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Publications:

Development of carbohydrate-based nano-microstructures loaded with fish oil by using electrohydrodynamic processing
The encapsulation of fish oil in carbohydrate-based nanomicrostructures obtained by electrohydrodynamic processing was investigated. Solutions of pullulan 200 kDa (15 wt%) and dextran 70 kDa (25 wt%) presented appropriate properties (viscosity, surface tension and conductivity) to allow the formation of nano-microfibers and nano-microcapsules, respectively. Although dextran 70 kDa exhibited antioxidant properties in solution, their capsules produced at lab and pilot-plant scales showed a low oxidative stability both with emulsified and neat oil. Phase separation of solution and opened capsules indicated a poor interaction between dextran and fish oil, which suggested that further optimization of the electrospraying solution is necessary. On the contrary, pullulan solutions were optimized to work even at pilot-plant scale. In this case, in spite of the prooxidant effect of pullulan in solution, oxidatively stable pullulan fibers (PV = 12.3 ± 0.9 meq O2/kg and 15.5 ± 5.1 ng/g of 1-penten-3-ol) were obtained when oil was incorporated as neat oil and when producing batches during short time (30 or 10 min). This superior oxidative stability when compared to fibers with emulsified oil is mainly attributed to a higher fish oil entrapment and to the location of the oil in large bead-structures with a reduced specific surface area. These results indicated the feasibility of producing omega-3 nanodelivery systems by encapsulating fish oil in pullulan nano-microfibers using electrospinning processing.

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Oxidative stability of pullulan electrospun fibers containing fish oil: Effect of oil content and natural antioxidants addition

The effect of oil content and addition of natural antioxidants on the morphology and oxidative stability of pullulan ultra-thin fibers loaded with fish oil and obtained by electrospinning was investigated. Pullulan sub-micron fibers containing 10 and 30wt% fish oil were prepared and both presented beads where the oil accumulated. The number of beads was significantly higher in 30wt% oil-loaded fibers. Moreover, fibers containing 30wt% fish oil had a higher oxidative stability when compared to 10wt% oil-loaded fibers, despite its lower encapsulation efficiency (EE) value (67.1±3.1%). The oxidative stability of fibers loaded with 10wt% fish oil (EE=88.5±0.7%) was significantly improved when adding δ-tocopherol (500ppm) and rosemary extract (500ppm) as antioxidants. However, higher concentration of antioxidants (2000ppm δ-tocopherol and 1000ppm rosemary extract) did not further improve the oxidative stability of 10wt% oil-loaded fibers, but had a pro-oxidant effect. Finally, the production of pullulan fibers containing 10wt% fish oil from formic acid solutions increased the oxidative stability of the fibers when compared to the same type of fibers obtained from water solutions. The latter was observed for fibers without and with antioxidants (500ppm of δ-tocopherol and 500ppm of rosemary extract). Practical applications: Encapsulation of omega-3 polyunsaturated fatty acids and addition of antioxidants are the most efficient strategies to protect these lipids against oxidation when incorporating them into food...
matrices. These results show the feasibility to encapsulate fish oil in pullulan ultra-thin fibers and to improve their oxidative stability by adding natural antioxidants such as δ-tocopherol and rosemary extract. Therefore, this study might open up new opportunities for further technological development in the production of omega-3 nanodelivery systems, which have potential applications in different types of fortified foods. Encapsulation of fish oil in electrospun pullulan fibers stabilized by natural antioxidants.

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**Organisations:** National Food Institute, Research Group for Bioactives – Analysis and Application, Research Group for Nano-Bio Science, Technical University of Denmark  
**Authors:** García Moreno, P. J. (Intern), Damberg, C. (Ekstern), Chronakis, I. S. (Intern), Jacobsen, C. (Intern)  
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Physical and oxidative stability of fish oil-in-water emulsions fortified with enzymatic hydrolysates from common carp (Cyprinus carpio) roe

Physical and oxidative stability of 5% (by weight) cod liver oil-in-water emulsions fortified with common carp (C. carpio) roe protein hydrolysate (CRPH) were examined. CRPH was obtained by enzymatic hydrolysis of discarded roe by using Alcalase 2.4 L for 30, 60, 90, and 120 min to yield different degrees of hydrolysis (DH). All the hydrolysates showed in vitro antioxidant activity in terms of radical scavenging and chelating properties. CRPH-containing emulsions had significantly smaller droplets than control (p

