JOURNAL OF OLEO SCIENCE

J. Oleo Sci., Vol. **53**, No. 11, 525-529 (2004)

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Efficacy of Malaysian Plant Extracts in Preventing Peroxidation Reactions in Model and Food Oil Systems

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Edited by I. Sugimoto, Nisshin OilliO Group, and accepted June 8, 2004 (received for review April 12, 2004)

Abstract: A study was conducted to investigate the antioxidative behaviour of the crude extracts of *pegaga* (*Centella asiatica*) leaves, and *limau purut* (*Citrus hystrix*) leaves, peels, and stems 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 Oxigen Consumption Method, it was found that among the samples evaluated, pegaga leaves had the longest time to reach the 50% oxygen in the chamber, with 90 min, meaning that this sample had the highest level of antioxidative activity. This was followed by the extracts of limau purut leaves (85 min), peels (60 min), and stems (39 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. Results from differential scanning calorimetry (DSC) analysis showed that addition of pegaga leaves and limau purut samples to the oil in the system reduced the oxidation as evidenced by longer T_o of antioxidants-treated samples. Statistical analysis from this study showed that there was no significant difference between T_o of pegaga leaves and those of rosemary and sage. This meant that the antioxidative activity of pegaga leaves was comparable to the activities of rosemary and sage. The antioxidative activities of limau purut leaves, peels and stems 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 words: antioxidative capacity, *Centella asiatica*, *Citrus hystrix*, DSC

1 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 (1,2). 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 (3) and accelerated loss of antioxidant vitamins, such as vitamin E (4,5) leading to growth depression and histologic changes in gastrointestinal tissues (6,7). 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 (8). Thus, to retard against undesirable changes in oil during storage and frying operations, antioxidants are required (9).

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Presently, butylated hydroxyanisole (BHA), butylated hydroxytoluene (BHT) and tertiary-butyl hydroquinone (TBHQ) are antioxidants commonly used in cooking oils in developing countries, including Malaysia. However, with the safety concerns that have been identified for these synthetic antioxidants (10), considerable recent interest in the use of natural antioxidants for frying purpose has occurred.

Pegaga (*Centella Asiatica*) and limau purut (*Citrus hystrix*) are two types of plant traditionally used in Malaysian local dishes. Leaves of these plants are normally incorporated into foods during the food preparation as ingredients. Pegaga leaves are also commonly consumed as "ulam", a local term for salad. Recent studies show that pegaga and limau purut leaves not only can be used as food ingredients, but also can benefit human health. Preliminary studies by Chin (11) indicated that pegaga has efficacy in alleviating cholesterol induced renal effects in rats, while Ruhani (12) has successfully extracted some bioactive compounds from limau purut leaves.

This present study was conducted to investigate the antioxidative behaviour of the crude extracts of pegaga leaves, and limau purut leaves, stems and peels 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.

2 Materials and Methods

2·1 Materials

Raw materials used in for this study were pegaga and limau purut. They were obtained from a supermarket in Selangor. All chemicals used were purchased from a local supplier.

2·2 Preparation of Antioxidant Extracts

The preparation of antioxidant extracts was conducted according the method of Chang *et al*. (13). The leaves of pegaga (PL) and the leaves, stems and peels of limau purut (LPL, LPS, and LPP, respectively) were washed with tap water before being dried at 30℃ for 24 hr. The samples were then extracted with 95% ethanol at an ethanol-samples ratio 12:5 (v:w) in a shaking water bath set at 50℃, 120 rpm for 24 hr. The mixture of solvent and samples were filtered using vacuum filter flask and the filtrate was concentrated under vacuum at 50℃ 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℃ for 15 min twice. The bleached solution was concentrated under vacuum at 50℃ in a rotary evaporator to yield the antioxidant extracts for further analysis.

2·3 Oxygen Consumption Measurement

Oxygen depletion in a linoleic acid emulsion system with added ferrous (Fe^{2+}) ions, and in the absence and presence of the antioxidant extracts, was measured according to the methods of McGookin and Augustin (14), Lingnert *et al*., (15), and Wijewickreme and Kitts (16) 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 $FeSO_4$ 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:

$$PI = \frac{\text{time for 50\% } O_2 \text{ depletion with test compound}}{\text{time for 50\% } O_2 \text{ depletion without test compound}}$$

where PI < 1 denotes prooxidant activity, PI = 1 denotes no activity, and PI > 1 denotes antioxidant activity (15).

