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Gull í greipar Ægis - Antioxidants from Icelandic marine sources

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Gull í greipar Ægis
Antioxidants from Icelandic marine sources

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Report summary

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Titill / Title	**Gull í greipar Ægis / Antioxidants from Icelandic marine sources**		
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Ágrip á íslensku:	Tilgangur þessa verkefnis var að skima fyrir þráahindrandi efnum úr íslensku sjávarfangi eins og þörungum, loðnu og sviljum, til að nota sem aukefni í matvæli, markfæði eða sem fæðubótarefni. Sérstaklega beindist athyglin að mögulegri notkun fjölfenóla úr þörungum sem náttúruleg andoxunarefni til að hindra þránun í fiskafurðum og fiskvöðvapróteinum (ísólöt). Þetta var gert með því að skima fyrir andoxunarvirkni með nokkrum tegundum af andoxunarprófum. Vænlegasta andoxunarefnið var valið til að rannsaka betur andoxunareiginleika þess í fæðulíkönum, þ.e. þvegnu þorskvöðvakerfi, þorskpróteinkerfi og í fiskiborgurum. Niðurstöður sýndu meðal annars að fjölfenól úr bóluþangi (*Fucus vesiculosus*) hafa mikla andoxandi eiginleika og eru vænleg til notkunar sem fæðubótarefni eða í matvæli til að stuðla að auknum stöðugleika, bragðgæðum og næringargildi.		
Lykilorð á íslensku:	*Andoxunarefni, lífvirk efni, þörungar, vökvafasi loðnu, svil*		
Summary in English:	The aim of this project was to explore the natural antioxidant activity of marine sources like seaweed, capelin and cod milt to use as food additives, functional ingredients or nutritional supplements. The potential application of algal polyphenols as novel natural antioxidants to prevent lipid oxidation of fish muscle and fish protein based products was of special interest. This was done by screening for antioxidant activity using different types of antioxidant assays. The most promising antioxidants were selected and their antioxidant properties studied further in fish model systems and fish patties. The results showed that phlorotannins isolated from bladderwrack (*Fucus vesiculosus*) had very high antioxidant properties and has a potential as nutritional supplements or food additive to enhance oxidative stability, flavor quality and nutritional value		
English keywords:	*Antioxidants, bioactivity, seaweed, aqueous capelin phase, milt*		

EXECUTIVE SUMMARY

Novel preservation techniques including natural antioxidative and functional additives need to be developed to maintain the prime quality of perishable seafood products. Natural antioxidants can be used to increase oxidative stability, flavor and nutritional value of food and can also be used as nutritional supplements to improve human health. The aim of this project was to explore the natural antioxidant activity of marine sources like seaweed, capelin and cod milt to be used as food additives, functional ingredients or nutritional supplements. The potential application of algal polyphenols as novel natural antioxidants to prevent lipid oxidation of fish muscle and fish protein based products was of special interest. This was done by screening for antioxidant activity using different types of antioxidant assays. The most promising antioxidants were selected and their antioxidant properties studied further in fish model systems and fish patties.

Seaweed

Seaweed, as a source of functional compounds with potential health benefits, is gaining worldwide interest. Results have shown that seaweeds are a rich source of various natural antioxidants such as polyphenols, which play an important role in preventing oxidation in foods and oxidative stress in humans. In our study, bladderwrack (*Fucus vesiculosus*) (í: bóluþang) was found to possess the highest total phlorotannin content and the greatest radical scavenging activities among ten different seaweed species that were tested. Subfractions rich in oligomeric and polymeric phlorotannins exhibited potent DPPH radical scavenging activity, comparable to or higher than several commercial antioxidants.

