EFFECT OF INDIAN GOOSEBERRY (*Phynanthus emblica*) ON FISH OIL ANTIOXIDATION

by

E.M.R.K.B. EDIRISINGHE[1], W.M.K. PERERA [2] AND A. BAMUNUARACHCHI [3]

[1] *Institute of Post Harvest Technology, National Aquatic Resources Research and Development Agency (NARA), Colombo-15, Sri Lanka.*

[2] *Department of Nutrition and Community Resources Management, Wayamba Campus, University of Rajarata, Kuliyapitiya, Sri Lanka.*

[3] *Department of Chemistry, University of Sri Jayawardenepura, Nugegoda, Sri Lanka.*

ABSTRACT

Fish oils are becoming important due to their nutritional implementations. Development of rancidity in fish oils is very fast and leads to reduction of the qualities of fish oils as well as fish. Prevention of development of rancidity in fish oils by the fruit of Indian Gooseberry (*Phynanthus emblica*) extracts was studied in detail. The ethanolic, methanolic and water extracts of Indian gooseberry were applied to fish oils and the activity of the different extracts was determined by measuring peroxide value (PV), free fatty acid value (FFA) and fatty acid composition (FAC) on eight occasions over forty four days of storage at room temperature (30°C). In the second experiment, active components of the ethanol extract were separated by hexane, carbon tetrachloride, chloroform, ethyl acetate and water, and the activity of these fractions were also measured by applying to fish oils.

In the first experiment, the ethanolic extract treatment recorded the highest activity in prevention of formation of peroxides, free fatty acids, and the conversion of fatty acids than the methanolic and water extracts. The amount of saturated and monounsaturated fatty acids increased whereas the polyunsaturated fatty acids decreased during the storage. The water extract treatment showed the lowest while the methanolic extract was intermediate. Results from the second experiment showed that the antioxidant activity of ethyl acetate fraction was higher than the other solvent fractions used in the study and this indicated that the active compounds might have a medium polarity. The study suggests the possibility of using Indian gooseberry to prevent rancidity of fish oils in industrial uses.

INTRODUCTION

The importance of antioxidants to prevent rancidity in fat and oils is increasing. Polyunsaturated fatty acids in fish oils tend to oxidize and hydrolyse due to their high unsaturation (Stansby, 1967; Khayat and Schwall, 1983). Most synthetic antioxidants cause some undesirable side effects and therefore there is an increasing demand for natural antioxidants (Haigh, 1986).

A number of plants have been recognized as natural antioxidants for a long time. Rosemary *(Rosemerinus officinalis L)* and sage (*Salvia officinalis*) are two with strong antioxidant efficiency. The antioxidant properties of the fruit of Indian gooseberry (*Phynanthus emblica*) was reported very recently (Edirisinghe *et al.*. 1996). This highly acidic, vitamin C rich, bitter tasting fruit has been used in traditional medicine for many purposes. In previous studies, the active components were extracted using two organic solvents, methanol and carbon tetrachloride, and the reported activity was high (Edirisinghe *et al.*. 1996). Methanol and carbon tetrachloride are poisonous and therefore the extracted product was suitable only for laboratory studies. The extraction of active components in non-poisonous solvents, i.e. ethanol and water, may

give opportunities for Indian gooseberry as an antioxidant in the food industry. This study was carried out to evaluate the activity of crude ethanol and water extracts of Indian gooseberry and to assess the activity of purified extracts in preventing oxidation in fish oils. The purification process may lead to identification of the active compounds present in this fruit.

EXPERIMENTAL

Materials

The dried fruit of Indian gooseberry was obtained from local market in Colombo, Sri Lanka. The fish oil of sudaya (*Sardinella albella*) was extracted by steam rendering (Edirisinghe *et al.*, 1998). The extracted fish oil was dried over anhydrous sodium sulphate for 24hrs. Analytical grade solvents and chemicals were obtained from Sigma Chemicals Co. Ltd, UK.

Experiment –1 Evaluation of anti-oxidant activity of Indian gooseberry using different solvent systems.

