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Food Chemistry 66 (1999) 471–476

Food Chemistry

www.elsevier.com/locate/foodchem

Antioxidant activity of water extract of four Harng Jyur (*Chrysanthemum morifolium* Ramat) varieties in soybean oil emulsion

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Received 30 June 1998; received in revised form and accepted 17 February 1999

Abstract

Antioxidant activities of the water extract of the four Harng Jyur (*Chrysanthemum morifolium* Ramat) varieties, which are commonly known as Huang Harng Jyur (HHJ), Bai Harng Jyur (BHJ), Gan Harng Jyur (GHJ), and Kung Harng Jyur (KHJ), were evaluated in soybean oil-in-water and water-in-oil emulsions after an accelerated oxidation at 60°C, which were examined by peroxide values and 2-thiobarbituric acid (TBA) tests. Results showed that the various soybean oil emulsions containing the water extract of the four Harng Jyur varieties were significantly ($p < 0.05$) more stable than the control. Moreover, 0.02% of each extract of the Harng Jyur variety had much more and better antioxidative properties than 0.02% of tocopherol (Toc) and butylated hydroxyanisole (BHA) in o/w (10:90, w/v), o/w (50:50, w/v), and w/o (10:90, v/w) emulsion systems. Each extract of the four Harng Jyur varieties tended to show better protection in the soybean o/w (10:90, w/v) emulsion system. No synergism was observed when each extract of the four Harng Jyur varieties was mixed with TBHQ in various emulsion systems in comparison to the single extract.

Keywords: Antioxidant activity; Butylated hydroxyanisole; *Chrysanthemum morifolium* Ramat; Peroxide value; Soybean oil emulsion; Synergism; Tertiary butyl hydroquinone; Thiobarbituric acid reactive substances; Tocopherol; Water extract of Harng Jyur

1. Introduction

The incorporation of antioxidants in fats and oils or in foods that contain fats and oils is effectively helpful in retarding the oxidation of lipids. There are many substances identified which prevent lipid peroxidation. Some of these compounds are synthetic antioxidants, whereas others occur as natural dietary constituents.

Antioxidative substances, present in spices, herbs and other plants, were extracted and systematically evaluated by Chipault, Mizuno, Hawkins and Lundberg (1952). Since then, numerous research works have been conducted to find, naturally effective antioxidants to substitute for synthetic antioxidants, which are of safety concern (Ito, Hagiwara, Shibata, Ogiso & Fukishima, 1982). Such naturally occurring antioxidative sources have been identified from oilseeds, grains, beans, vegetables, fruits, bark, roots etc. (Duh, Yeh & Yen, 1992; Duh, Yen, Du & Yen, 1997; Duh & Yen, 1997a; Namiki, 1990; Yen, Wu, & Duh, 1996).

Chrysanthemum morifolium Ramat, which is commonly known as Harng Jyur in Taiwan, has been used as a folk medicine for many centuries. In the past decade, beverages made from herbs are quite popular. Traditionally, four varieties of Harng Jyur, known as Huang Harng Jyur (HHJ), Bai Harng Jyur (BHJ), Gan Harng Jyur (GHJ), and Kung Harng Jyur (KHJ), are selected to process drinks or to be extracted as medicine. Investigations of the flower *Chrysanthemum morifolium* Ramat on linoleic acid peroxidation and its toxic side effects have been reported (Duh & Yen, 1997b). Linoleic acid has been used to evaluate natural antioxidants. However, Yi, Meyer and Frankel (1997) reported that linoleic acid may not completely reflect the lipid peroxidation due to its unique physical properties in aqueous micelles. Hence, vegetable oils, such as soybean oil including unsaturated triglycerides and phospholipids, may be considered as being more relevant substrates for evaluating antioxidant activity in lipid food systems. Thus, the objective of this work was to examine the

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0308-8146/99/$ - see front matter
PII: S0308-8146(99)00081-3

antioxidant efficacy of the water extract of four Harng Jyur varieties on soybean oil emulsions.

2. Materials and methods

2.1. Materials

Four dried Harng Jyur (*Chrysanthemum morifolium* Ramat) varieties, Huang Harng Jyur (HHJ), Bai Harng Jyur (BHJ), Gan Harng Jyur (GHJ), and Kung Harng Jyur (KHJ), were selected for the study. The flowers of the four Harng Jyur varieties were purchased from the local oriental herb stores in Tainan, Taiwan, Republic of China. All the dried samples were commercial varieties which are consumed in Taiwan.

