博　士（水産科学）　バスカル　ナラヤン

学 位 論 文 題 名

Lipids of tropical and temperate seaweed species with special reference to conjugated fatty acids and food applications

（共役脂肪酸の探索と食品への利用を目的とした
熱帯及び温帯海域での海藻脂質に関する研究）

学位論文内容の要旨

Seaweeds are macro-algae living in sea or brackish water. They are referred to as "*benthic marine algae*" which means *attached algae that live in the sea.* They are often referred to as seaweeds or marine macroalgae with some authors even branding them as sea vegetables. They are simple organism with unique properties. Seaweed cultivation has become a major industry in the Asia, thereby making the region utilizing these plants the most. Seaweeds form a part of the staple diet in Japan, Korea and China apart from being used as delicacies in some of the western world. They are traditionally consumed in the Far East countries and in Hawaiian Islands, while in the West the principal uses of seaweeds are as sources of phycocolloids, thickening and gelling agents for various industrial applications including uses in foods.

Although, seaweeds have been studied for long, for production of industrially important polysaccharides like agar, carrageenan, fucoidan etc; they have not been looked upon as sources of lipids as these are found in relatively small quantities. Since lipids would be wasted as a major by-product during the production of these phycocolloids, it would be worthwhile to utilize them as sources of nutritious lipids and other compounds that have profound health benefits. Comprehensive reviews are available on the occurrence and significance of bioactive molecules in seaweeds by various authors. In this regard, an attempt was made in the current study to highlight the prospects of seaweed lipids as sources of useful bioactive molecules that have a potential for biomedical and food applications. The major objectives, as listed in Chapter 1, of the present study, were –

(a) To analyse different brown and red seaweeds for the occurrence of conjugated polyenoic fatty acids and any other unusual fatty acids.
(b) To comparatively evaluate the fatty acid composition of a brown seaweed (*Sargassum* sp) harvested from cold and tropical waters.
(c) To assess the efficacy of seaweed extracts for their anticancerous potential; and,
(d) To evaluate the fatty acid, amino acid, fucoxanthin and fucosterol contents in seaweed incorporated food products with emphasis on fucoxanthin stability.

Chapter 2 reviews the available literature on the composition, functionality and potential applications of seaweed lipids. The chapter emphasizes the importance of seaweed lipids as biofunctional materials and their possible recovery and application methods.

Chapter 3 is on the occurrence of conjugated fatty acids in some of the Indian red seaweeds. Three species of red marine macro algae (Rhodophyta) from the Indian Ocean were analysed for the occurrence of conjugated polyenes. The composition of different lipid classes in these seaweeds along with their fatty acid composition has also been reported. Analysis of lipid classes of these seaweeds revealed that both *Acanthophora spicifera* (Ceramiales, Rhodophyta) and two species of *Gracilaria, viz. G. edulis* and *G. folifera* (Gracilariales, Rhodophyta) were rich in

glycolipids followed by neutral- and phospholipids. The fatty acid composition of these seaweeds revealed C16:0 as the predominant fatty acid in all three species. However, *A. spicifera* had significantly higher amounts of eicosapentaenoic acid (EPA) and arachidonic acid (AA) as compared to negligible amount of these fatty acids in both species of *Gracilaria*. The red seaweed *Acanthophora spicifera* contained conjugated eicosapentaenoic acid (CEPA) and conjugated arachidonic acid (CAA) in all lipid classes except glycolipids. One interesting observation was that though both species of seaweeds *viz.*, *Acanthophora* and *Gracilaria* were from the same geographical sampling site, the former showed occurrence of conjugated polyenes while the latter did not. We hypothesize that *Acanthophora* may be harboring enzymes, similar to that of those reported in *Ptilota* sp from the northern hemisphere, that could aid in isomerisation of the normal EPA/AA to CEPA/CAA. Relatively higher amount of EPA and AA in this seaweed may aid in this.

Chapter 4 of the work comparatively evaluates lipid composition and profile of three brown seaweed species of genus *Sargassum*. Three different species of brown algae of genus *Sargassum* (Fucales, Phaeophyta), collected from temperate and tropical waters, were analysed for their lipid class and fatty acid composition. Two species–Umitorano (*Sargassum thunbergii*) and Fushi sujimoku (*S. confusum*) – harvested from the Northwest Pacific Ocean off Usujiri, Hokkaido, Japan and one species (*S. marginatum*) harvested from the Arabian Sea (Indian Ocean) off Goa, west coast of India, formed the materials of the study. In all the seaweeds glycolipids formed the major lipid class followed by neutral lipids and phospholipids. Palmitic acid (C16:0) was found to be the predominant fatty acid among all the three species with *Sargassum marginatum* recording the highest quantity. However, the species of *Sargassum* harvested from the cold waters of NE Pacific Ocean were found to contain more of polyunsaturated fatty acids (PUFAs; > 50% of total fatty acids) as compared to the higher content of saturated fatty acids (about 53% of total fatty acids) in the seaweed harvested from the tropical waters of the Indian Ocean. However, the degree of unsaturation (DU) and mean chain length (MCL) of PUFAs were not significantly different ($p>0.05$) among the cold water species. A non-methylene interrupted (NMI) fatty acid, eluting just before 20:2 n-6, was found to be a characteristic feature of *Sargassum* and could well be used as a taxonomic marker for seaweeds of the genus *Sargassum*. The higher PUFA content in *S. thunbergii* and *S. confusum* make them potential sources in the preparation of PUFA-rich nutraceutical and/or functional-food formulations.