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Physical and oxidative stability of high fat fish oil-in-water emulsions stabilized with combinations of sodium caseinate and sodium alginate

A systematic study was carried out in order to evaluate the physical and oxidative stability of high fat omega-3 delivery fish oil-in-water emulsions stabilized with combinations of sodium caseinate (NaCas) and sodium alginate (NaAlg). The influence of 3 factors related to emulsion composition (fish oil content: 50, 60 and 70%; total amount of NaCas and NaAlg: 1.4, 2.1 and 2.8 %; and ratio NaCas:NaAlg: 0.4, 1.2 and 2) on physical (droplet size, viscosity and zeta potential) and oxidative (primary and secondary oxidation products) parameters was evaluated. It was possible to produce emulsions with a combination of NaCas and NaAlg, except when the ratio between NaAlg and aqueous phase was high (0.047 or 0.054). Viscosity of the emulsions significantly increased with increasing fish oil and total stabilizer content. Zeta potential was significantly affected by total stabilizer content. The content of primary oxygenation products in the emulsions was very low (0.93 meq peroxides/kg oil). Secondary oxidation products were detected in small amounts (<60 ng/g emulsion). Even though the optimum formulation concerning physical parameters was suggested as 61.8% fish oil content, 1.4% total stabilizer and 1.2 ratio NaCas:NaAlg by Box-Behnken's design, the formulae 70%-1.4%-1.2 was decided due to high fish oil content's decreasing effect on droplet size and peroxide value. Practical applications: Physically and oxidatively stable...
high fat (50-70%) omega-3 delivery fish oil-in-water emulsions are of high interest to food industry for the production of omega-3 fortified products. Our results show the feasibility to stabilize high fat delivery fish oil-in-water emulsions using combinations of NaCas and NaAlg. As these emulsions had high amount of fish oil, food products can be enriched with smaller amounts of high fat emulsions when compared to low fat delivery emulsions. This results in minor changes of the product's original structure. Examples for enrichment of food products with omega-3 are dressings, cream cheese, yoghurt and mayonnaise.

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Lipid oxidation represents a severe challenge in food engineering because it deteriorates quality of foods, especially those containing high contents of polyunsaturated fatty acids (PUFAs). One way to overcome this barrier is application of synthetic antioxidants such as butylated hydroxyanisole (BHA), butylated hydroxytoluene (BHT), propyl gallate (PG), and tertiary butylhydroquinone (TBHQ) in PUFA-rich foods. However, recently there have been concerns over health-related risks posed by these synthetic agents. Therefore, obtaining safe antioxidants from natural sources, especially those which are discarded with no use, with potency to retard lipid oxidation has gained sizable attention. Therefore, the present study aimed at obtaining “green” antioxidants from discarded common carp roe via the so-called hydrolysis process by using alcalase and determining their antioxidant activity both in vitro and in food model systems. Four common carp roe protein hydrolysates (CRPH) obtained at different reaction times (i.e. 30, 60, 90, and 120 min) were assayed. In vitro antioxidant activity of the hydrolysates was measured through three different assays (i.e. DPPH radical scavenging, metal ion chelating, and reducing power). Furthermore, the oxidative stability of 5% fish oil-in-water emulsions containing or not the hydrolysates was investigated by monitoring their content of hydroperoxides and volatiles markers. The hydrolysates exhibited high DPPH radical scavenging activity and reducing power when compared to positive controls, i.e. BHT and ascorbic acid, respectively. However, Fe2+ chelating capacity of the hydrolysates was relatively lower than that of Ethylenediaminetetraacetic acid (EDTA), applied as positive control. The antioxidant activity of hydrolysates was changed with DH and increased in a dose dependent manner. Unlike CRPH-fortified emulsions, the emulsions without CRPH exhibited significantly increased peroxide value (PV) during the storage period (p<0.05), reaching from 4.7±0.1 at day 0 to 79.2±11.3 at day 7. Moreover, all CRPH-containing emulsions showed negligible amounts of most secondary oxidation products (e.g. 1-penten-3-one and (E,E)-2,4-heptadienal) when compared to the emulsion control at day 7. These results indicate that common carp roe protein hydrolysates exert antioxidant activity both in vitro and in fish oil-in-water emulsions, and can thus be considered as alternative antioxidants to the synthetic ones.
Effect of digestive enzymes on the bioactive properties of goat milk protein hydrolysates