Refined, bleached, and deodorized soybean oil, with 915 ppm of total tocopherol content [determined by high performance liquid chromatography (HPLC)], was obtained from commercial sources (Tainan, Taiwan).

2.2. Extraction

Each flower of the four Harng Jyur varieties (20 g) was boiled in water (600 mL) for 10 min. The extract was filtered and the residue was re-extracted under the same conditions. The combined filtrate was evaporated in a vacuum below 70°C on a rotary evaporator. The yields of the crude extract for HHJ, BHJ, GHJ, and KHJ were 10.05, 7.23, 10.69, and 10.35 g, respectively. The crude extract was dissolved in distilled water and used for the determination of its antioxidant activity.

2.3. Oil storage tests

Oxidation tests were run on triplicate emulsion preparations: (1) oil-in-water (o/w, 10:90, w/v), 10 g oil plus 90 ml water and 1 g Tween 20; (II) oil-in-water (o/w, 50:50, w/v), 50 g oil plus 50 ml water and 1 g Tween 20; (III) water-in-oil (w/o, 10:90, v/w), 10 ml water plus 90 g oil and 1 g Span 60. Each extract (0.02%) of the four Harng Jyur varieties or antioxidants (0.02%), including tocopherol, butylated hydroxyanisole (BHA), or tertiary hydroquinone (TBHQ) was added to each treatment mixture and homogenized (Ystral, Germany) for 5 min to form stable emulsions. A control treatment with no additives was also determined. Two g of weighed emulsion was kept in an open beaker (50 ml). Each beaker contained 2.0 g emulsion stored in an oven at 60°C. The stability of emulsions to oxidation was evaluated periodically by analyzing the samples for their peroxide and TBA values by AOCS (1990) Official Method Cd 8-53 and by the method of Nair and Turner (1984), respectively. All the tests were replicated three times, and their mean values are reported.

2.4. Tocopherol analysis

Tocopherol in oil was determined by HPLC as previously reported by Duh and Yen (1997).

2.5. Statistical analyses

Statistical analyses were conducted with the SAS (1985) software package of the replicate test data. Analyses of variance were performed by ANOVA procedures. Significant difference ($p < 0.05$) between the means was determined by Duncan's multiple range tests.

3. Results and discussion

Edible fats and oils, such as vegetable oils or lard, are usually used as substrates for evaluating the antioxidant activity from natural sources (Duh & Yen, 1997a; Tian & White, 1994). Lipid oxidation, however, is basically a surface phenomenon, and complex foods contain a multitude of surface active components and they are needed to have a better understanding of their interfacial effects in multiphased food systems (Frankle, 1994). Thus, in the present study, the soybean emulsion systems were prepared for testing the antioxidative efficacy of water extract of the four Huang Harng Jyur varieties.

Emulsifiers are compounds containing both hydrophobic and hydrophilic groups; hence, an oil emulsion system selection is based on the hydrophilic–lipophilic balance (HLB) concept. As a rule, emulsifiers with HLB values in the range of 3–6 promote w/o emulsion, whereas values between 8 and 18 promote o/w emulsions (Nawar, 1985). In the present study, the HLB value for Tween 20 and Span 60 is 16.7 and 4.7, respectively, and they were selected as emulsifiers.

Fig. 1 shows the peroxide values of a soybean o/w (10:90, w/v) emulsion system with 0.02% of water extract from the four Harng Jyur varieties and 0.02% of commercial antioxidants. During 10 days of storage at 60°C, the treatments that contained a water extract of the four Harng Jyur varieties had significantly ($p < 0.05$) lower PV than did the control [Fig. 1(A)]. On the ninth day, PV of the control increased markedly, and reached a maximum PV of 138 meq/kg. However, the PV of the soybean o/w (10:90, w/v) emulsion was 19.5, 16.1, 12.0, 16.4, 72.0, and 45.0 meq/kg for HHJ, BHJ, GHJ, KHJ, BHA, and TBHQ, respectively. These samples had 85.9, 88.4, 91.3, 88.2, 48.0, and 67.5% inhibition of oxidation after 9 days of storage, compared with the control. Significant differences ($p < 0.05$) in antioxidant activity were found between antioxidants (BHA and TBHQ) and each extract of the four Harng Jyur varieties. These results indicate that each extract of