Chapter 5 details the preliminary work done with regards to the anticancerous activity of lipid extracts of Indian brown seaweed *S. marginatum*. An effort was made to screen the growth inhibitiory/cytotoxic activity of lipid extracts on the human pro-melocytic leukemia (HL-60) cells. Phospholipids (PL) were found to be the most effective compared to the other lipid classes in terms of cytotoxic activity. PL exhibited cytotoxic activity at concentrations as low as 20 μg/ml. PL were found to be higher in poly unsaturated fatty acids (PUFA) among all the lipid classes analysed. This study indicated the possibility of seaweeds as potential sources of anticancer substances.

Chapter 6 compares the fatty acid compositions of brown seaweeds harvested from tropical and temperate waters. Lipid class and fatty acid composition were determined in *Padina tetrastomatica* (a tropical seaweed) harvested from the Arabian Sea (Indian Ocean) off Goa, west coast of India and *Desmerestia ligulata* (commonly called as urushigusa, a cold water seaweed) harvested from the Northwest Pacific Ocean off Usujiri, Hokkaido, Japan. Glycolipids formed the major lipid class followed by neutral- and phospholipids. Palmitic acid (C16:0) was found to be the predominant fatty acid, with saturated fatty acids contributing to the major portion of the total fatty acids in case of tropical species. However, among the unsaturated fatty acids poly unsaturated fatty acids (PUFAs) formed the major portions and were highest in phospholipids (PL). The degree of unsaturation (DU) and mean chain length (MCL) of PUFAs are also presented.

Chapter 7 is on the influence of edible seaweeds (Wakame) in food products (pasta). Wakame, an edible Japanese seaweed and pasta as the food product Wakame (*Undaria pinnatifida*) is edible seaweed rich in fucoxanthin; while, pasta is an important dish from nutritional and gastronomic point of view. Pasta was prepared with wakame as an ingredient at different levels. *In-vitro* antioxidant properties, total phenolic content, fatty acid composition, fucoxanthin and fucosterol contents formed the major bio-functional characteristics analysed. Pasta with 10% wakame was acceptable sensorily. The total phenolic content varied between 0.10 and 0.94 mg gallic acid equivalents (GAE)/g, while total antioxidant activity varied from 0.16 to 2.14 mg ascorbic acid equivalents (AAE)/g, among different samples. 2,2-diphenyl-1-picrylhydrazyl (DPPH) and superoxide radical scavenging activities of sensorily acceptable pasta were 7.71 and 4.56 %, respectively. The sensorily acceptable pasta had a mild seaweed flavor with taste similar to control pasta, as assessed by panelists. The ratio of n-3 to n-6 fatty acid in seaweed-incorporated pasta was 1:3.4 as compared to 1:15.2 in the control. Heat process involved in

pasta preparation and cooking did not destroy fucoxanthin. Microstructure studies revealed the enhanced interaction between starch granules and protein matrix in pasta containing seaweeds up to 20%.

The brown seaweeds had a balanced fatty acid composition especially with reference to n-3/n-6 FA ratio pointing to their usefulness as sources of PUFA. Overall, these seaweeds have considerable quantities of functional lipids making them suitable candidates for recovery of beneficial phyto-nutraceuticals. Fucoxanthin being a valuable carotenoid, pasta products can be used for popularizing seaweed consumption in populations that does not traditionally consume seaweed in their diets. Technologies to effectively recover total lipids from these seaweeds and methods to utilize recovered biofunctional materials in food, pharamaceutical and biomedical applications need to be researched further.

