Antioxidant activity of cod (Gadus morhua) protein hydrolysates: Fractionation and characterisation of peptide fractions

This study aimed to characterise peptide fractions (>5 kDa, 3–5 kDa and <3 kDa) with antioxidative activity obtained from a cod protein hydrolysate. The free amino acids in all fractions were dominated by Ala, Gly, Glu and Ser. The total amino acid composition had high proportions of Lys, Ala and Glu. The 3–5 kDa and <3 kDa fractions were further fractionated by size exclusion chromatography. All sub-fractions showed high Fe\(^{2+}\) chelating activity. The DPPH radical-scavenging activity of the 3–5 kDa fraction was exerted mainly by one sub-fraction dominated by peptides with masses below 600 Da. The DPPH radical-scavenging activity of the <3 kDa fraction was exerted by sub-fractions with low molecular weight. The highest reducing power was found in a sub-fraction containing peptides rich in Arg, Tyr and Phe. Both free amino acids and low molecular weight peptides thus seemed to contribute to the antioxidative activity of the peptide fractions, and Tyr seemed to play a major role in the antioxidant activity.
Antioxidant Activity of Seaweed Extracts: In Vitro Assays, Evaluation in 5 % Fish Oil-in-Water Emulsions and Characterization

In this study the antioxidant activity of absolute ethanol, 50 % ethanol and water extracts of two species of seaweeds, namely Fucus serratus and Polysiphonia fucoides, were evaluated both in in vitro assays and in 5 % fish oil-in-water (o/w) emulsions. The 50 % ethanolic extracts of P. fucoides showed higher antioxidant activity both in in vitro assays and in 5 % oil-in-water emulsion in the presence or absence of iron. In spite of the higher phenolic content and very good antioxidant activity in some of the in vitro assays, the absolute ethanol extracts of both the species showed a pro-oxidative tendency in 5 % fish oil-in-water emulsion in the presence or absence of iron. In order to investigate the reason for the higher antioxidant activity of 50 % ethanolic extracts of P. fucoides, these extracts were further fractionated into polyphenol-rich, protein-rich, polysaccharide-rich and low-molecular-weight fractions. These fractions were tested both in in vitro and in 5 % oil-in-water emulsions. The results of the present study showed that the main effect was due to the phenolic compounds. In conclusion, the 50 % ethanolic extracts of P. fucoides can be a potential source of natural antioxidants as these extracts have antioxidant activities similar to those of synthetic antioxidants such as BHT.

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Contributors: Farvin Habebullah, S., Jacobsen, C.
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Antioxidant activities and functional properties of protein and peptide fractions isolated from salted herring brine

In the present study proteins isolated from herring brine, which is a by-product of marinated herring production were evaluated for their functional properties and antioxidant activity. Herring brine was collected from the local herring industry and proteins were precipitated by adjusting the pH to 4.5 and the obtained supernatant was further fractionated by using ultrafiltration membranes with molecular weight cut offs of 50, 10 and 1kDa. The obtained >50kDa, 50–10kDa, 10–1kDa fractions and pH precipitated fraction were studied for their functional properties and antioxidant activity. Functional properties revealed that >50kDa polypeptides showed good emulsion activity index when compared to the other fractions. However all fractions had low emulsion stability index. The pH precipitated fraction showed the highest foaming capacity and stability at pH 10. The 50–10kDa and 10–1kDa peptide fractions showed good radical scavenging activity and reducing power at a concentration of 0.5mg protein/ml. All the fractions demonstrated low iron chelating activity and did not inhibit oxidation in a soybean phosphatidylcholine liposome model system. However all the fractions were to some extent able to delay iron catalyzed lipid oxidation in 5% fish oil in water emulsions and the 10–50kDa fraction was the best. These results show the potential of proteins and peptide fractions recovered from waste water from the herring industry as source of natural antioxidants for use in food products.
Antioxidant activity of Cod (Gadus morhua) protein hydrolysates: In vitro assays and evaluation in 5% fish oil-in-water emulsion

Cod protein hydrolysates were fractionated according to the molecular mass into three fractions of >5kDa, 3–5kDa and...
effect increased in all samples after digestion with GI proteases whereas the antioxidative capacity decreased. The effect on the caspase activity depended on the proteases used in the preparation of hydrolysates. In conclusion, the caspase activity and antihypertensive activity are maintained during digestion with GI proteases, while the antioxidative capacity seems to be reduced.