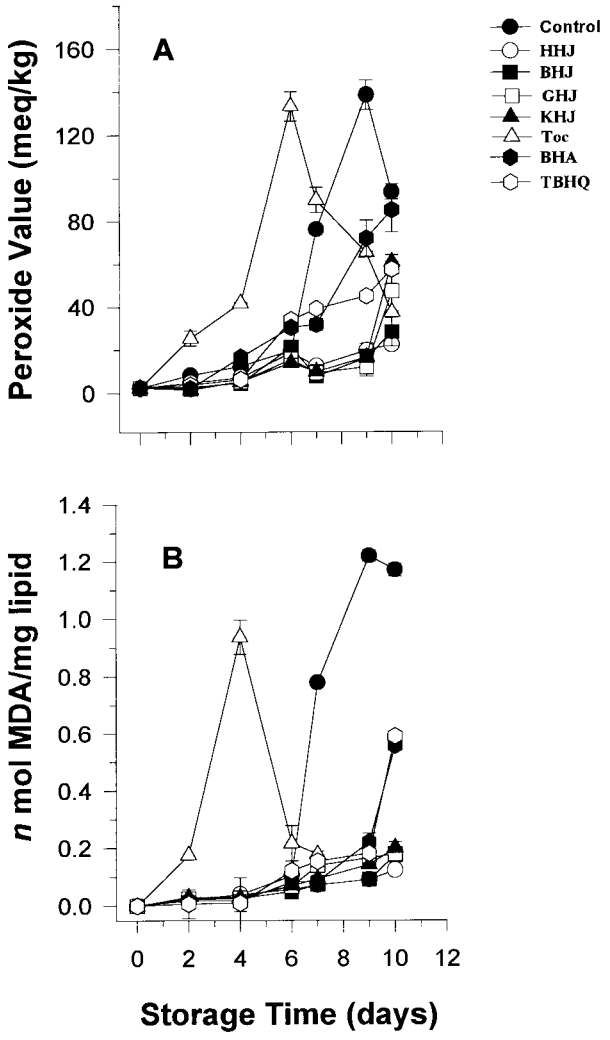

Fig 1. Antioxidative action of the water extract of the Harng Jyur in soybean o/w (10:90, w/v) emulsion stored at 60°C, as measured by (A) peroxide value and (B) TBARS. HHJ: Huang Harng Jyur; BHJ: Bai Harng Jyur; GHJ: Gan Huang Jyur; KHJ: Kung Huang Jyur; Toc: α-tocopherol; BHA: butylated hydroxyanisole; TBHQ: tertiary butyl hydroquinone. The concentration of each sample was 200 ppm.

the four Harng Jyur varieties can effectively inhibit the peroxidation of a soybean o/w (10:90, w/v) emulsion system. The antioxidant activity of the commercial antioxidants tested followed the order of TBHQ > BHA > Toc. Addition of 0.02% Toc to the soybean o/w (10:90, w/v) emulsion system accelerated the oxidation. Toc acts as a prooxidant with soybean oil containing 915 ppm total Toc. These results agree with the findings of Dziezak (1986) who reported that unsaturated vegetable oils with their inherent Toc contents, do not benefit much from additional Toc.

The primary products of lipid peroxidation are hydroperoxides. Therefore, the results from determining the concentration of peroxides for oxidized oil are a clear index of lipid peroxidation (Gray, 1978). However, measurement of peroxides only provides information regarding the initial oxidation potential of the oil. To elucidate the antioxidant effect of the extract on other stages of lipid oxidation, it is necessary to find whether the extract has an inhibitory effect at a later stage of peroxidation. Satue, Huang and Frank (1995) reported that antioxidants showed different activities toward hydroperoxide formation and decomposition; hence it is important that more than one method be used to monitor the oxidation process.

The effects of the extract and the antioxidants on the TBARS values of soybean o/w (10:90, w/v) emulsion after accelerated oxidation at 60°C are shown in Fig. 1(B). The TBARS formation of the control increased with an increase in the storage time, and increased rapidly on the sixth day. However, the values of the samples treated with the four Harng Jyur varieties and antioxidants, except Toc, were found to be significantly ($p < 0.05$) lower than the control. On the ninth day, the values of the TBARS were found to be 1.22, 0.091, 0.091, 0.165, 0.142, 0.224, and 0.181 *n* mol MDA/mg lipid for the control, HHJ, BHJ, GHJ, KHJ, BHA, and TBHQ, respectively. These samples had 92.5, 92.5, 86.5, 88.4, 81.6, and 85.2% of inhibition on the MDA formation after 10 days storage, compared with the control. Among the four varieties, the extract of HHJ and BHJ showed the greatest inhibitory effect on the MDA formation in soybean o/w (10:90, v/w) emulsion. Moreover, on the tenth day, it was observed that the inhibitory effect of the four extracts of Harng Jyur on MDA formation is much superior to commercial antioxidants (Toc, BHA, and TBHQ).

