ABSTRACT

The beneficial health effects of n-3 polyunsaturated fatty acids (PUFA) are closely related to their protection against cardiovascular diseases, autoimmune disorders, diabetes, arthritis, and arrhythmia. The oils from fish and algae have been so far recognized as rich sources of n-3 PUFAs, while new research findings have predicted liver lipids of pelagic sharks as a highly potential source. Even though nearly 100 million sharks are annually caught in the world for their meat and other body parts, the liver of shark is currently regarded as a waste in many countries. In Sri Lanka 11% of total marine catch is contributed by sharks and the shark liver is an underutilized material.

Oxidative stability of n-3 PUFA is one of the major challenges to be faced by the lipid food industry as the oxidation of n-3 PUFA leads to undesirable chemical and physical changes and decreases the shelf-life of the products. In this regard, natural antioxidants such as tocopherols and extracts of aromatic and medicinal herbs are preferred over the artificial antioxidants that are commonly used as radical scavengers for lipid containing foods.

The research described in this thesis focuses on the evaluation of several pelagic shark liver lipids as a possible source of n-3 PUFAs and an aromatic plant (sweet basil; *Ocimum basilicum* L.) grown in Sri Lanka for the potential of antioxidant in foods. The thesis is consisted of five chapters. Various health beneficial aspects of n-3 PUFA, of current status of shark fishing industry in Sri Lanka, oxidation of unsaturated lipids, natural sources of radical scavenging antioxidants, and the aims of the thesis are outlined in Chapter 1.

The Chapter 2 deals with the chemical, and structural characteristics of pelagic shark liver lipids in order to understand and identify inter- and intra-species changes and possible utilization avenues. The livers of five dominant shark species such as silky (*Carcharhinus falciformis),* thresher (*Alopias superciliosus*), white tip (*Carcharhinus longimanus*), blue (*Prionace glauca*), and hammerhead (*Sphyrna lewini*), dwelling in the Indian ocean were studied for their lipid classes, fatty acid and triacylglycerol (TAG) molecular species compositions. In general, the livers showed 30.0–60.0 % lipid content, 65.2–86.1% TAG and appeared to be an excellent source of n-3 PUFA (25.0-49.0 mol %), especially docosahexaenoic acid (DHA) (15.0-43.0 mol %). The observed high DHA content (43.9 %) for the hammerhead (male) sharks liver lipids has not been reported for any fish oil previously. The salmon shark (*Lamna ditropis*) dwelling in the Pacific Ocean showed that DHA as the most abundant fatty acid (22.7-32.4 mol %) and presence of more than 10 % of the newly identified vitamin E marine derived tocopherol (MDT) (2-10mg/g oil) in their liver lipid. The POD (12.5–19.9 %),

and PPD (8.4–15.4 %) (where, P: palmitic acid; O: oleic acid and D: DHA) were the predominant fatty acid combinations consisted in TAG. Tridocosahexaenoin (DDD) (0.1-3 %), a very rare fatty acid combination found in shark liver lipids was reported for the first time by this study. Furthermore, it was observed that 30- 50 % of the molecular species was consisted of at least one DHA in their glycerol moiety, and DHA was preferentially positioned into the *sn*-2 in shark liver TAGs.

The Chapter 3 enumerates the phenolic composition and antioxidant activity of sweet basil. The antioxidant activity of methanolic extract of sweet basil leaves was examined using 2,2'-diphenyl-1-picrylhydrazyl (DPPH) free radical and soy phosphatidylcholine (PC) liposome model systems. Fractions I-VI that were obtained by Sephadex LH-20 column chromatography showed different radical scavenging activities in both model systems, however, fraction IV had the strongest activity. The mass fragmentation patterns observed by Atmospheric Pressure Chemical Ionization–Mass Spectroscopy (APCI-MS) suggested that the major antioxidative compound in fraction IV was rosmarinic acid ((*m/z* 360) and it was confirmed by the results obtained by ^{13}C NMR and ^{1}H NMR. Moreover, 15 compounds that were responsible for the antioxidant activity of sweet basil have been identified by the characteristic fragmentations obtained by APCI-MS. Presence of three flavonoid glycosides such as dihydroxy kaempferol 3-*O*-glucoside (*m/z* 466), luteolin acetyl glucuronide (*m/z* 504) and dihydroxy kaempferol 3-*O*-glycoside (*m/z* 434) that was confirmed by this study in sweet basil has not been reported previously. The native of antioxidant activity of rosmarinic acid in the soy and fish PC liposome systems was closely examined in order to clarify the potential applications and further developments for the food uses. It appeared that even though fish PC consists n-3 PUFA (55 mol %) (DHA, EPA and DPA), the oxidation rate of fish PC was six times lower than the oxidation rate of soy PC (n-6 PUFA, 65 mol %, linoleic acid). The calculated radical scavenging number of rosmarinic acid per second for both soy and fish PC liposome systems was observed as 1.5. Furthermore, the existence of strong synergistic effect between α-tocopherol and rosmarinic acid was observed in the liposome systems.

Chapter 4 contains the evaluation study of the methanolic extracts of plant materials such as leaves of sweet basil and fruits of Indian gooseberry (*Embilica officinalis*), which were evaluated for their antioxidant activities in the shark liver oil-in-water emulsion. The progress of oxidation in emulsion systems was evaluated by measuring the formation of conjugated diene by spectrophotometry and volatile compounds by solid phase micro extraction method. Without any antioxidant, n-3 PUFA rich oil-in-water emulsion was stable for 48 h. The findings implied that the oxidative stability of oils containing high amount of PUFAs may not be limiting factor in their utilization in foods if it is used in aqueous

dispersions such as emulsions. The antioxidant activity of these extracts were in the order of α-tocopherol (500ppm)> sweet basil > Indian gooseberry.

The present study is concluded in the Chapter 5 and the emphasis is on the potential of liver lipids of sharks as a rich source of n-3 PUFA. This study clearly brought out significant information on stabilization of n-3 PUFAs rich oil against auto-oxidation with methanolic extract of sweet basil as a natural source of antioxidant. Potential food applications (e.g., oil-in-water emulsions) of sweet basil extract as antioxidant was also confirmed.