The aim of this research was to study the influence of the gastrointestinal digestion on the bioactivity of goat milk protein hydrolysates prepared with subtilisin, trypsin and a combination of these two enzymes. All hydrolysates had excellent angiotensin converting enzyme (ACE) inhibitory activity, antioxidant activity and bile acid-binding capacity. Peptide profiles and bioactivities were mainly altered during the intestinal digestion, whereas the effect of the gastric digestion was negligible. The influence of the intestinal digestion varied depending on the hydrolysate and the bioactivity studied. In the case of ACE inhibitory activity, it exclusively decreased when peptides were produced with trypsin. In contrast, antioxidant activity and bile acid-binding capacity improved after the gastrointestinal digestion, regardless the enzymatic treatment conducted. Hydrolysis employing mixtures of subtilisin and trypsin is considered a good approach to produce peptides that maintain, or even enhance, their bioactivity after digestion. (C) 2015 Elsevier Ltd. All rights reserved.
The encapsulation of fish oil in poly(vinyl alcohol) (PVA) nanofibers by emulsion electrospinning was investigated. Independently of the emulsifier used, whey protein isolate (WPI) or fish protein hydrolysate (FPH), PVA concentration had a high influence on fiber morphology. Fibers without bead defects were only produced for solutions with 10.5% (w/w) PVA, which presented sufficient number of polymer chain entanglements. On the other hand, increasing oil load from 1.5 to 3% (w/w) resulted in fibers with larger diameters containing spindle-like enlargements interspersed. High omega-3 encapsulation efficiency (92.4 ± 2.3%) was obtained for fibers produced from 10.5% (w/w) PVA-5% (w/w) emulsion blend stabilized with WPI, resulting in an oil load capacity of 11.3 ± 0.3%. Moreover, the encapsulated oil was randomly distributed as small droplets inside the fibers. However, the electrospun fibers presented a higher content of hydroperoxides and secondary oxidation products (e.g. 1-penten-3-ol, hexanal, octanal and nonanal) compared to emulsified and unprotected fish oil.
Evaluation of the antioxidant activity in food model system of fish peptides released during simulated gastrointestinal digestion

In the last decade, increasing evidences of the occurrence of lipid oxidation during digestion have been reported, in either in vivo or in vitro studies (1,2,3). As a result, the nutritional quality and safety of foodstuffs could be affected by the decrease of certain lipidic compounds of interest and the generation of potentially toxic oxidation products. Regarding fish composition, the high content in polyunsaturated ω-3 acyl groups renders its lipids especially prone to oxidation. However, fish is also a major source of protein, which could greatly influence the extent of oxidation reactions taking place in the gastrointestinal tract. In fact, several studies have reported antioxidant activity of fish protein hydrolysates, coming from fish industry waste by-products (3,4). Thus, the potential release of peptides showing antioxidant properties during fish
digestion cannot be ruled out. In order to shed light on these aspects, in vitro digestates of European sea bass were submitted to ultrafiltration using membranes with different cut off size. Afterwards, the potential antioxidant activity of the peptide fractions obtained was evaluated by comparing the oxidative stability of fish oil-in-water emulsions (5%), containing or not the isolated fractions (2 mg/ml final protein concentration). For this purpose, the occurrence of volatile markers of lipid oxidation, the tocopherol content, and the lipid composition of the emulsions during storage were studied. At day 12 of storage, emulsions made with digested protein fractions showed a higher content of docosahexanoic acyl groups and tocopherol than the emulsion control (without fractions), and also a lower content of volatile oxidation markers.