The PV development showed that the water extract of the four Harng Jyur varieties and the antioxidants, except for Toc, retarded the oxidation in o/w (50:50, w/v) emulsion [Fig. 2(A)]. On the seventh day, the PV of the control increased markedly, and reached a maximum of 536 meq/kg. The PV of soybean o/w (50:50, w/v) emulsion was 97.1, 75.8, 55.5, 100, 220, and 81.9 meq/kg for HHJ, BHJ, GHJ, KHJ, BHA, and TBHQ, respectively. These samples had 81.9, 85.9, 89.7, 81.3, 59.0, and 84.7% of oxidation inhibition after 7 days of storage, compared with the control.

The results of the water extract of the four Harng Jyur varieties and the antioxidants on the TBARS values of soybean o/w (50:50, w/v) emulsion after accelerated oxidation at 60°C are shown in [Fig 2(B)]. The TBARS values of the samples treated with extract and the antioxidants except for Toc were significantly ($p < 0.05$) lower than the control. On the seventh day, the TBARS values were 0.929, 0.203, 0.119, 0.159, 0.241, 0.636, and 0.152 *n* mol MDA/mg lipid for the control, HHJ, BHJ, GHJ, KHJ, BHA, and TBHQ, respectively. These samples had 78.2, 87.2, 82.9, 74.1, 31.5, and 83.4% inhibition on MDA formation of soybean o/w (50:50, w/v)(or w/o, 50:50, v/w) emulsion, whereas BHJ showed the greatest inhibition of MDA

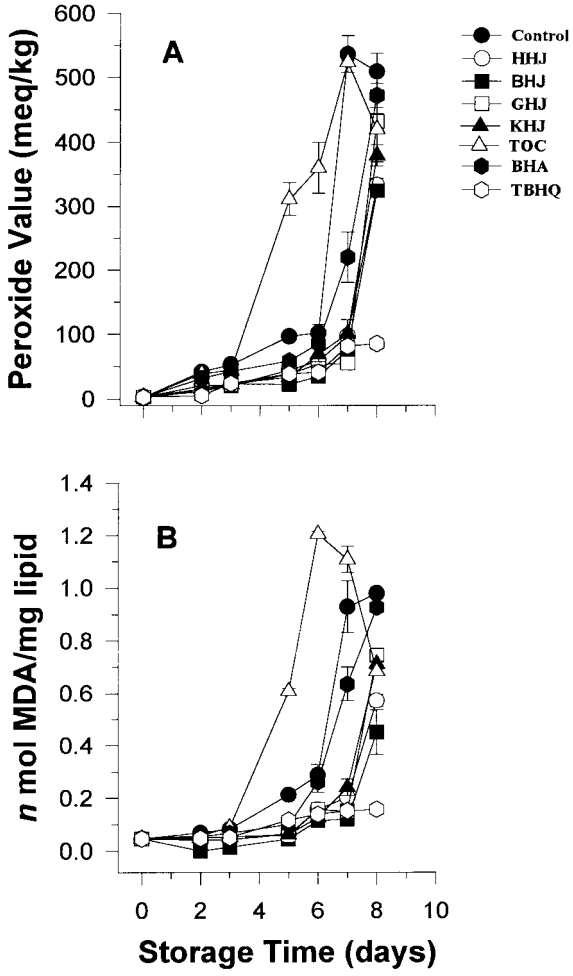

Fig. 2. Antioxidative action of the water extract of the Harng Jyur in soybean o/w (50:50, w/v) emulsion stored at 60°C, as measured by (A) peroxide value and by (B) TBARS. Abbreviations as in Fig. 1. The concentration of each sample was 200 ppm.