Enzyme-assisted extraction by protease treatment enhanced the yield of polyphenols and other hydrophilic antioxidant components from *Palmaria palmata* (í: söl), but was not effective for other species. The integrated use of *in vitro* antioxidant tests, monocyte-based bioassay and fish model systems verified the complex mechanisms involved in the antioxidant effect of algal polyphenols in different systems and gave a more comprehensive antioxidant profile than a single test. Semi-purified phlorotannin subfractions from *F.*

vesiculosus exhibited superior *in vitro* antioxidant activity, comparable to or higher than several commercial antioxidants. In washed cod model system and in cod protein isolates, the oligomeric subfractions were highly effective while polymeric subfractions only had slight retarding effect. The studies provide valuable information on antioxidant properties of phlorotannin fractions and first evidence of their antioxidant effects in fish model systems.

Collection of seaweeds in Hvassahraun

Capelin

The potential use of capelin (*Mallotus villosus*) into value added products is based on antioxidant properties that have been linked to components in the aqueous fraction of fishmeal from capelin, which undergoes heating during processing[1][2]. Characterization of antioxidant capacities of different aqueous fractions related to the content of peptides, amino acids or other components with antioxidant properties is therefore of interest to gain information on possible further use into valuable products. Capelin protein hydrolysates are a

[1] Bragadóttir, Margrét, Judith Reichert, Rósa Jónsdóttir and Guðrún Ólafsdóttir. 2006. Characterisation and antioxidant properties of aqueous extracts from capelin (Mallotus villosus), Rf-skýrsla 37/06. 26 bls.)
[2] Guðrún Ólafsdóttir, Rósa Jónsdóttir, Margrét Bragadóttir, 2006. The role of volatile compounds in odor development during haemoglobin-mediated oxidation of cod muscle membrane lipids. TAFT 2006, 2nd Joint Trans Atlantic Technology Conference, Quebec City, Oct 29th –Nov 1st 2006

good candidate to help reduce oxidation in foods and to regulate blood pressure, especially the smaller fractions. This study suggested that it is better to use the aqueous extract of capelin muscle hydrolyzed with Protamex rather than Cryotin-F. Indeed, Protamex produces a larger amount of the more bioactive smaller peptides, at the same degree of hydrolysis.

Capelin (Mallotus villosus)

Cod milt

Furan fatty acids were first discovered in seed oil of *Exocarpus cupressiformis* by Morris and others (1966)[3]. Two series of propyl- and pentyl-substituted furan fatty acids in the 5-position of the furan ring have been described in fish, crayfish, soft coral and various plants and their presence was more recently detected in mammals including man[4]. In fish, furan fatty acids are found in liver cholesterol esters and in testis triglycerides, but are also present in phospholipids. Shirasaka and others[5] have proposed that those detected in marine fish are derived from marine plants and/or intestinal bacteria of fishes. Furan fatty acids have been suggested to play an important role as radical scavengers in membranes[6]. In our study, cod milt was studied as a possible source of furan fatty acids based antioxidant. The cod milt

[3] Morris, L.J., Marshall, M.O. and Kelly. W.1966. A Unique Furanoid Fatty Acid from Exocarpus Seed Oil, Tetrahedron Lett. 36:4249–4253.
[4] Puchta, V., Spiteller, G. and Weidinger, H. 1988. F-acids – a new component of the phospholipids of human blood. Liebigs Annalen Der Chemie, Vol 1988 (1), 25-28.
[5] Shirasaka, N., Nishi, K., and Shimizu, S. 1995. Occurrence of a furan fatty acid in marine bacteria. Biochimica et Biophysica Acta 1258, 225-227.
[6] Spiteller G. 2005 Furan fatty acids: Occurrence, synthesis, and reactions. Are furan fatty acids responsible for the card ioprotective effects of a fish diet?

sample did not show high antioxidant activity although the metal chelating activity was rather high. The cod milt did not show high ability to scavenge ROS compared to the control sample.

Using natural antioxidants from marine source is an innovative alternative to enhance oxidative stability, flavor quality and nutritional value of food products, as well as for utilization in functional foods or nutritional supplements. The market for antioxidants is big and the demand for natural antioxidants is increasing. The opportunity for niche products, for example Icelandic seaweed extracts and purified polyphenols from seaweed with beneficial health effects is therefore great interest.