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**Functional, bioactive and antigenicity properties of blue whiting protein hydrolysates: Effect of enzymatic treatment and degree of hydrolysis**

BACKGROUND: Fish discards represent an important under-utilisation of marine resources. This study evaluated the upgrading of the protein fraction of blue whiting (Micromesistius poutassou) discards by the production of fish protein hydrolysates (FPHs) exhibiting functional, antioxidant, angiotensin-I converting enzyme (ACE)-inhibitory and antigenicity properties. RESULTS: FPHs with low DH (4%) showed better emulsifying, foaming and oil binding capacities, particularly those obtained using only trypsin. FPHs with DH 4% exhibited also the stronger antioxidant activity, especially the one obtained using only subtilisin (IC50 = 1.36mg protein mL-1). The presence of hydrophobic residues at the C-terminal of the FPH produced using subtilisin also led to the stronger ACE-inhibitory activity. However, FPHs with high DH (12%), which implies a higher proportion of short peptides, was required to enhance ACE-inhibition (IC50 = 172μg protein mL-1). The antigenic levels of the FPH were also reduced with DH independently of the enzymatic treatment. Nevertheless, the highest degradation of fish allergens (e.g. parvalbumin) was also obtained when using only subtilisin. CONCLUSION: These results suggest that added-value products for food applications can be produced from the protein fraction of discards.

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Authors: García Moreno, P. J. (Intern), Pérez-Gálvez, R. (Ekstern), Espejo-Carpio, F. J. (Ekstern), Ruiz-Quesada, C. (Ekstern), Pérez-Morilla, A. I. (Ekstern), Martínez-Agustín, O. (Ekstern), Guadix, A. (Ekstern), Guadix, E. M. (Ekstern)
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Nutritional indexes, fatty acids profile, and regiodistribution of oil extracted from four discarded species of the Alboran Sea: Seasonal effects

The new EU fisheries regulations state that all catches from pelagic fisheries must be landed from the January 1, 2015 on. Consequently, the development of new techniques for the up-grading of these discards is required. The aim of this work was to study the variations of the lipid profile and nutritional value of the oil extracted from four discarded species: axillary seabream (Pagellus acarne), sardine (Sardina pilchardus), horse mackerel (Trachurus mediterraneus), and blue whiting (Micromesistius poutassou). To that end, the proximate composition, the lipid profile, the regiospecific distribution of fatty acids within the glycerol backbone, the nutritional parameters (thrombogenic and atherogenicity indexes and hypcholesterolemic/hypercholesterolmic ratio), and the peroxidability index were determined in each season. All species presented polyunsaturated fatty acids (PUFA) as the major fraction in both the global and the sn-2 profile. DHA presented highly regiospecifity to position sn-2 whereas the tendency of EPA was to occupy sn-1(3) positions. The relative concentration of PUFA occupying sn-2 position varied from 30 to 60% (molar basis).
Oxidative Stability of Nano-Microstructures containing fish oil

Electrohydrodynamic processing is a straightforward and versatile encapsulation technique suitable for the production of nano-microstructures (NMS) (e.g. fibers and capsules) containing bioactive compounds. The process is very gentle and does not require the use of heat, avoiding deterioration of thermolabile active compounds such as fish oil. Moreover, encapsulates produced present a decreased size, which allows their incorporation into food systems without affecting product sensory qualities.

In this work, electrohydrodynamic processing and oxidative stability of NMS containing fish oil were investigated. For that purpose, three different biopolymers namely pullulan, dextran and whey protein concentrate (WPC) were evaluated as encapsulating materials. First, the influence of biopolymer concentration on the physical properties (e.g. viscosity, conductivity and surface tension) of the biopolymer solutions and on the morphology of NMS was assayed. Secondly, the oxidative stability of the biopolymer solutions containing emulsified fish oil during storage (14 days at 40 °C) and of NMS loaded with fish oil (e.g. pullulan fibers and dextran and WPC capsules) was determined. Finally, to improve the oxidative status of the NMS, pullulan fibers, dextran capsules and WPC capsules were produced by adding neat fish oil instead of emulsified fish oil to the biopolymer solutions. These latter NMS presented a higher oxidative stability, which may be due to a better entrapment of the fish oil into biopolymer encapsulates.

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Oxidative stability of pullulan nanofibers loaded with fish oil: effect of oil content and antioxidants addition

Electrospinning processing is a promising technique for the encapsulation of thermolabile bioactive compounds (e.g. fish oil) since it does not require the use of heat, which makes them easier to disperse in food matrices compared to traditional encapsulates (e.g. microcapsules produced by spray-drying). Biopolymers such as proteins and polysaccharides are required for the production of food-grade NMF. In this sense, pullulan, which is a food-approved polysaccharide, is an interesting encapsulating material due to its high electrospinnability and low oxygen permeability.