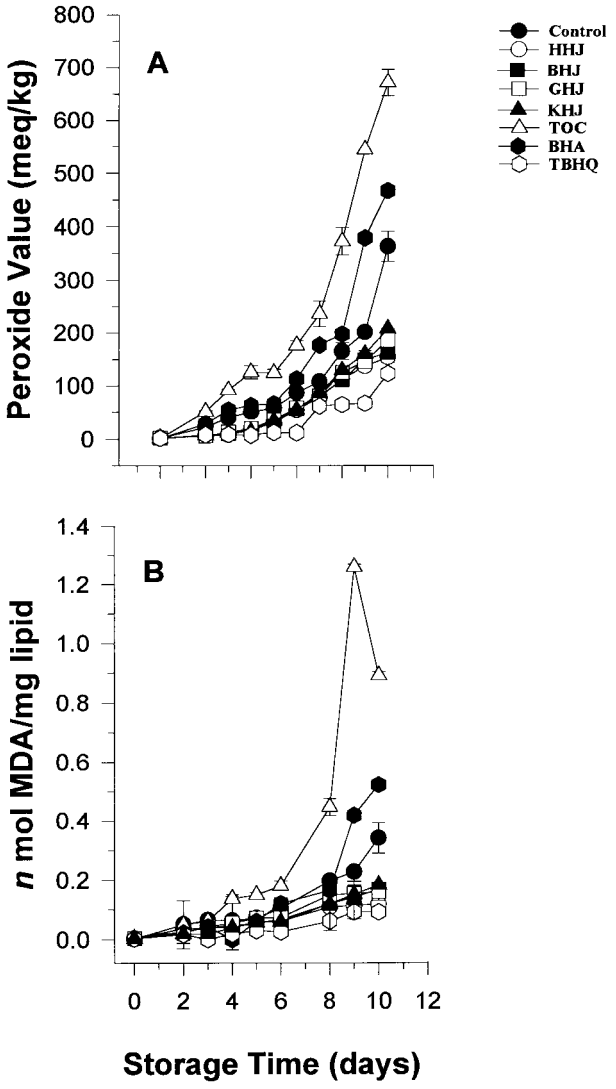

Fig. 3. Antioxidative action of the water extract of the Harng Jyur in soybean w/o (10:90, v/w) emulsion stored at 60°C, as measured by (A) peroxide value and by (B) TBARS. Abbreviations as in Fig. 1. The concentration of each sample was 200 ppm.

formation among all the test samples. Moreover, the inhibitory effect of the water extract of the four Harng Jyur on MDA formation was found to be much more effective than that of BHA and Toc during 7 days of storage. On the eighth day, however, TBHQ showed the greatest antioxidant activity, followed by BHJ > HHJ > GHJ > KHJ > BHA.

Fig. 3(A) shows the PV development of soybean w/o (10/90, v/w) emulsion, with accelerated oxidation at 60°C, treated with four extracts of Harng Jyur varieties and the antioxidants. BHA and Toc showed prooxidant effect in this study. The control had a higher PV than the four Harng Jyur varieties as well as TBHQ indicated the antioxidative properties of the four extract. In general, no significant difference ($p > 0.05$) in PV development was observed among the four extracts of the four Harng Jyur varieties during 9 days of storage. On the tenth day, the PV of soybean w/o (10/90, v/w) emulsion was 364, 154, 163, 186, 208, and 123 meq/kg for the control, HHJ, BHJ, GHJ, KHJ, and TBHQ, respectively. These results had 57.6, 55.2, 48.8, 42.9, and 66.1% of oxidation inhibition after 10 days of storage, compared with the control. In other words, the antioxidant effect was in the following decending order: TBHQ > HHJ > BHJ > GHJ > KHJ.

The TBARS values [Fig. 3(B)] showed that the water extract of the four Harng Jyur varieties and TBHQ retarded MDA formation in the soybean w/o (10/90, v/w) emulsion. Toc and BHA, however, did not inhibit the MDA formation but displayed prooxidative action in this model system. On the tenth day, the values of TBARS were 0.342, 0.118, 0.167, 0.159, 0.183, and 0.092 *n* mol MDA/mg lipid for the control, HHJ, BHJ, GHJ, KHJ, and TBHQ, respectively. These samples showed 65.5, 51.2, 53.6, 46.5, and 73.1% oxidation inhibition of MDA formation after 10 days of storage, compared to