2·4 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 (17) 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 tempera-

ture was programmed at 150℃ and purified oxygen (99.8%) was passed through the sample enclosure at 50 ml/min. 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 (**Fig. 1**).

3 Results and Discussion

3·1 Assessment of Antioxidant Activity by 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 PI values of these antioxidants. **Figure 2** shows percentage of oxygen consumption by the pegaga and limau purut 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 percent oxygen in the chamber.

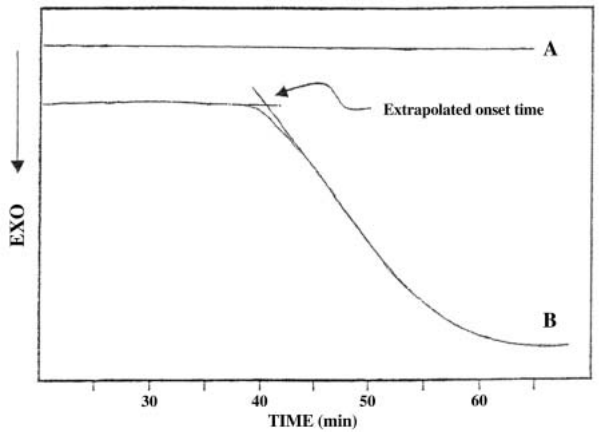

Fig. 1 Differential Scanning Calorimetric Oxidation Curve of Refined, Bleached and Deodorized (RBD) Palm Olein without Antioxidant. (A) Isothermal curve at 150℃ with nitrogen (99.999%) flowed at 50 ml/min; and (B) isothermal curve at 150℃ with oxygen (99.8%) flowed at 50 ml/min.

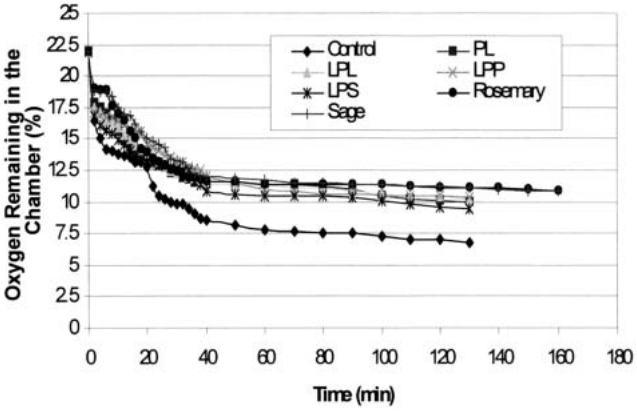

Fig. 2 Percentage of Oxygen Consumption by Natural Antioxidant Extracts in a Model Linoleic Acid Emulsion System. PL = pegaga leaves; LPL = limau purut leaves; LPP = limau purut peels; LPS = limau purut stems

Among the samples evaluated, pegaga leaves were found to have the longest time to reach the 50% oxygen in the chamber, with 90 min, meaning that this sample had the highest level of antioxidative activity. This was followed by the extracts of limau purut leaves (85 min), peels (60 min), and stems (39 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-suplemented linoleic model 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. Sample pegaga leaves was found to be the sole sample with more than 4-times antioxidant activity higher than control. The PI value for the pegaga leaves was 4.1. The values for the extracts of limau purut leaves, peels, and stems were 3.9, 2.9, and 1.9, respectively. However, results from this study showed that no significant difference between pegaga and limau purut leaves was observed. The results also showed that the antioxidant capacity of these two samples were significantly ($P < 0.05$) higher than those of limau purut peels and stems (**Table 1**).

Table 1 Protective Index (PI) and Onset Time (T_o) of 15 Different Antioxidant-treated Samples*.