In light of the above, the aim of this work was to investigate the oxidative stability of omega-3 enriched pullulan NMF. First, the influence of fish oil content (10-20-30 %) on the properties of the electrospinning solutions (e.g. viscosity, conductivity and surface tension) as well as on the morphology of NFM and oxidative stability of NMF during storage (20 days at 20 °C and relative humidity of 33%) was studied. Secondly, the effect on the oxidative stability of the NMS of incorporating hydrophilic antioxidants (e.g. EDTA) to pullulan solutions and/or lipophilic antioxidants (e.g. tocopherols) to fish oil was evaluated. Preliminary results show that neat fish oil can be incorporated into pullulan NMS by adding 30% Tween20 (by
weight to respect to fish oil content), leading to NMS not containing antioxidants with a peroxide value lower that 20 meq O2/kg oil at day 0.

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**Physical and Oxidative Stability of Fish Oil-In-Water Emulsions Stabilized with Fish Protein Hydrolysates**

The emulsifying and antioxidant properties of fish protein hydrolysates (FPH) for the physical and oxidative stabilization of 5% (by weight) fish oil-in-water emulsions were investigated. Muscle proteins from sardine (Sardina pilchardus) and small-spotted catshark (Scyliorhinus canicula) were hydrolyzed to degrees of hydrolysis (DH) of 3-4-5-6% with subtilisin. Sardine hydrolysates with low DH, 3% and 4%, presented the most effective peptides to physically stabilize emulsions with smaller droplet size. This implied more protein adsorbed at the interface to act as physical barrier against prooxidants. This fact might also be responsible for the higher oxidative stability of these emulsions, as shown by their lowest peroxide value and concentration of volatiles such as 1-penten-3-one and 1-penten-3-ol. Among the hydrolysates prepared from small-spotted catshark only the hydrolysate with DH 3% yielded a physically stable emulsion with low concentration of unsaturated aldehydes. These results show the potential of FPH as alternative protein emulsifiers for the production of oxidatively stable fish oil-in-water emulsions.

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Ice creams produced with unsaturated fats rich in oleic (OO, 70.7% of oleic) and linoleic (LO, 49.0% of linoleic) fatty acids, were compared to ice cream based on saturated coconut oil (CO, 50% of lauric acid). The globule size distribution of OO mix during aging (72 h at 4°C) followed a similar trend to CO mix, being stable after 48 h; whereas LO mix destabilized after 24 h. CO mix showed higher destabilization during ice cream production, but no significant differences among fats were observed in the particle size of the ice cream produced. The overrun was also lower for OO and LO ice creams (34.19 and 27.12%, respectively) compared to CO based ice cream (45.06%). However, an improved melting behavior, which gradually decreased from 88.69% for CO to 66.09% for LO ice cream, was observed.

Production and characterization of ice cream with high content in oleic and linoleic fatty acids
Ice creams produced with unsaturated fats rich in oleic (OO, 70.7% of oleic) and linoleic (LO, 49.0% of linoleic) fatty acids, were compared to ice cream based on saturated coconut oil (CO, 50% of lauric acid). The globule size distribution of OO mix during aging (72 h at 4°C) followed a similar trend to CO mix, being stable after 48 h; whereas LO mix destabilized after 24 h. CO mix showed higher destabilization during ice cream production, but no significant differences among fats were observed in the particle size of the ice cream produced. The overrun was also lower for OO and LO ice creams (34.19 and 27.12%, respectively) compared to CO based ice cream (45.06%). However, an improved melting behavior, which gradually decreased from 88.69% for CO to 66.09% for LO ice cream, was observed.