Extraction of antioxidants from Indian gooseberry

Three samples of 5 g of dried, powdered Indian gooseberry were extracted with 150ml of ethanol, methanol and water, respectively by Soxhlet extractor for 4hrs. After the extraction, the solvents were removed by rotavapor at 38°C. The products obtained were dried in an oven (40°C) for 24hrs and stored in a freezer (-18°C) until use.

Assess of activity

Each of the three products as extracted above was (weight : 1g) dissolved in three sets of 1ml of ethanol and added to the fish oil separately at concentrations of 2000 ppm and the reference samples were treated with 200ppm BHT. Treated samples were stored at room temperature (30°C). The quality of the fish oil was measured by peroxide value (AOAC 1980), free fatty acid value (AOAC 1980), and fatty acid composition (by gas chromatography) over a 44 day storage period. The quality of the samples was compared with the control.

Experiment –2. Purification of crude Indian gooseberry extract and the examination of preservative action of purified fractions

Crude ethanol extracts of Indian gooseberry were obtained by Soxhlet extraction as described in experiment -1. The dried ethanol extract (10 g) was dissolved in 100 ml of warm water (45 - 50°C). This aqueous solution was extracted with 100 ml of hexane, carbon tetrachloride, chloroform and ethyl acetate in order to increase the polarity. The final residue remained in the water. The solvents were removed using a rotavapor at 38°C. The procedure is as follows:

Dried Indian gooseberry

↓ Soxhlet extraction with ethanol

↓

10g of crude extract (dried)

↓ dissolved in 100ml of water (slightly hot ~ 50°C)

↓

aqueous solution

↓ extraction with 100ml of hexane

↓

mixture → separation of hexane phase, removal → residue 1
of solvent using rotavapor

↓

residue in aqueous phase

↓ extraction with 100ml of carbon tetrachloride

↓

mixture → separation of carbon tetrachloride phase, removal → residue 2

↓ of solvent using rotavapor

residue in aqueous phase

↓ extraction with 100ml of chloroform

↓

mixture → separation of chloroform phase, removal → residue 3

↓ of solvent using rotavapor

residue in aqueous phase

↓ extraction with 100ml of ethyl acetate

↓

mixture → removal of ethyl acetate phase, removal → residue 4

↓ of solvents using rotavapor

↓

residue in water → removal of water using rotavapor → residue 5

The five extracts were dried at 40°C for 4 hrs and at ambient temperature (30°C) for 24 hrs. The final residues were mixed with fish oil in 1000ppm concentrations using ethanol and stored in screw capped vials ambient temperature (30°C). The efficiency of the treatments were determined by measuring PV, FFA and FAC as experiment -1 over a 16 days storage period, and compared with the treatment with 200 ppm BHT.

Determination of fatty acid composition:

The fatty acid methyl esters, for fatty acid analysis, were prepared (AOCS 1989 and FF Method 1991) and these methyl esters were separated by packed column gas chromatography on a Shimadzu GC-14A gas chromatograph using GP 10% SP 2330 on chromasorb WAW (100-120 mesh) packed glass column (2.1 m * 3.2 mm). The temperature programming used was 20 min. at 175°C, then up to 190°C at a rate of 1°C/min, 10 min at 190°C, then up to 240° at a rate of 3°C/min and 10 min of holding time at 240°C. Helium was used as the carrier gas at a flow rate of 35 ml/min at 175°C with a flame ionization detector (FID). The temperature of the detector and injector was maintained at 270°C. The peaks were identified by comparing retention times of the methyl esters in standard mixtures from Larodane Fine Chemicals AB, Sweden and Neu-Chek pack, U.S.A. The weight of individual fatty acids was calculated as mg by using a C 17:0 internal standard.

Four replicate analysis were carried out to determine PV and FFA and the results were statistically analyzed ($p < 0.05$). Fatty acid analysis were carried out in duplicate and the results were given as mean values

RESULTS AND DISCUSION

Experiment –1.

The quality of the fish oil samples which were treated with the three different extracts and BHT were found to be different from each other and from the blank sample.