**General information**

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Organisations: National Food Institute, Division of Industrial Food Research,UIT The Arctic University of Norway, Marinova A/S, University of Copenhagen
Contributors: Jensen, I., Andersen, L. L., Ossum, C. G., Jakobsen, G., De Gobba, C., Farvin, S., Johansson, I., Hoffmann, E., Oddny Ellevoll, E., Jessen, F., Nielsen, H. H.
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**Antioxidant Effect of Seaweed Extracts in Vitro and in Food Emulsion Systems Enriched With Fish Oil**

Natural antioxidants derived from marine algae have a high content of bioactive components with potential for improving oxidative stability of lipids in food systems. Bioactive components like polyphenols have been identified in marine algae. In this presentation we will discuss results from our ongoing work on the brown algae Fucus vesiculosus. This seaweed contains a wide range of polyphenols with potential antioxidant activity. Thus, in vitro antioxidant properties of F. vesiculosus extracts have been found to be related to the total polyphenolic content. It has been suggested that the primary antioxidant activity comes from secondary metabolites such as phlorotannins, a dominant polyphenolic compound. However, studies on the effectiveness of seaweed extracts in food model systems are sparse, therefore there is a need to look further into this area. Results obtained in our lab with different extracts of F. Vesiculosus in a range of different food models will be presented.

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Contributors: Larsen, D. B., Farvin, S., Jacobsen, C.
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Source: dtu
Source-ID: u::8387
Research output: Contribution to conference › Conference abstract for conference – Annual report year: 2013 › Research

**Antioxidant potential of water hyacinth (Eichornia crassipes): In vitro antioxidant activity and phenolic composition**

The aims of the present study were (a) to extract and quantify the main phenolic acids and tocopherols from the petiole, leaves, and flowers of Eichornia crassipes; (b) to evaluate the antioxidant capacity of the extracts in four in vitro systems (1,1-diphenyl-2-pycryl-hydrazyl [DPPH] radical scavenging ability, iron chelating activity, reducing power, and prevention of oxidation in a liposome model system); and (c) its effectiveness in retarding lipid peroxidation in fish oil by accelerated stability test. Significant differences were observed in total and individual phenolic contents and in the antioxidant activities of extracts from the various parts of E. crassipes. Out of the 11 phenolic acids analyzed, ethanolic extracts contained high amounts of gallic, protocatechuic, gentisic, and p-hydroxybenzoic acid, whereas, water extracts contained less amounts of a varied number of phenolic acids. Ethanolic extracts of flower, which contained the highest total phenolic content, were found to have high DPPH radical scavenging activity and reducing power. However, ethanolic extracts of leaf exerted a high Fe2+ chelating activity and also inhibited lipid peroxidation process both in liposomes and fish oil. Our results demonstrate that E. crassipes, an underutilized aquatic weed, could be a potential natural antioxidant source for food, feed, and pharmaceutical applications. © 2013 Copyright Taylor & Francis Group, LLC.

**General information**

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Organisations: National Food Institute, Division of Industrial Food Research, Institute of Food and Dairy Technology, Central Institute of Fisheries Technology
Contributors: Surendraraj, A., Farvin, S., Anandan, R.
Phenolic compounds and in vitro antioxidant activity of selected species of seaweeds from Danish coast

Water and ethanolic extracts of 16 species of seaweeds collected along the Danish coasts were screened for antioxidant activities using four in vitro antioxidant assays (2,2-diphenyl-1-picrylhydrazyl radical scavenging activity, reducing power, ferrous ion-chelating and liposome model system). Furthermore their effectiveness in retarding lipid peroxidation in fish oil was evaluated by an accelerated stability test. Significant differences were observed in total and individual phenolic content and the antioxidant activities of seaweed species evaluated. Ethanol was more efficient for polyphenol extraction than water. Polysiphonia fucoïdes and all the Fucus species tested showed highest radical scavenging activity, reducing power, inhibition of oxidation in liposome model system and in fish oil and were high in phenolic content. These seaweeds could be potential rich sources of natural antioxidants for protection of foods against oxidation. In general, the various antioxidative assays correlated well with the total phenolic content, indicating that algal polyphenols are active components in these extracts. However, in some of the antioxidative assays some species with low total phenolic content also showed good antioxidative effects indicating that some other co-extracted active compounds such as pigments and tocopherols in ethanolic extracts and sulphated polysaccharides, proteins or peptides in water extracts may also contribute to the overall antioxidant properties and this needs further investigation.

