differential scanning calorimetry (DSC) analysis showed that addition of red spinatch and Chinese cabbage extract samples to the oil in the system reduced the oxidation as evidenced by longer Tₒ of antioxidants-treated samples. The onset time (Tₒ) of the oxidation reaction corresponded closely to the intersection of the extrapolated baseline and the tangent line (leading edge) of the exotherm, meaning that the longer Tₒ, the greater the antioxidative activity of a sample. Statistical analysis from this study showed that there was no significant difference between Tₒ of red spinatch and those of rosemary and sage. This meant that the antioxidative activity of red spinatch samples was comparable to the activities of rosemary and sage. The antioxidative activities of Chinese cabbage, “kucai” and “kesom” were also much higher than that of control. The finding from this study indicated that all samples used in this study had very good potential to be explored as sources of natural antioxidants.

Key word: Oxidative behavior, edible plants, model system, red spinatch.

INTRODUCTION

Many lipids are particularly labile when exposed to a combination of heat, air and light. Under conditions of heating or frying, the acceleration of both thermal and oxidative decomposition reactions will occur (Grandgirard et al., 1984, Sebedio et al., 1987). It is well established that the excessive heating of oils or fats can result in the formation of compounds that possess antinutritional properties, such as enzyme inhibitors (Ruiz-Gutierrez and Muriana, 1992) and accelerated loss of antioxidant vitamins, such as vitamin E (Sheehy et al., 1994; Lü and Lee, 1998) leading to growth depression and histologic changes in gastrointestinal tissues (Alexander, 1983; Clark...
and Serbia, 1991). Notwithstanding these potential adverse health effects of thermally oxidized oil, it is important to note that lipid oxidation also influences the acceptability of the fried product (Jacobson, 1991). Thus, to retard against undesirable changes in oil during storage and frying operations, antioxidants are required (Cuvelier et al., 1994).

Presently, butylated hydroxyanisole (BHA), butylated hydroxytoluene (BHT) and tertiary-butyl hydroquinone (TBHQ) are antioxidants commonly used in cooking oils. However, with the safety concern that have been identified for these synthetic antioxidants (Wichi, 1988), considerable recent interest in the use of natural antioxidants for frying purpose has occurred.

Various types of natural antioxidants have been explored for long for many purposes, including for health reasons (Uchiyama et al., 2004; Tian et al., 2007; Takahashi et al., 2006). Some of the natural antioxidants are even used traditionally for dishes or healing diseases (Umeno et al., 2005; Yamaguchi et al., 2006).

Red spinach (Amaranthus gangeticus), “kucai” (Allium odorum), Chinese cabbage (Brassica chinensis), and “kesom” (Polygonum minus) are edible plants traditionally used in Malaysian local dishes. These plants are normally incorporated into foods during the food preparation as ingredients.

This present study was conducted to investigate the antioxidative behaviour of these edible plant extracts in palm olein system and in a linoleic acid model system. The antioxidative capacities of these local plants were compared to the activity of rosemary and sage, two antioxidants commonly found in the market.

MATERIALS AND METHODS

Raw materials used for this study includes red spinach (A. gangeticus), “kucai” (A. odorum), Chinese cabbage (B. chinensis), and “kesom” (P. minus). All of them were used in fresh form. They were all obtained from a supermarket in Selangor. All chemicals used were purchased from a local supplier.

Preparation of antioxidant extracts

The preparation of antioxidant extracts was conducted according the method of Chang et al. (1978). The leaves of each plant were washed with tap water before being dried at 30°C for 24 h. The samples were then extracted with 95% ethanol at an ethanol:sample ratio 12.5 (v:w) in a shaking water bath set at 50°C, 120 rpm for 24 h.

The mixture of solvent and samples were filtered using vacuum filter flask and the filtrate was concentrated under vacuum at 50°C in a rotary evaporator to yield crude antioxidant extracts. These extracts were further dissolved in the ethanol and bleached with activated carbon (60% by weight of samples) and stirred at 60°C for 15 min twice.