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Organisations: National Food Institute, Research Group for Nano-Bio Science, Research Group for Bioactives – Analysis and Application, University of Granada
Authors: Marín-Suárez, M. (Ekstern), García Moreno, P. J. (Intern), Padial-Dominguez, M. (Ekstern), Guadix, E. M. (Ekstern)
Fish protein hydrolysates (FPH), produced from the six main discard species from the West Mediterranean Sea (sardine, horse mackerel, axillary seabream, bogue, small-spotted catshark and blue whiting) were tested for their bile acid binding capacity. This capacity is directly linked to the ability to inhibit bile reabsorption in the ileum and therefore to lower cholesterol levels in the bloodstream. From each species, FPH were obtained by three different enzymatic treatments employing two serine endoproteases (subtilisin and trypsin) sequentially or in combination. The results show statistically significant differences among the fish species, attaining interesting average values of bile acid binding capacity for blue whiting (27.32% relative to cholestyramine on an equal protein basis) and horse mackerel (27.42% relative to cholestyramine on an equal protein basis). The enzymatic treatments did not significantly affect the ability of a given species to bind bile acids. These results are similar to other protein sources, such as soy protein or casein, of proven hypocholesterolemic effect. It can be concluded that fish protein hydrolysates from these discard species are suitable as ingredients in the formulation of cholesterol-lowering supplements.
Biodiesel production from mixtures of waste fish oil, palm oil and waste frying oil: Optimization of fuel properties

The present work studies the influence of waste fish oil, palm oil and waste frying oil as raw material on biodiesel properties. The experimental planning was executed through acid esterification (6:1 methanol to oil ratio, 1 wt.% sulfuric acid, at 60 degrees C, 1 h) followed by transesterification (9:1 methanol to oil ratio, 0.5 wt.% sodium hydroxide, at 60 degrees C for 1 h). Biodiesel samples showed yield higher than 82%, reaching 90% for palm oil (33.3 wt.%) and waste frying oil (66.7 wt.%) biodiesel. FAME content was higher than 92.3% and had a maximum of 98.5% for waste fish oil (333 wt.%) and palm oil (66.7 wt.%) biodiesel. Special cubic models were used to fit experimental data, and were optimized by response surface methodology and multi-objective optimization. Viscosity (4.3 mm(2)/s) and COM (2.5 degrees C) were minimized when pure fish oil was used as raw material, whereas IP maximum (22.0 h) was found for palm oil biodiesel.

Multi-objective optimization evidenced that although the use of the pure oils as feedstock presented more advantages to biodiesel properties, the waste fish oil (42.1 wt.%) and waste frying oil (57.9 wt.%) mix is beneficial, if the aim is IP (20%) and COM (80%) improvement.

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BFI (2014): BFI-level 2
Scopus rating (2014): SJR 1.629 SNIP 2.161 CiteScore 3.96
BFI (2013): BFI-level 2
Scopus rating (2013): SJR 1.636 SNIP 2.142 CiteScore 3.83
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Emulsifying and antioxidant properties of fish protein hydrolysates obtained from discarded species: evaluation on fish oil-in-water emulsions

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Organisations: National Food Institute, Research Group for Bioactives – Analysis and Application, Research Group for Nano-Bio Science, University of Granada
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Multiobjective optimization of a pilot plant to process fish discards and by-products on board
The current application of the zero discards policy in EU fisheries poses economic and logistic problems to fishing vessels, which are obliged to retain and preserve unwanted catches on board. The installation of compaction devices on board can effectively reduce the space requirements and the refrigeration loads to store these materials on board. In the current work, the performance of a pilot hydraulic press was optimized to attain a maximal volume reduction (i.e., maximal yield of press liquor) while keeping a reduced pollution load of the liquid effluents. To this end, a designed experiment was conducted where the yield (Y), the content of suspended solids (SS), and the chemical oxygen demand (COD) of the press liquor were related to the main operation parameters of the pilot plant (i.e., number of pressing steps and time of relaxation between consecutive steps). Statistical modeling, coupled to a multiobjective optimization technique (i.e., the weighted-sum method), was employed to find a set of optimal solutions meeting three objectives: maximal Y (i.e., maximal volume reduction of the press cake), and minimal SS and COD of the press waters. This approach concluded that 5 pressing steps and a time of relaxation between 105 and 120 s ensured a low content of SS (0.0170–0.0185 kg solids/kg sardine), while the yield was high (0.1942–0.2001 kg liquid/kg sardine) and COD remained below 24.4 g O2/kg sardine.

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Authors: Pérez-Gálvez, R. (Ekstern), García Moreno, P. J. (Intern), Huong, N. T. (Ekstern), Guadix, E. M. (Ekstern), Guadix, A. (Ekstern), Bergé, J. (Ekstern)
Number of pages: 14
Publication date: 2015
Optimization of α-tocopherol and ascorbyl palmitate addition for the stabilization of sardine oil.