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Publication date: 2013
Peer-reviewed: Yes

Phenolic compounds and in vitro antioxidant activity of selected species of seaweeds from Danish coast

Water and ethanolic extracts of 16 species of seaweeds collected along the Danish coasts were screened for antioxidant activities using four in vitro antioxidant assays (2,2-diphenyl-1-picrylhydrazyl radical scavenging activity, reducing power, ferrous ion-chelating and liposome model system). Furthermore their effectiveness in retarding lipid peroxidation in fish oil was evaluated by an accelerated stability test. Significant differences were observed in total and individual phenolic content and the antioxidant activities of seaweed species evaluated. Ethanol was more efficient for polyphenol extraction than water. Polysiphonia fucoïdes and all the Fucus species tested showed highest radical scavenging activity, reducing power, inhibition of oxidation in liposome model system and in fish oil and were high in phenolic content. These seaweeds could be potential rich sources of natural antioxidants for protection of foods against oxidation. In general, the various antioxidative assays correlated well with the total phenolic content, indicating that algal polyphenols are active components in these extracts. However, in some of the antioxidative assays some species with low total phenolic content also showed good antioxidative effects indicating that some other co-extracted active compounds such as pigments and tocopherols in ethanolic extracts and sulphated polysaccharides, proteins or peptides in water extracts may also contribute to the overall antioxidant properties and this needs further investigation.

General information
Publication status: Published
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Contributors: Farvin, S., Jacobsen, C.
Pages: 1670-1681
Publication date: 2013
Peer-reviewed: Yes
Antioxidant effect of seaweed extracts in food emulsion systems enriched with fish oil
Natural antioxidants derived from marine algae have a high content of bioactive components with potential for improving oxidative stability of lipids in food systems. In this presentation we will discuss results from our ongoing work on the brown algae Fucus vesiculosus. This seaweed contains a wide range of polyphenols with potential antioxidant activity. Thus, in vitro antioxidant properties of F. vesiculosus extracts have been found to be related to the total polyphenolic content. It has been suggested that the primary antioxidant activity comes from secondary metabolites such as phlorotannins, a dominant polyphenolic compound. However, studies on the effectiveness of seaweed extracts in food model systems are sparse, therefore there is a need to look further into this area. Results obtained in our lab with different extracts of F. Vesiculosus in a range of different food models will be presented.

Antioxidative effect of seaweed extracts in 5% fish oil-in-water emulsion

Antioxidative Effect of Seaweed Extracts in Minced Mackerel- Effect on Lipid and Protein oxidation

Composition and health benefits of potato peels
New natural antioxidants for protecting omega-3 rich products.

The long-chain (LC) highly unsaturated omega-3 fatty acids, EPA (eicosapentaenoic acid, 20:5) and DHA (docosahexaenoic acid, 22:6) are vital for a wide range of biological functions and are implicated in the prevention of numerous diseases. However, these fatty acids are highly susceptible to oxidation because of their unsaturated nature. Addition of antioxidants is one method to prevent lipid oxidation. As synthetic antioxidants may have carcinogenic effects at higher levels, the replacement of synthetic antioxidants with natural antioxidants is now in demand. We have isolated natural antioxidants from yoghurt, potato peel, fish protein hydrolysates and seaweed, which were able to protect highly unsaturated fish oil from oxidation. These antioxidant extracts may have potential for commercial exploitation. Copyright © 2012 WILEY-VCH Verlag GmbH & Co. KGaA, Weinheim.