Peroxide and free fatty acid values

Variation of peroxides and free fatty acids in these treatments were shown in Tables 1 and 2.

It was observed that there were significant differences in peroxide values between the blank sample and the other four treatments. When comparing the three treatments, the ethanol extract was found to have very low PV up to the 30th day and after that period this value rapidly increased. The treatment with methanolic extract showed a low peroxide value up to the 24th day and the water extract showed the lower level up to nearly 16 days. The peroxide value of the fish oil sample treated with 200 ppm BHT maintained a lower level than the blank but higher than all the other three treatments of Indian gooseberry extracts up to the 16th day. The values of final peroxide levels showed that two observations were nearly equal and very high.

Table 1. Variation of peroxides in fish oil during storage with different solvent extracts of Indian gooseberry. (mean ± S.D, n= 4, $p < 0.05$)

Peroxide Value (P.V) m.eq/kg)	**Storage Time / Days**							
Sample No.	**1**	**4**	**9**	**16**	**24**	**30**	**37**	**44**
1	10.2 ±0.2	17.8^{b} ±3.8	66.1^{b} ±5.4	165.7^{b} ±9.7	500.4^{b} ±13.9	661.8^{ab} ±54.0	705.7^{c} ±27.3	778.7^{a} ±46.5
2	10.2 ±0.2	1.0^{c} ±0.0	24.5^{b} ±1.2	54.4^{d} ±7.9	108.0^{c} ±9.7	143.1^{c} ±10.7	834.2^{ab} ±63.4	841.1^{a} ±52.9
3	10.2 ±0.2	15.3^{b} ±0.0	25.2^{b} ±1.5	67.0^{cd} ±4.8	126.2^{c} ±17.7	678.1^{ab} ±48.8	775.8^{bc} ±49.6	833.1^{a} ±39.8
4	10.2 ±0.2	16.5^{b} ±0.7	43.3^{b} ±1.5	85.7^{c} ±8.5	536.8^{b} ±25.7	560.6^{b} ±163.5	856.7^{a} ±47.9	822.0^{a} ±30.9
5	10.2 ±0.2	34.3^{a} ±2.5	487.9^{a} ±12.9	543.7^{a} ±23.2	660.2^{a} ±36.8	763.9^{a} ±118.3	748.8^{c} ±32.7	682.6^{a} ±32.1

1 – Sample treated with BHT (200ppm); 2 – Sample treated with Indian gooseberry extracted in EtOH (2000ppm)
3 – Sample treated with Indian gooseberry extracted in MeOH (2000ppm)
4 – Sample treated with Indian gooseberry extracted in Water (2000ppm) 5 – Control

Table 2. Variation of free fatty acids in fish oil during storage with different solvent extracts of Indian gooseberry. (mean ± S.D, n= 4, $p < 0.05$)

Free fatty acid value (FFA) (%)	Storage Time / Days							
Sample No	**1**	**4**	**9**	**16**	**24**	**30**	**37**	**44**
1	3.5 ±0.3	4.0^{ab} ±0.0	5.1^{b} ±0.2	5.2^{b} ±0.4	5.8^{b} ±0.3	7.2^{b} ±1.1	8.6^{b} ±0.5	10.3^{b} ±0.3
2	3.5 ±0.3	3.6^{b} ±0.5	5.5^{c} ±0.3	4.4^{c} ±0.3	5.6^{b} ±0.2	5.0^{b} ±0.2	5.5^{d} ±0.7	8.0^{d} ±0.4
3	3.5 ±0.3	4.1^{ab} ±0.3	5.3^{b} ±0.2	4.5^{c} ±0.2	5.7^{b} ±0.3	5.6^{b} ±0.5	7.6^{c} ±0.3	9.6^{c} ±0.3
4	3.5 ±0.3	4.1^{ab} ±0.1	5.5^{ab} ±0.3	4.7^{bc} ±0.5	5.8^{b} ±0.1	7.0^{b} ±0.3	8.6^{b} ±0.2	10.3^{bc} ±0.5
5	3.5 ±0.3	4.5^{a} ±0.4	5.8^{a} ±0.3	6.7^{a} ±0.5	11.4^{a} ±0.1	11.4^{b} ±1.9	12.6^{a} ±0.7	14.4^{a} ±0.5