In this final report of the project Gull í greipar Ægis, a summary is given of the overall work, materials and methods and the main results. This three year project was initiated as part of research activities in the area of lipid oxidation and antioxidants. The project provided an opportunity for a challenging research that was part of a doctoral thesis from the University of Iceland. The outcome of the studies have been published as scientific papers as part of the thesis, as well as additional publications and chapters in scientific books. The expertise gained has been invaluable to motivate further projects funded by national and Nordic research funds[7][8].

FUTURE PERSPECTIVES

- Continue the screening of bioactive properties of seaweed and other underutilized marine resources
- Test the antioxidant activities and other bioactivities *in vivo*
- Scale up the extraction, isolation and purification process of bioactives in seaweed
- Test the antioxidants from seaweed extracts in other food models like emulsions
- Assess the risk-benefit of consuming seaweed bioactives
- Marketing analysis for bioactives extracted from marine sources

[7] Tækniþróunarsjóður Rannís, Forverkefni no 081505008. Íslensk matarrmenning með þörungum og plöntum, 2008
[8] Safefood ERA /Rannsóknasjóður Rannís. Safe transportation of marine bioactive´s from source to active site, 2008

MATERIAL & METHODS

The project was divided into four main work packages (Figure 1). The first work package was on screening the antioxidant activity of different types of Icelandic seaweed. Ten species of seaweed were collected in Hvassahraun, total amount of polyphenols determined and antioxidant activity tested using three different antioxidant assays. The efficiency of different extraction technique, including enzyme hydrolysis, was evaluated and the antioxidative activity in the different types of edible seaweed extracts was screened.

The second work package was on the antioxidant activity of cooked aqueous capelin phase, with and without enzyme hydrolysis. The chemical content of the aqueous phase of capelin mince was characterized, fractions of aqueous phase of different molecular size were prepared by ultrafiltration and the antioxidative activity of aqueous phase and different fractions screened.

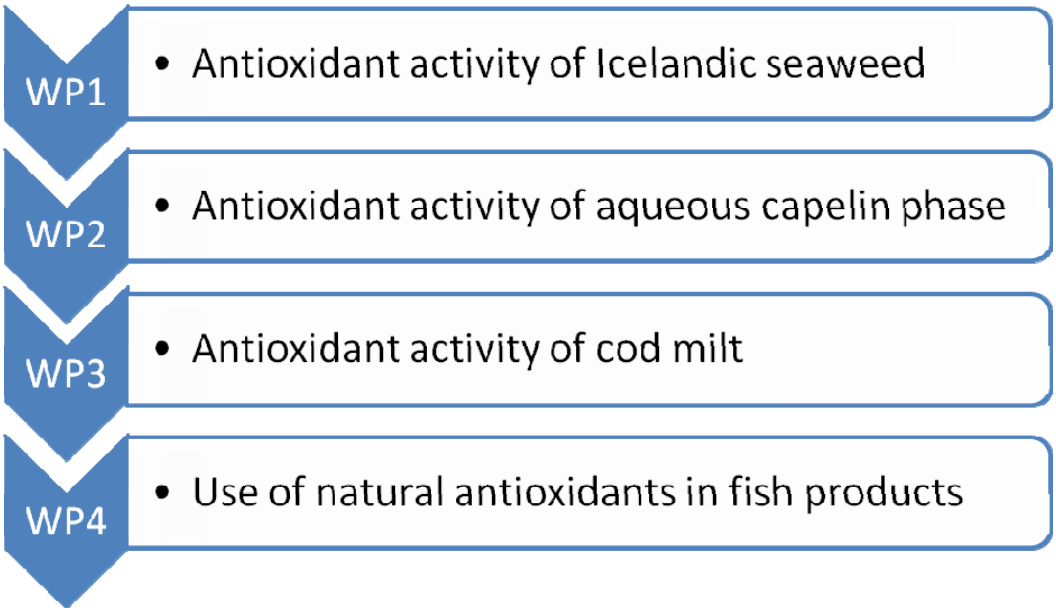

Figure 1. Overview of the work packages of the project.