Potato peel extract as a natural antioxidant in chilled storage of minced horse mackerel (Trachurus trachurus): Effect on lipid and protein oxidation

The present work was undertaken to examine the utilisation of potato peel, a waste material, as a source of natural antioxidants for retarding lipid and protein oxidation in minced mackerel. Mackerel mince with two different concentrations (2.4 or 4.8g/kg) of water or ethanol extracts of potato peel and a control with no added extracts were prepared. The samples were stored at 5°C for 96h and the sampling was done at time points 0, 24, 48 and 96h. The ethanol extracts, which contained high amounts of phenolic compounds, was found to be very effective in retarding lipid and protein oxidation as it resulted in low levels of peroxide value, volatiles, carbonyl compounds and protected against the loss of α-tocopherol and tryptophan and tyrosine residues. Water extracts was less efficient especially at higher concentrations, which might be due to lower phenolic content or due to the pro-oxidative nature of some of the phenolic acids/co-extracted compounds.
Antioxidant Activity of Fish Protein Hydrolysates in in vitro Assays and in Oil-in-Water Emulsions.
The aim of this study was to screen different protein hydrolysates with respect to their antioxidative properties in order to select the most promising extracts for further evaluation in oil-in-water emulsions. Three fractions of protein hydrolysates (Crude, >5kDa and 5kDa, 3-5kDa and...

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Organisations: Division of Industrial Food Research, National Food Institute
Contributors: Farvin, S., Andersen, L. L., Jacobsen, C., Nielsen, H. H., Jessen, F.
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Source: orbit
Source-ID: 312475
Research output: Contribution to conference › Conference abstract for conference – Annual report year: 2011 › Research

Antioxidant potential of water hyacinth (Eichornia crassipes): In vitro antioxidant activity and phenolic composition
The aims of the present study were (a) to extract and quantify the main phenolic acids and tocopherols from the petiole, leaf and flowers of Eichornia crassipes, (b) to evaluate the antioxidant capacity of the extracts in four in vitro systems (DPPH radical scavenging ability, iron chelating activity, reducing power and prevention of oxidation in a liposome model system) and its effectiveness in retarding lipid peroxidation in fish oil by accelerated stability test. Significant differences were observed in total and individual phenolic content and antioxidant activities of extracts from the various parts of E. crassipes. Out of the 11 phenolic acids analysed, ethanolic extracts contained high amounts gallic, protocatechuic, gentisic and phhydroxybenzoic acid, whereas, water extracts contained less amounts of varied number of phenolic acids. Ethanolic extracts of flower, which contained the highest total phenolic content, were found to have high DPPH radical scavenging activity and reducing power. Ethanolic extracts of leaf were found to have high Fe2+ chelating activity and inhibited lipid peroxidation in liposomes and fish oil. Our results demonstrate that E. crassipes, an underutilized aquatic weed, could be a potential natural antioxidant source for food, feed and pharmaceutical applications.

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Organisations: Division of Industrial Food Research, National Food Institute, Institute of Food and Dairy Technology, Biochemistry and Nutrition Division
Contributors: Surendraraj, A., Farvin, S., Anandan, R.
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Effect of sunlight on the survival of pathogenic E. coli in freshwater and sea water

An enteropathogenic group of E. coli are the emerging category of pathogen of public health significance. Several recent pathogenic E. coli outbreaks are associated with drinking water. Aquaculture, the fast emerging food production sector also poses a pathogenic EHEC outbreak risk, as it regularly uses cow dung, a reservoir of this organism. Hence, a experiment was set up to study the duration of survival of pathogenic E. coli under sunlight and darkness. Eight pathogenic E. coli isolates from clinical (EPEC, ETEC, EHEC, EAEC), veterinary (CTE3, CTE4) and environmental sources (ASHE3, Rao II) were studied for their survival under sunlight and darkness in fresh water and seawater. Effect of direct sunlight on the viable but nonculturable (VBNC) state of cultures was also studied. The results of the study indicated a distinct pattern between freshwater system and seawater system. Pathogenic E. coli from different sources showed significantly higher level of destruction under direct sunlight than in complete darkness. A reduction of 1.1 to 5.7 log CFU was seen in fresh water after 90 to 105 min under direct sunlight and only 0.2 to 2 log reduction was observed in complete darkness in 5 to 96 h period. The effect of sunlight was severe in seawater and in selective media, where a higher cell reduction to an extent of 3 log CFU was observed. Survival period was least for environmental pathogens followed by veterinary and finally clinical pathogens with an exception of Shiga-toxigenic E. coli (STEC) from veterinary sources. Findings of this study, may aid the public health specialist to evolve better strategies to control these organism in the above systems.