The percentage of FFA in the blank sample was high from the beginning but the values from other treatments were considerably lower than the blank sample. The lowest value in the oil sample treated with ethanol extract varied from 3.5 to 8% during storage. The treatments with methanolic and water extracts showed lower free fatty acid values. There is no significant difference between free fatty acid values of treatments of BHT and water extracts during storage. This indicated that the activity of the water extract in preventing the formation of FFA's was comparable with BHT at this concentration. This results showed that the ethanol extract has the highest ability to prevent formation of peroxides and free fatty acids, but methanolic and water extracts also showed considerable activity.

Fatty acid composition

The fatty acid composition results showed the stability of individual fatty acids during the experiment. The stability of the saturated fatty acids (SFAs) were very high during the storage (Fig. 1). During storage the amounts of SFAs increased at varying rates in all five treatments. The highest rate was recorded in the blank sample and the lowest in the sample treated with ethanol extract. The SFAs of the other three treatments varied between these two treatments.

The variation of MUFAs was observed with respect to palmitoleic acid, oleic acid and with minor quantities of myristoleic acid, eicosenoeic acid, eruc acid and nervoneic acid. The variation of fatty acids of five treatments was different from each other (Fig. 2). During the study MUFAs increased at different rates but similar to the changes observed in SFAs. The ethanol treatment of Indian gooseberry showed the lowest rate of increase while the control sample showed the highest rate of increase of monounsaturated fatty acids.

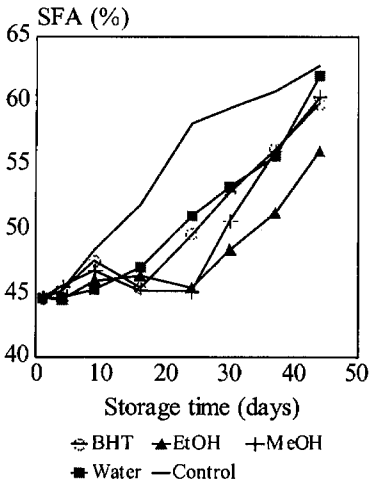

Fig. 1. Variation of saturated fatty acids of fish oils treated with different solvent extracts of Indian gooseberry during storage at 30°C.

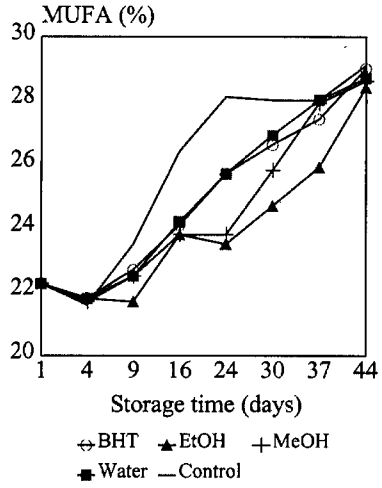

Fig. 2. Variation of monounsaturated fatty acids of fish oils treated with different solvent extracts of Indian gooseberry during storage at 30°C.

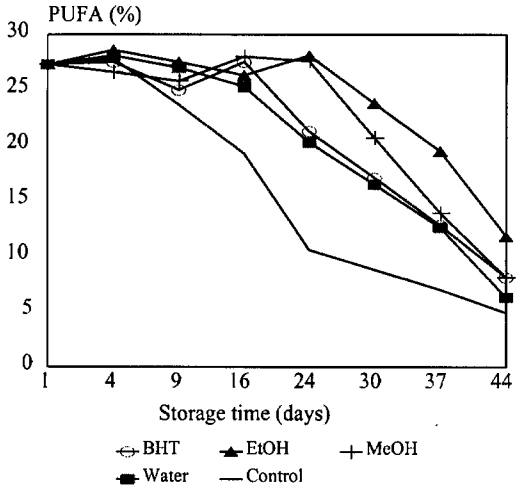

Fig. 3. Variation of polyunsaturated fatty acids of fish oils treated with different solvent extracts of Indian gooseberry during storage at 30°C.