Samples	PI	T_o (min)
Control	1 ± 0^{e}	39.8 ± 2.6^{d}
PL	4.1 ± 0.2^{b}	71.3 ± 3.1^{a}
LPL	3.9 ± 0.1^{b}	58.0 ± 2.7^{b}
LPP	2.9 ± 0.3^{c}	56.0 ± 3.0^{b}
LPS	1.9 ± 0.3^{d}	49.5 ± 2.7^{c}
Rosemary	7.4 ± 0.4^{a}	79.3 ± 3.2^{a}
Sage	6.6 ± 0.3^{a}	75.6 ± 3.1^{a}

[1]Mean of three replications

[+]: PL = pegaga leaves; LPL = limau purut leaves; LPP = limau purut peels;
LPS = limau purut stems

[a-e]: Means within a column with different letters are significantly different ($P < 0.05$)

As comparison, the PI values of rosemary and sage were 7.4 and 6.6, respectively, slightly but statistically ($P < 0.05$) higher than the values of pegaga and limau purut leaves (**Table 1**).

3·2 Monitoring Effect of Natural Antioxidants by DSC

The use of DSC for measuring the stability of oil was first reported by Cross (18) and Hassel (19). The tests were carried out at isothermal modes with a 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 (19), 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 **Fig. 1** (curve B). No exothermic peak was detected when the oil sample was scanned under nitrogen (**Fig. 1**, curve A). The extrapolated onset time (T_o) was taken as a measure of the relative stability of the oil toward oxidation. **Figure 3** shows the DSC oxidation curves of 4 samples evaluated, compared to the curves of rosemary, sage and control. It is clearly seen that addition of pegaga leaves and limau purut 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, pegaga leaves once again showed the highest activity of antioxidant. Pegaga leaves had the longest T_o of 71.3, followed by limau purut leaves, peels, and stems with 58.6, 56.0,14 and 49.5, respectively.

Statistical analysis from this study showed that there was no significant difference between T_o of pegaga

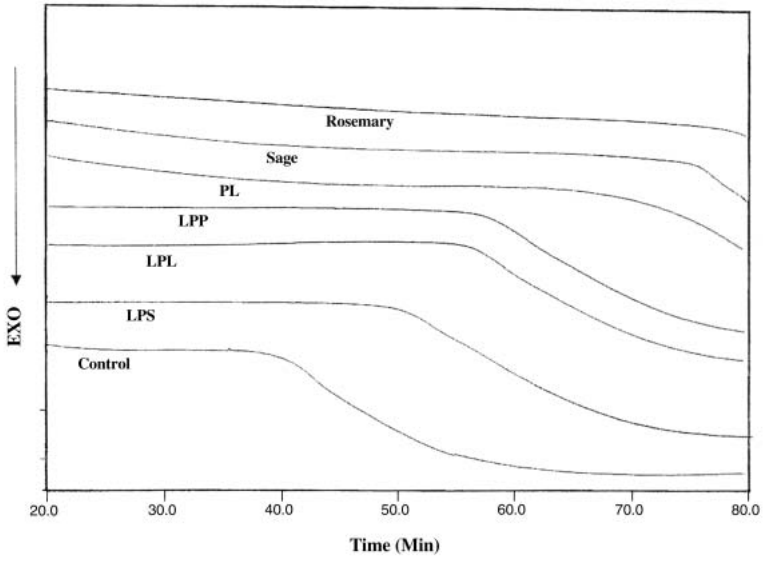

Fig. 3 DSC Oxidation Curves of RBD Palm Olein with Added Natural Antioxidants.
For abbreviations see **Fig. 2**.

leaves and those of rosemary and sage (**Table 1**). This meant that the antioxidative activity of pegaga leaves was comparable to the activities of rosemary and sage. The antioxidative activities of limau purut leaves, peels and stems 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.

4 Conclusion

From the analysis using Oxigen Consumption Method, it was found that among the samples evaluated, pegaga leaves had the longest time to reach the 50% oxygen in the chamber, with 90 min, meaning that this sample had the highest level of antioxidative activity. This was followed by the extracts of limau purut leaves (85 min), peels (60 min), and stems (39 min). Results from differential scanning calorimetry (DSC) analysis showed that addition of pegaga leaves and limau purut samples to the oil in the system reduced the oxidation as evidenced by longer T_o of antioxidant-treated samples. This study revealed that all plant samples used in this study had very good potential to be explored as sources of natural antioxidants.

Acknowledgements

The authors thank the Research Centre of the International Islamic University Malaysia for funding this research project.

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