The bleached solution was concentrated under vacuum at 50°C in a rotary evaporator to yield the antioxidant extracts for further analysis.

Oxygen consumption measurement

In this study, oxygen consumption measurements were undertaken as a chemical measure for evaluating lipid oxidation reactions. The percentage of oxygen remaining in the reaction chamber over a period of time, in the absence and presence of the plant extracts, was recorded as a measure of calculating protective index (PI) values of these antioxidants.

Oxygen depletion in a linoleic acid emulsion system with added ferrous (Fe²⁺) ions, and in the absence and presence of the antioxidant extracts, was measured according to the methods of McGookin and Augustin (1991), Lingnert et al. (1979), and Wijewickreme and Kitts (1997) using a YSI Model 5300 biological oxygen monitor (Yellow Springs, OH). Each sample of the antioxidant extracts were first dissolved in a linoleic acid emulsion [1.5 g of linoleic acid mixed with 0.1 g of Tween 20 in 40 ml of 0.1 M potassium phosphate buffer (pH 6.8)]. One milliliter of this mixture was then mixed with 5 ml of buffer (0.1 M potassium phosphate buffer, pH 6.8), and 0.6 ml of 2 mM FeSO₄ dissolved in 0.1 M potassium phosphate buffer (pH 6.8). The reaction mixture was pumped into a jacketed reaction vessel containing an oxygen electrode at room temperature. Oxygen depletion was recorded immediately after the reaction mixture was introduced into the vessel. Both antioxidant and prooxidant activity of natural antioxidant mixtures were expressed in terms of protective index (PI), defined as:

\[
\text{PI} = \frac{\text{Time for 50% O}_2\text{ depletion with test compound}}{\text{Time for 50% O}_2\text{ depletion without test compound}}
\]

where PI < 1 denotes prooxidant activity, PI=1 denotes no activity, and PI>1 denotes antioxidant activity (Lingnert et al., 1979).

Differential scanning calorimetric (DSC) analysis

The oxidative stability of oil treated with the plant extracts was determined by a Perkin-Elmer differential scanning calorimeter DSC-7 (Norwalk, CT), according to the method of Gupta and Jaworski (1991) with minor modifications. The equipment was calibrated with pure indium and the baseline was obtained with an empty open aluminum pan. An empty open aluminum pan was used as a reference. Oil sample of 5.0±0.5 mg was weighed in an open aluminum pan and placed in the equipment’s sample chamber. The isothermal temperature was programmed at 150°C and purified oxygen (99.8%) was passed through the sample enclosure at 50 ml/min. The onset time (Tₜₒ) of the oxidation reaction corresponded closely to the intersection of the extrapolated baseline and the tangent line (leading edge) of the exotherm (Figure 1).

RESULTS AND DISCUSSION

Assessment of antioxidant activity by oxygen consumption

Figure 2 shows percentage oxygen consumption by the plant sample extracts, as well as rosemary and sage extracts in a linoleic emulsion system. It is clear that the percentage of oxygen remaining in the vessel depleted more rapidly in the control emulsion.
compared to the other emulsions containing the plant extracts. It indicated that no extract samples with a potential prooxidant activity were observed. For the control sample, it was required 21 min to reach 50% oxygen in the chamber.

Among the samples evaluated, red spinach was found to have the longest time to reach the 50% oxygen in the chamber, with 110 min, meaning that this sample had the highest level of antioxidative activity. This was followed by the extracts of Chinese cabbage (80 min), “kucai” (60 min), and “kesom” (40 min). The antioxidative activities of these plants, however, were lower than those of rosemary and sage. These commercial antioxidants were found to have 155 and 145 min time, respectively, to reach the 50% oxygen in the chamber.