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Organisations: Division of Industrial Food Research, National Food Institute, Institute of Food and Dairy Technology, Central Institute of Fisheries Technology
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the potato peel extracts was evident from in-vitro systems as they showed strong reducing power, radical scavenging ability, ferrous ion chelating activity and prevented oxidation in a liposome model system. The Sava variety, which showed strong antioxidant activity in in-vitro systems, was tested in oil and oil-in-water emulsions. Ethanolic extracts of Sava (C1,600 mg/kg) prevented lipid oxidation in emulsions and in oil. Water extracts showed no antioxidant activity in oil whereas it showed pro-oxidant activity in emulsions. Thus, the results of the present study show the possibility of utilizing waste potato peel as a promising source of natural antioxidants for retarding lipid oxidation.

Antioxidant activity of yoghurt peptides: Part 1-in vitro assays and evaluation in omega-3 enriched milk

The aim of the present study was to investigate important factors contributing to the high oxidative stability of fish-oil-enriched yoghurt, with particular emphasis on the possible antioxidative effects of peptides released during yoghurt fermentation. Yoghurt samples were stripped from sugars and lactic acid and subsequently fractionated by ultrafiltration using membranes with cut off sizes of 30 kDa, 10 kDa and 3 kDa. The fractions were tested for antioxidant activity by investigating the inhibition of oxidation in liposome model system, 1,1-diphenyl-2-picrylhydrazyl radical-scavenging activity, iron-chelating activity, and reducing power. The lower molecular weight fractions were found to be more effective antioxidants than higher molecular weight fractions. The lower molecular fractions were further tested as antioxidants in fish-oil-enriched milk. On the basis of peroxide value, volatiles, tocopherol and sensory characteristics, the lower molecular weight fractions 3–10 kDa and
Antioxidant activity of yoghurt peptides: Part 2 – Characterisation of peptide fractions

The aim of the present study was to elucidate previous findings showing that peptide fractions isolated from yoghurt had antioxidant effects. Therefore, peptides and free amino acids released during fermentation of milk were characterised. Yoghurt samples were stripped from sugars and lactic acid and subsequently fractionated by ultra filtration using membranes with cut off sizes of 30, 10 and 3 kDa. The peptides in these fractions were identified by LC–MS/MS. The identified peptides comprised a few N-terminal fragments of αs1-, αs2-, and β-casein, and several fragments from β-casein. Almost all the peptides identified contained at least one proline residue. Some of the identified peptides included the hydrophobic amino acid residues Val or Leu at the N-terminus and Pro, His or Tyr in the amino acid sequence, which is characteristic of antioxidant peptides. In addition, the yoghurt contained a considerable amount of free amino acids such as His, Tyr, Thr and Lys, which have been reported to have antioxidant properties. Thus, our findings confirm that the antioxidant effects of the peptide fractions from yoghurt are due to the presence of certain peptides and free amino acids with recognised antioxidant activity in these fractions.

Phenolic composition and in vitro antioxidant activities of selected species of seaweeds from Danish coast.

This study was aimed to evaluate the preventive role of squalene on free amino acids and lysosomal alterations in experimentally induced myocardial infarction in rats. The levels of lysosomal enzymes (β-glucuronidase, β-
galactosidase, beta-glucosidase, acid phosphatase and cathepsin D) in plasma and lysosomal fractions, hydroxyproline content and free amino acids in heart tissue were determined. Isoprenaline administration to rats resulted in decreased stability of the membranes which was reflected by significantly (p

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Contributors: Farvin, S., Surendraraj, A., Anandan, R.
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**Antioxidant activities and phenolic content of some of the selected species of seaweeds from Danish coast**

**General information**
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Organisations: Section for Aquatic Lipids and Oxidation, National Institute of Aquatic Resources
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Source: orbit
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**Potato peel extract - A natural antioxidant for retarding lipid oxidation in bulk fish oil and oil in water emulsions**

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Organisations: Section for Aquatic Lipids and Oxidation, National Institute of Aquatic Resources
Contributors: Farvin, S., Nielsen, N. S., Jacobsen, C.
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**Antioxidant activity of potato peel extracts in bulk fish oil and oil in water emulsions**

**General information**
Publication status: Published
Organisations: Section for Aquatic Lipids and Oxidation, National Institute of Aquatic Resources
Contributors: Farvin, S., Nielsen, N. S., Jacobsen, C.
Publication date: 2008
Peer-reviewed: No
Investigation of dairy components responsible for resistance of omega-3 enriched yogurt to lipid oxidation

**General information**
- **Publication status:** Published
- **Organisations:** Section for Aquatic Lipids and Oxidation, National Institute of Aquatic Resources
- **Contributors:** Farvin, S., Baron, C., Nielsen, N. S., Jacobsen, C.
- **Publication date:** 2008
- **Peer-reviewed:** No
- **Event:** Poster session presented at 6th EuroFedLipid Congress, Athens, Greece, Sept.