The third work package involved the study of antioxidant activity of furan fatty acids. Due to difficulties in getting standard compounds of furan fatty acids the goal of this work was changed and decided to study the antioxidative activity of freeze dried cod milt that can possibly contain relatively high amount of furan fatty acids.

Total polyphenol content (TPC) of the different seaweed species was measured. The *in vitro* antioxidant assays used in these three work packages were oxygen radical absorbance capacity (ORAC), 2,2-diphenyl-1-picrylhydrazyl (DPPH) radical scavenging, reducing power, and metal ion chelating activity assays. In some instances, the ability of the bioactives to reduce blood pressure was evaluated by angiotensin converting enzyme inhibition (ACE) assay. Ability of seaweed extracts and cod milt to scavenge reactive oxygen species (ROS) generated by mononuclear cells was also studied.

Finally, the aim of the fourth work packages was to incorporate natural antioxidants into fish model systems and fish product to study their influence on storage stability. The antioxidative stability was tested using different methods, i.e. thiobarbituric acid reactive substances (TBARS), peroxide value (PV), sensory analysis and gas chromatography analysis.

Detailed description of materials and methods can be found in published peer reviewed journals, scientific research manuscript and reports from this project. List of publications is given in the chapter on main results.

MAIN RESULTS

The main results of each work package are given here below. For more detailed information, see list of publications and presentations.

1. Antioxidant activity of Icelandic seaweed

Among the ten species screened by *in vitro* antioxidant tests, bladderwrack (*Fucus vesiculosus*) was found to possess the TPC and the greatest radical scavenging activities (Figure 2). Subfractions rich in oligomeric and polymeric phlorotannins exhibited potent DPPH radical scavenging activity, comparable to or higher than several commercial antioxidants. Ultra-filtration showed that phlorotannins in *F. vesiculosus* are mainly composed of high molecular weight polymers. The oligomeric subfractions at 300 mg/kg were highly effective in inhibiting lipid oxidation in fish model systems while polymeric subfractions only had slight retarding effect. All the subfractions separated by Sephadex LH-20 column and ultra-filtration showed potent in vitro reactive oxygen species (ROS) scavenging ability in a mononuclear cell-based bioassay as well as high ACE inhibitory activities, demonstrating the multiple bioactive properties of phlorotannins.

Figure 2. Fucus vesiculosus (e. Bladder wrack)

Different types of enzyme extractions were tested in order to extract polyphenols and other antioxidant components from the different seaweed species. Protease treatment enhanced the extraction of polyphenols and other hydrophilic antioxidant components from *P. palmata* but negative effects were observed for *F. vesiculosus* and *Laminaria hyperborea* (í: stórþari).

The effects of *F. vesiculosus* extract and fractions towards haemoglobin-catalyzed lipid oxidation in washed cod muscle system and cod protein isolates during ice storage were examined. Progression of oxidation was followed by determining rancid odor, TBARS, redness and volatile oxidation compounds by gas chromatography (GC). In both washed cod muscle and protein isolates, phlorotannin-enriched ethyl acetate (EtOAc) fraction showed higher inhibitory effect than crude 80% ethanol (EtOH) extract. The addition of oligomeric phlorotannin-rich subfraction (LH-2) separated by Sephadex LH-20 chromatography, completely inhibited the initiation of lipid peroxidation in both systems throughout the entire study period (8 days). Its effectiveness at 300 mg/kg level was comparable to that of 100 mg/kg propyl gallate (PG), a highly effective synthetic antioxidant in muscle foods. Although polymeric phlorotannin-rich subfraction (LH-5) had similar level of TPC and chemical antioxidant activities as oligomeric subfraction LH-2, it was far less efficient in model systems. These results suggest that other factors rather than the intrinsic reactivity toward radicals could be responsible for the inhibitory effect of phlorotannins on lipid oxidation in fish muscle.