The PI values calculated for the plant extracts studied and the commercial antioxidants are given in Table 1. The PI values calculated in this study, employing an iron-supplemented linoleic acid emulsion system in which iron ions act as a promoter of lipid oxidation reactions, were useful to distinguish between antioxidant and prooxidant activities of various mixture samples. It is seen that the PI values of all sample extracts studied were statistically higher than the control PI. Samples of red spinach and Chinese cabbage were found to be more than 4-times antioxidant activity higher than control. The PI value for the red spinach was 4.5, while Chinese cabbage was 4.0. The values for the extracts of “kucai” and “kesom” were 2.9 and 2.2, respectively. However, results from this study showed that no significant difference between red spinach and Chinese cabbage was observed. The results also showed that the antioxidant capacity of these two samples were significantly (P<0.05) higher than those of other plants (Table 1). As mentioned earlier, The PI values reflect the antioxidative capacity of each sample. The greater the value, the higher the antioxidative activity of a sample.

As a comparison, the PI values of rosemary and sage were 7.4 and 6.6, respectively, but slightly higher than the values of red spinach and Chinese cabbage statistically (P<0.05) (Table 1).

### Monitoring effect of natural antioxidants by DSC

The use of DSC for measuring the stability of oil was first reported by Cross (1987) and Hassel (1976). The tests were carried out at isothermal modes with purge oxygen. The end point of DSC was taken at the time where a rapid exothermic reaction of oil and oxygen occurred. According to Hassel (1976), the use of DSC could shorten the time needed to analyse the oxidative stability of oil samples from 14 days by Schaal oven test (SOT) to less than 4 h.
Results from this study showed that oxidation reactions produce traces as shown in Figure 1 (curve B). No exothermic peak was detected when the oil sample was scanned under nitrogen (Figure 1, curve A). The extrapolated onset time ($T_o$) was taken as a measure of the relative stability of the oil toward oxidation. As mentioned previously, the onset time ($T_o$) of the oxidation reaction corresponded closely to the intersection of the extrapolated baseline and the tangent line (leading edge) of the exotherm (Figure 1). In this study, Figure 3 shows the DSC oxidation curves of 4 samples evaluated in this study, compared to the curves of rosemary, sage and control. It is clearly seen that addition of red spinach and Chinese cabbage extract samples to the oil reduced the oxidation as evidenced by longer $T_o$ of antioxidants-treated samples. The control sample had the shortest $T_o$ of 39.8 min, while the longest one belonged to rosemary sample, with 79.3 min. The $T_o$ values for all treatment samples and control are given in Table 1. Among the local plant samples evaluated, red spinach once again
showed the highest activity of antioxidant. Red spinach sample had the longest $T_o$ of 71.4, followed by Chinese cabbage, “kucai”, and “kesom” with 58.5, 57.0 and 49.7, respectively.

Statistical analysis from this study showed that there was no significant difference between $T_o$ of red spinach and those of rosemary and sage (Table 1). This meant that the antioxidative activity of red spinach sample was comparable to the activities of rosemary (79.3) and sage (75.6). The antioxidative activities of Chinese cabbage, “kucai”, and “kesom” were also much higher than that of control (39.8). The finding from this study indicated that all samples used in this study had very good potential to be explored as sources of natural antioxidants.

**Conclusion**

From the analysis using oxygen consumption method, it was found that among the samples evaluated, red spinach had the longest time to reach the 50% oxygen in the chamber, with 110 min, meaning that this sample had the highest level of antioxidative activity. This was followed by the extracts of Chinese cabbage (80 min), “kucai” (60 min), and “kesom” (40 min). Results from differential scanning calorimetry (DSC) analysis showed that addition of the sample extracts in this study to the oil in the system reduced the oxidation as evidenced by longer $T_o$ of antioxidants-treated samples. Statistical analysis from this study revealed that there was no significant difference between $T_o$ of red spinach and those of rosemary and sage, meaning that the antioxidative activity of red spinach was comparable to the activities of commercially available antioxidants of rosemary and sage. The antioxidative activities of Chinese cabbage, “kucai” and “kesom” were also much higher than that of control. The finding from this study indicated that all samples used in this study had very good potential to be explored as sources of natural antioxidants.

**REFERENCES**