The changes in the polyunsaturated fatty acids (PUFAs) of these five treatments were totally different from the variation of saturated and monounsaturated fatty acids during storage (Fig. 3). The amounts of PUFAs in the five samples were found to decrease at different rates during storage. The amount of PUFAs remained high up to the 24th day and then decreased slowly in the ethanol and sharply in the methanolic treatment. PUFA's decreased sharply in the control from the beginning of storage.

The ethanol extract of Indian gooseberry showed the highest ability of preventing the formation of peroxides, free fatty acids and showed the highest stability of fatty acids. The methanolic and water extracts also showed considerable activity against oxidation and hydrolysis. The different types of solvents were used to extract antioxidant components from different sources and their activity was different from each other. Wu *et al.* (1994) reported that the antioxidant activity of ethanol and methanol extracts of wild rice (*Z. aquatica L.*) were higher than the activity of an ethyl acetate extract. The antioxidant activity of 2000 ppm methanolic extract of ajowan seed (*Carum coptimum*) was a little lower than the activity of 200 ppm of BHT and this was measured by measuring peroxide values of stored soybean oil (Mehta *et al.*, 1994). Results from the current study showed that the activity of methanolic extract was higher than the BHT at the same concentration. The activity of these crude extracts depends on the other methanol soluble materials which do not have antioxidant activity. Chang *et al.* (1977) used seven solvents with different polarities (except ethanol) to extract antioxidant compounds of rosemary and sage and concluded that the methanolic extract had the highest activity. Both Chang et al (1977) and Wu et al (1994) concluded that high polar solvents extract active compounds more efficiently. The activity of the extracts depends on the polarity of the active compounds present in the material, and solvents with similar polarity to the compounds extract them. Other compounds with similar polarity, but no antioxidant activity may be extracted with the active ones and change the overall performance. This can be minimized by purification after extraction.

Experiment –2. Activity of purified compounds from Indian gooseberry

Variations of peroxides and free fatty acids in the treatments are shown in Figs. 4 and 5. The significantly lower PV ($P < 0.05$) for the ethyl acetate fraction than for the other treatments indicated that the antioxidant components were extracted more by ethyl acetate than other solvents. The variation of free fatty acids was very similar to the variation of peroxides. The highest and the lowest values of FFA were recorded in the control and the ethyl acetate extracts, respectively. The other solvent extracts also recorded lower PV's than the control and therefore showed some antioxidant activity. Among the Indian gooseberry treatments, the hexane extract showed the lowest effect on preventing formation of peroxides and free fatty acids. According to the present results the highest antioxidant activity was recorded in the ethyl acetate fraction (1000ppm) and this activity was higher than the crude extract as well as 200ppm BHT. There is no significant difference, in both PV and FFA, between the ethyl acetate extract treatment and the BHT treatment.

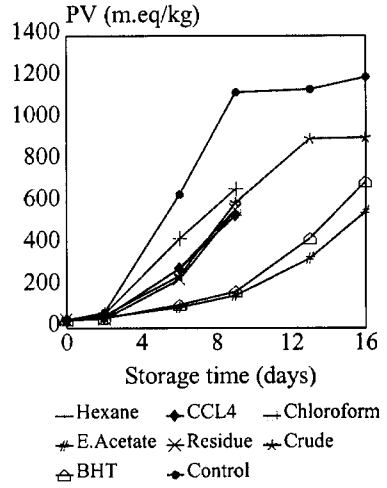

Fig. 4. Changes in the levels of peroxides in fish oils during storage with purified Indian gooseberry.