2. Antioxidant activity of aqueous capelin phase

Two samples of capelin (*Mallotus villosus*) were obtained from the third (3wks) and seventh week (7wks) of the year 2007 (Figure 3). The fat content of the 3wks and 7wks samples were 10.6 % and 5.7 %, respectively. Aqueous extracts were prepared from cooked capelin and the aqueous phase centrifuged. Ultrafiltration of capelin broth, protein determination, ACE inhibitory activity test and peptide analysis using capillary electrophoresis was done. The results suggested that fractionated capelin broth has the potential to inhibit the ACE even at low concentration of 1 mg/ml. It was concluded that hydrolysis in addition to ultra-filtration might improve the bioactivity of the capelin aqueous extraction.

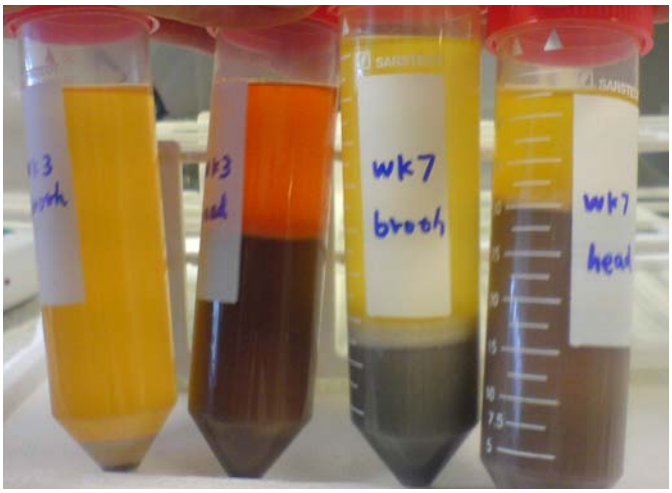

Figure 3. Lipid phases of broth and broth from head of capelin caught in 3 week and 7 week of the year.

One year later, sample of capelin was obtained in February. The aim of that work was to determine the in-vitro bioactive properties of aqueous extracts of capelin hydrolyzed with two different enzymes and to investigate the effect of the best aqueous extract in fish product. Headed and gutted capelin was cooked in a conventional oven, and then it was filtrated and centrifuged. The supernatant was collected as an aqueous extract which was then subjected to hydrolysis using cold adapted cod proteases (Cryotin®) and the enzyme mixture Protamex®. The reaction was terminated when degree of hydrolysis (DH %) reached 8.0%. The hydrolysate was then ultra-filtrated using membranes of different molecular weight cut-offs (30 kDa, 10 kDa, and 5 kDa). The different hydrolysate fractions were subjected to oxygen radical absorbance capacity (ORAC), DPPH radical scavenging, reducing power, ferrous-ion chelating ability assays and ACE assay. The fractionated capelin protein hydrolysates showed an increase in ORAC, DPPH radical scavenging, reducing power and ferrous-ion chelating values with decreasing protein/peptide molecular size. All fractions however demonstrated strong antioxidant activity based on these assays. All fractionated capelin hydrolysates demonstrated good ACE inhibition. The highest ACE inhibitory activity was in fractions having peptides with molecular weight lower than 5 kDa. This study suggests that fractionated capelin may have the potential to regulate the blood pressure system and help reduce oxidation.

3. Antioxidant activity of cod milt

Sample of cod milt was obtained for fatty acids analysis and evaluation of antioxidant properties and monocytes. The milt was homogenized, freeze dried and kept as powder until analyzed. The total amount of unknown fatty acids was 5.9% which is similar to cod liver oil. Some of the unknown fatty acids can possibly be furan fatty acid but further identifications are needed to confirm their existence in cod milt. The sample did not show high antioxidant activity although the metal chelating activity was rather high. The cod milt did not show high ability to scavenge ROS compared to the control sample.

4. Use of natural antioxidants in fish products

The effect of phlorotannin-enriched ethyl acetate (EtOAc) fraction isolated from *F. vesiculosus* as natural antioxidant in fish products was studied. Fish patties produced form cod was selected in this study. The fish was blended with 300 and 600 mg/kg of EtOAc fraction. All the samples were cooked at 70°C for 30 min as patties and stored at refrigerated temperature for 28 days. The fish patties were taken every 7 days for analysis. Lipid oxidation during storage was analyzed by measuring PV and TBARS. The results suggested that 300 or 600 mg/kg of EtOAc fraction added to fish patties can reduce lipid oxidation.