学位論文審査の要旨

主　査　　教　授　　宮　下　和　夫

副　査　　教　授　　板　橋　　豊

副　査　　准教授　　細　川　雅　史

学位論文題名

Lipids of tropical and temperate seaweed species with special reference to conjugated fatty acids and food applications

(共役脂肪酸の探索と食品への利用を目的とした熱帯及び温帯海域での海藻脂質に関する研究)

海藻は加工食品の重要な素材として広く活用されてきた。国連食糧農業機関の統計によれば、1995年度の海藻類の生産量は680万トン程度であったが、2004年度になると約1400万トンに達した。これは、海藻から得られる粘性多糖類の需要が高くなったためと予想される。また、日本を始めアジア各国では、海藻を食材として古くから利用してきた。海藻中の栄養成分としては多糖類が最も多く、ついで、タンパク質、脂質、無機塩類などが含まれている。海藻タンパク質には必須アミノ酸が多く、その栄養価は、他の良質な陸上植物タンパク質にひけをとらない。また、海藻脂質には、アラキドン酸やエイコサペンタエン酸（EPA）などの高度不飽和脂肪酸も多く含まれている。しかし、海藻に含まれる多糖類の食物繊維としての働きや、一部の粘性多糖類（フコイダン）の免疫能向上作用と抗腫瘍活性などの報告はあるものの、陸上植物中に含まれるファイトケミカルに相当する栄養機能成分や脂質についての知見は少なかった。こうしたなか、海藻カロテノイド、フコキサンチンの独特な機能性が示され、海藻脂質の成分の詳細と栄養機能性、さらには、その食品への活用が望まれている。本研究では、温帯海域と熱帯海域から得られる海藻の化学的性質とその機能性食品への応用について以下の成果を得た。

１．インド洋沿岸で採取した紅藻Rhodophytaに属する３種の海藻(*Acanthophora spicifera*; *Gracilaria edulis*; *G. folifera*)は、ω３系の機能性高度不飽和脂肪酸（PUFA）としてEPAを、ω６系の機能性PUFAとしてアラキドン酸を相当量含むが、その含量は種によって異なることを明らかにした。特に、*A. spicifera*はEPAとアラキドン酸を多く含むことを見出した。さらに、*A. spicifera*脂質中には共役型のアラキドン酸(CAA)とEPA(CEPA)を含むことも明らかにした。このことより、*Acanthophora*には共

役異性化酵素の存在が示された。また、その脂質組成を分析したところ、糖脂質が主要な脂質であり、ついでリン脂質が多いことが分かった。

２．温帯海域と熱帯海域から採取した褐藻*Sargassum*３種の脂質組成と脂肪酸組成について検討した。すべての褐藻において主要な脂質は糖脂質であり、ついで、中性脂質、リン脂質の順となった。これらの脂質中の脂肪酸組成について分析したところ、温帯海域の褐藻中にはPUFAが50%以上含まれているのに対し、熱帯域で採取した場合には、主な脂肪酸は飽和型のパルミチン酸であることを明らかにした。また、*Sargassum*は非メチレンインターラプテッド型不飽和脂肪酸を含むことも明らかにした。非メチレンインターラプテッド型不飽和脂肪酸は他の海藻には含まれておらず、この脂肪酸は*Sargassum*に特有の脂肪酸であることを明らかにした。

３．インド沿岸で採取した*Sargassum marginatum*脂質のガン細胞（ヒト大腸ガン細胞）に対する増殖抑制効果を検討したところ、同海藻脂質中のリン脂質に強い細胞増殖抑制効果のあることを見出した。*Sargassum marginatum*リン脂質には高度不飽和脂肪酸が多量に含まれており、こうした脂肪酸組成がその生理活性と深く関わっていることも明らかにした。

４．熱帯域で採取された褐藻（*Padina tetrastomatica*）と温帯域で採取された褐藻（*Desmerestia ligulata*）の脂肪酸組成について検討した。両褐藻共、主要な脂質は糖脂質であり、ついで中性脂質、リン脂質の順となった。これらの海藻においても熱帯性の褐藻では、パルミチン酸などの飽和脂肪酸が多いのに対し、温帯期の海藻中の脂肪酸は不飽和度の高いものが多かった。

５．褐藻の機能性食品への活用を検討するため、ワカメ粉末（５－３０%）を含むパスタを試作した。その結果、ワカメ粉末は２０%まで添加してもパスタの食品科学的性状に影響を与えないこと、ワカメ粉末中に含まれる機能性脂質（フコキサンチンとフコステロール）の含量は加工中並びに調理中に減少しないこと、パスタ抽出物には高い抗酸化作用が認められ、これはワカメ粉末中に含まれるポリフェノールに由来することを明らかにした。また、パスタ脂質は、ワカメ粉末１０%添加によりその脂肪酸組成が変化した。すなわち、小麦粉中ではn-6系高度不飽和脂肪酸とn-3系高度不飽和脂肪酸の比率が1:15.2であったのに対し、ワカメ粉末添加により、ワカメ粉末脂質中に含まれるEPAや18:4n-3の影響により同比率が1:3.4に変化した。WHOによるn-6/n-3比は1:4以下とされており、ワカメ粉末添加によりパスタの栄養効果が向上することを明らかにした。

以上の成果は、温帯域と熱帯域での海藻の脂肪酸組成の違いを明らかにすると共に、栄養学的に重要な知見を与えた。また、海藻の機能性食品等への活用にも大きく寄与するものと判断できた。よって審査員一同は本研究の申請者が博士（水産科学）の学位を授与される資格のあるものと判定した。