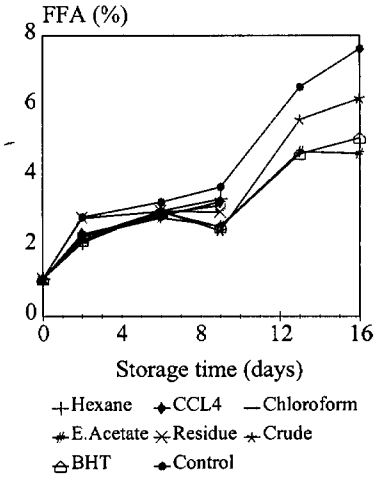

Fig. 5. Changes in the levels of free fatty acids in fish oils during storage with purified Indian gooseberry .

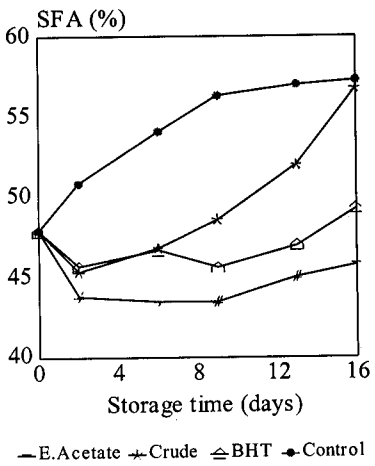

Fig. 6. Changes in the levels of saturated fatty acids in fish oils during storage with purified Indian gooseberry.

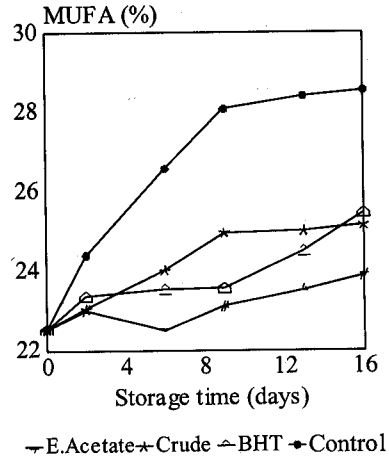

Fig. 7. Changes in the levels of monounsaturated fatty acids in fish oils during storage with purified Indian gooseberry.

The variation of fatty acids of stored fish lipids with antioxidant treatments were plotted in Figs. 6, 7 and -8. During storage, the amount of saturated and monounsaturated fatty acids increased and the amount of polyunsaturated fatty acids decreased. When treated with the antioxidant, the increasing rate of SFAs and MUFAs, and the decreasing rate of PUFAs was low and remained stable. The ethyl acetate treatment gave the highest stability of the fatty acids of fish lipids. The crude Indian gooseberry treatment also showed some activity, but lower than the treatment of BHT at this particular concentration.

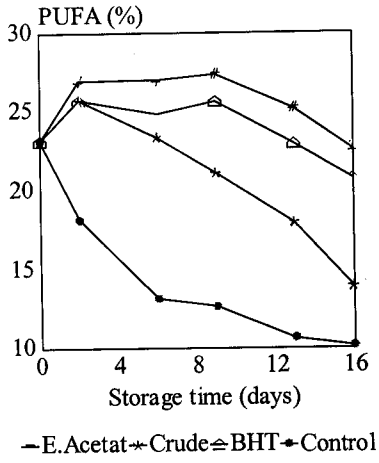

Fig. 8. Changes in the levels of polyunsaturated fatty acids in fish oils during storage with purified Indian gooseberry.

All these quality parameters indicate that the antioxidant components of Indian gooseberry can be easily separated by ethyl acetate and this indicated that this active compounds might have a medium polarity (relative polarity of ethyl acetate = 0.58).

CONCLUSIONS

The fruit of Indian gooseberry is reported to have considerable antioxidant activity in preservation of fish oils. The active components of this fruit can be extracted by ethanol and water and the activity of these two solvent extracts showed the possibility of using Indian gooseberry extracts as an antioxidant on a large scale. The antioxidant components extracted to these non-toxic solvents can be used without removing solvent or even without purification of the obtained product. The active components of Indian gooseberry can be easily separated and purified by ethyl acetate and the active compounds might have a medium polarity. The study suggests the possibility of using Indian gooseberry for industrial uses.

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