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Investigation of dairy components responsible for resistance of omega-3 enriched yogurt to lipid oxidation

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- **Publication status:** Published
- **Organisations:** Section for Aquatic Lipids and Oxidation, National Institute of Aquatic Resources
- **Contributors:** Farvin, S., Baron, C., Nielsen, N. S., Jacobsen, C.
- **Publication date:** 2008
- **Peer-reviewed:** No
- **Event:** Abstract from 6th Euro Fed Lipid Congress, Athens, Greece.

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Projects:

**Glycoprotein: Ekstraktion af glycoprotein fra tang**
The project is based on a patent application regarding a new technology for extraction of glycoproteins with antioxidative properties from brown seaweed. The aim of the project is to upscale the technology and to evaluate whether it can be used on red seaweed, too. Furthermore, the antioxidative activity of the glycoprotein will be evaluated in selected food and skin care products.

Farvin Habebullah, S., Project Participant
Jacobsen, C., Project Participant, National Food Institute, Research group for Bioactives – Analysis and Application
Kryger, K., Project Participant, National Food Institute

**GAP:** DKK543,000.00
15/08/2014 → 15/02/2015
**Award relations:** Ekstraktion af glycoprotein fra tang
**Project:** Research

**Potential natural antioxidants: Aquatic Resources as a Source of Potential Natural Antioxidants for Food Industry**
- It is well documented that long-chain polyunsaturated omega-3 fatty acids (omega-3 PUFA) have a range of beneficial health effects such as reducing atherosclerosis, prevention and treatment of numerous disorders like cardiovascular disease, cancer, diabetics, mental illness etc.
- At the same time they are very susceptible to lipid oxidation that not only causes deterioration of food sensory quality, but also contributes to carcinogenesis, atherosclerosis and aging processes in humans. Hence, the oxidative instability of omega-3 fatty acids often limits their use as nutritionally beneficial lipids in fish oil enriched foods. Addition of antioxidants that scavenge free radicals and control pro-oxidative metals is used to retard lipid oxidation.
- Many of the most commonly used antioxidants are synthetic compounds, which have been reported to possess carcinogenic effect in humans and there is, therefore, a need to find potent and safer natural antioxidants.
- Many living organisms in the marine environment are rich in omega-3 PUFA. Our hypothesis is therefore that these marine organisms are rich in natural antioxidants that are able to protect them against lipid oxidation and that these antioxidants can be used to protect foods against oxidation.
Objectives
The overall goal of the project is to identify natural compounds with antioxidant activity from aquatic resources such as marine algae, bacteria, fungi, peptides isolated from fish waste and to evaluate potential applications of these novel compounds to enhance oxidative stability, flavor quality and nutritional value of foods enriched with omega-3 fatty acids and seafood based products.
This will be achieved by:
Screening extracts from aquatic resources like marine algae, bacteria, fungi and peptides isolated from fish waste for their antioxidative mechanisms and properties and identifying the most promising sources of antioxidants.
Evaluating the antioxidant properties of the most promising antioxidant sources in different foods systems enriched with omega-3 fatty acids such as milk, dressing and seafood.
Project financing:
Danish research council for Technology and production (FTP)
Farvin Habeullah, S., Project Manager, National Food Institute, Division of Industrial Food Research
Jacobsen, C., Contact Person, National Food Institute, Division of Industrial Food Research
Gram, L., Project Participant, Department of Systems Biology, Bacterial Ecophysiology and Biotechnology
Jessen, F., Project Participant, National Food Institute, Division of Industrial Food Research
Nielsen, H. H., Project Participant
Nielsen, K. F., Project Participant, Department of Systems Biology, Metabolomics Platform
01/01/2009 → 31/12/2012
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