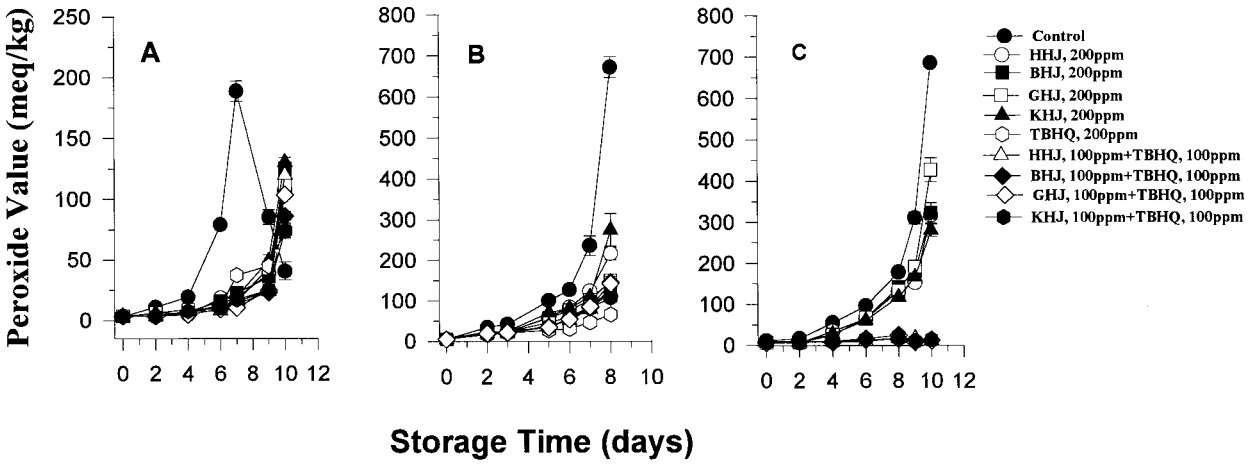

Fig. 4. Synergistic antioxidative action of the water extract of the Harng Jyur with tertiary butyl hydroquinone (TBHQ) in soybean o/w (10:90, w/v) (A), o/w (50:50, w/v) (or w/o, 50:50,v/w) (B), w/o (10:90, v/w) (C) emulsions stored at 60°C, as measured by peroxide value. Abbreviations as in Fig. 1.

the control. Among the four varieties, HHJ showed the greatest antioxidant activity and retarded MDA formation in soybean w/o (10/90, v/w) emulsion, followed by GHJ, BHJ, and KHJ.

The relatively high TBARS values of the samples may arise due to the presence of carbonyl groups, which react with TBA reagent, that may contribute to off-flavor of oxidized oil. As shown in Figs. 1–3, the water extract of the four Harng Jyur varieties obviously suppressed the primary and secondary oxidation products in the various soybean emulsion systems.

On the other hand, TBHQ showed the greatest antioxidant activity among the commercial antioxidants in various emulsion systems. Hence, TBHQ was selected as an antioxidant for determining the synergistic action with the water extract of the four Harng Jyur varieties. Fig. 4 shows the synergistic effect of the water extract of the four Harng Jyur varieties on the antioxidant activity of TBHQ. No synergistic action of the water extract of four Harng Jyur varieties on the inhibitory effect of TBHQ in various emulsion systems [Figs. 4(A)–(C)] was observed. These results indicated that the water extract of the four Harng Jyur varieties can effectively inhibit the oxidation of soybean emulsion, but they can not enhance the antioxidant activity of TBHQ.

On the basis of the data presented, it is observed that the water extract of four Harng Jyur varieties tended to give better protection to the soybean o/w (10/90, w/v) emulsion system. In addition, the water extract of the four Harng Jyur varieties was more useful as an antioxidant in emulsion than was BHA and Toc. This effect may be due to the presence of several antioxidative components in the extract which gave a range of solubilities in different emulsion systems, thus allowing some compounds to be more soluble at the oil/water (or water/oil) interphase in the oil and some more soluble in the water (Tian & White, 1994).

In conclusion, chemical data of the soybean oil emulsion, exposed to the Schaal oven test, indicated that the water extract of the four Harng Jyur varieties can effectively retard peroxidation, as determined by peroxide values and TBA values. It was found that the antioxidant activity of the water extract of the four Harng Jyur varieties is much superior to that of BHA and Toc. Moreover, natural antioxidants are preferred over synthetic antioxidants from the food safety view point. Thus, the water extract of the four Harng Jyur varieties can be recommended as a potential source of natural antioxidants.

Acknowledgements

This research work was supported by the National Science Council, Republic of China, under grant NSC 86-2314-B-041-001.

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