Hydrolyzed capelin fractions lower than 5 kDa were selected to apply in fish product. As before fish patties produced form cod was selected in this study and lipid oxidation evaluated using PV and TBARS. Hydrolysis with Protamex improved the capability of capelin aqueous extract to inhibit the early stage lipid oxidation (formation of hydroperoxides) as well as retard propagation of the oxidation process (degradation of hydroperoxides leading to TBARS formation). However, hydrolysis with Cryotin-F had smaller effect on the antioxidative activity of capelin aqueous extract in the present study. This study suggests that capelin aqueous extract hydrolyzed with Protamex may improve quality of fish products by reducing oxidation.

ACKNOWLEDGEMENTS

Thanks to the AVS fund of Ministry of Fisheries in Iceland for financing this project and thanks to the United Nations University - Fisheries Training Programme (UNU-FTP) for funding a scholarship for the doctoral student Tao Wang who performed the main work on seaweed. We also thank Eyjólfur Friðgeirsson and the company Íslensk hollusta for good collaboration in the project.

LIST OF PUBLICATIONS AND PRESENTATIONS

Work package 1: Antioxidant activity of Icelandic seaweed

Scientific papers:

Tao Wang, Rósa Jónsdóttir, Guðrún Ólafsdóttir. 2009. Total phenolic compounds, radical scavenging and metal chelation of extracts from Icelandic seaweeds. *Food Chemistry*, 116, 240-248. http://dx.doi.org/10.1016/j.foodchem.2009.02.041

Tao Wang, Rósa Jónsdóttir, Hördur G. Kristinsson, Gudjon Thorkelsson, Charlotte Jacobsen, Patricia Yuca Hamaguchi, and Gudrun Ólafsdóttir. 2009. Inhibition of haemoglobin-mediated lipid oxidation in washed cod muscle and cod protein isolates by *Fucus vesiculosus* extract and fractions. *Food Chemistry*. Article in Press. http://dx.doi.org/10.1016/j.foodchem.2010.04.038

Tao Wang, Rósa Jónsdóttir, Hördur G. Kristinsson, Guðmundur Óli Hreggviðsson, Jón Óskar Jónsson, Guðrún Ólafsdóttir. 2009. Free radical scavenging and metal chelating activities of enzymatic extracts from the red algae *Palmaria palmata*. *LWT – Food Science and Technology*. Accepted for publication.

Tao Wang, Rósa Jónsdóttir, María Jesús González, Isabel Medina, Hördur G. Kristinsson, Sivakumar Raghavan and Gudrun Ólafsdóttir. 2010. Antioxidant properties of solvent extracts and fractions from the brown algae *Fucus vesiculosus*. Manuscript.

PhD Thesis:

Tao Wang. Enhancing the quality of seafood products through new preservation techniques and seaweed based antioxidants – Algal polyphenols as novel natural antioxidants. Faculty of Food Science and Nutrition, School of Health Sciences, University of Iceland, Reykjavík, Doctoral thesis, December 2009. http://skemman.is/bitstream/1946/4139/1/Final_fixed.pdf

Book chapter:

Tao Wang, Guðrún Ólafsdóttir, Rósa Jónsdóttir, Hördur G. Kristinsson, Ragnar Jóhannsson. Functional and nutraceutical ingredients from marine macroalgae. *In:* Seafood quality, safety and health effects. Editors: Cesarettin Alasalvar, Kazuo Miyashita, Fereidoon Shahidi, Udaya Wanasundara. Blackwell Publishing, UK. In Press.

Presentation:

Tao Wang, Rósa Jónsdóttir, Hördur G. Kristinsson, Gudjon Þorkelsson, Charlotte Jacobsen, Guðmundur Óli Hreggviðsson and Guðrún Ólafsdóttir. 2009. Algal polyphenols as novel natural antioxidants. Presentation at the 3rd Joint Trans-Atlantic Fisheries Technology Conference Sept. 15 – 18. Copenhagen, Denmark. http://www.matis.is/media/utgafa/krokur/TAFT-2009_Presentation_Algal-polyphenols.pdf

Hörður G. Kristinsson og Margrét Geirsdóttir. Lífvirk efni úr íslenskri náttúru. Erindi flutt á Matvæladegi Matvæla- og næringarfræðafélagi Íslands, 15. október 2009.

Report:

Lára Valsdóttir. The antioxidant effect of seaweed extracts in protein isolate. Report to The Icelandic Student Innovation Fund. 2009.

Posters:

Rósa Jónsdóttir, Tao Wang, María Jesús Gonzalez, Isabel Medina, Hordur G. Kristinsson, Guðrún Ólafsdóttir. Bioactivity of phlorotannins in brown seaweed, *Fucus vesiculosus.*

3nd Joint Trans-Atlantic Fisheries Technology Conference Sept. 15 – 18. Copenhagen, Denmark. http://www.matis.is/media/utgafa/krokur/Poster-seaweead-Taft-2009.pdf

Tao Wang, Rósa Jónsdóttir, Hordur G. Kristinsson, Guðmundur Óli Hreggviðsson, Jón Óskar Jónsson, Guðrún Ólafsdóttir. 2009. Evaluation of antioxidant activity of enzymatic extracts from the red algae *Palmaria palmata*. Poster at the IFT annual meeting. Anaheim, CA, June 2009.
http://www.matis.is/media/utgafa/veggspjold/2009_IFT_Palmaria-palmata.pdf

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http://avs.is/frettir/2008/08/29/nr/1825. Gull í greipar Ægis. Frétt á heimasíðu AVS, 29.8.2008.

http://avs.is/frettir/2009/06/11/nr/1921 AVS rannsóknir kynntar á alþjóðlegri ráðstefnu í Bandaríkjunum. Frétt á heimasíðu AVS, 11.6.2009.

Work package 2: Antioxidant activity of aqueous capelin phase

Scientific papers:

Patricia Hamaguchi, Annabelle J. C. Vrac, Rósa Jónsdóttir, Hólmfríður Sveinsdóttir, Hordur G. Kristinsson. In vitro bioactivities of fractionated capelin (*Mallotus villosus*) protein hydrolysates by two different commercial proteases. Manuscript.

Poster:

Patricia Hamaguchi, Rósa Jónsdóttir, Annabelle J. C. Vrac, Matís, Hordur G. Kristinsson, Bioactivity properties of fractionated capelin (*Mallotus villosus*) protein. Poster at the IFT annual meeting. Anaheim, CA, June 2009.
http://www.matis.is/media/utgafa/veggspjold/2009_IFT_Capelin.pdf

Online news:

http://avs.is/frettir/2009/06/11/nr/1921 AVS rannsóknir kynntar á alþjóðlegri ráðstefnu í Bandaríkjunum. Frétt á heimasíðu AVS, 11.6.2009.

Work package 3: Antioxidant activity of cod milt

Report:

Rósa Jónsdóttir, Patricia Y. Hamaguchi, Annabelle Vrac. Antioxidant activity of cod milt. Experimental report. April 2010.

Work package 4: Use of natural antioxidants in fish products

Scientific papers:

Patricia Hamaguchi, Annabelle J. C. Vrac, Rósa Jónsdóttir, Hólmfríður Sveinsdóttir, Hordur G. Kristinsson. Capelin (*Mallotus villosus*) protein hydrolysates as natural antioxidant in fish products. Manuscript.

Rósa Jónsdóttir, Patricia Y. Hamaguchi. Hörður G. Kristinsson, Tao Wang. Seaweed extract as antioxidants in fish product. Manuscript.

Poster:

Guðrún Ólafsdóttir, Tao Wang, Rósa Jónsdóttir, Hörður Kristinsson, Guðmundur Óli Hreggviðsson, Guðjón Þorkelsson. *Palmaria palmata* in food formulations as natural antioxidant and functional ingredient. Poster at the Innovation in the Nordic Marine Sector, Reykjavik, May 12th 2009.
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