The purpose of the present work was to optimize the addition of natural antioxidants (α-tocopherol and ascorbyl palmitate) for the stabilization of sardine oil rich in omega-3 PUFA. The optimal values for peroxide value (PV), which minimizes primary oxidation products, were obtained at low concentrations of α-tocopherol (50-207 ppm), high content of ascorbyl palmitate (450 ppm) and 50 ppm citric acid. On the other hand, optimal values for p-anisidine value (AV), which minimizes secondary oxidation products, were found at medium concentrations of α-tocopherol (478-493 ppm), high contents of ascorbyl palmitate (390-450 ppm) and 50 ppm citric acid. The conflicting effect of α-tocopherol on the individual optimization of PV and AV motivated the generation of a Pareto front (set of non inferior solutions) employing the weighted-sum multi-objective optimization technique.

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Authors: Morales-Medina, R. (Ekstern), García Moreno, P. J. (Intern), Munio, M. M. (Ekstern), Guadix, A. (Ekstern), Guadix, E. M. (Ekstern)
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Oxidative stability of electrospun nanofibers loaded with fish oil

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Scopus rating (2009): SJR 0.437 SNIP 0.785
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Production and identification of angiotensin I-converting enzyme (ACE) inhibitory peptides from Mediterranean fish discards

The production of peptides exhibiting Angiotensin I-converting enzyme (ACE)-inhibitory activity from discarded Mediterranean fish species such as sardine, horse mackerel, axillary seabream, bogue and small-spotted catshark was studied. The evolution of the ACE-inhibitory activity with the degree of hydrolysis (DH) of protein hydrolysates was also investigated. Hydrolysates of horse mackerel and small-spotted catshark, both obtained with the simultaneous addition of subtilisin and trypsin, showed the highest antihypertensive activity (IC50, of 279 and 302 μg/mL, respectively). For horse mackerel hydrolysate, fraction B (130-2350 Da) exhibited the highest ACE-inhibitory activity (IC50 = 85 μg/mL). In the case of small-spotted catshark hydrolysate, fraction D (
Production of omega-3 nanodelivery systems by emulsion electrospinning

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Organisations: National Food Institute, Research Group for Bioactives – Analysis and Application, Research Group for Nano-Bio Science
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Seasonal variations in the regiodistribution of oil extracted from small-spotted catshark and bogue
The aim of this work was to seasonally characterize the nutritional quality of oil extracted from small-spotted catshark (Scyliorhinus canicula) and bogue (Boops boops). The proximate composition, lipid profile and regiodistribution of the fatty acid in the glycerol backbone were analyzed. In addition, three nutritional indexes were calculated (atherogenicity and thrombogenicity indexes and the hypocholesterolaemic/hypercholesterolaemic ratio). Both species presented PUFA as the predominant fraction, the most abundant being DHA. Healthy values of the aforementioned indexes were maintained throughout the year. Moreover, the relative composition of omega 3 fatty acids at the sn-2 position ranged from 47.3 to 66.8 mol%, attracting interest in the employment of these oils as the raw source for the production of 2-monacylglycerols. Regarding the individual behavior of each fatty acid, DHA presented a high tendency to occupy the sn-2 bond, whereas EPA presented the opposite behavior.

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Number of pages: 7
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Publication date: 2015
Antioxidant activity of protein hydrolysates obtained from discarded Mediterranean fish species

In this study, five discarded species in the Mediterranean Sea, namely sardine, horse mackerel, axillary seabream, bogue and small-spotted catshark, were evaluated as raw material for obtaining fish protein hydrolysates exhibiting antioxidant activity. The DH of the hydrolysates ranged from 13.2 to 21.0%, with a protein content varying from 60.7 to 89.5%. The peptide profile of all hydrolysates was very similar, except for the hydrolysate of small-spotted catshark. Their lipid content was found to be between 4.6 and 25.3%. The highest DPPH scavenging activity was found for the hydrolysates of sardine and horse mackerel with EC50 values varying from 0.91 to 1.78 mg protein/mL. Sardine and small-spotted catshark hydrolysates exhibited the highest ferrous chelating activity with an EC50 value of 0.32 mg protein/mL. Moreover, sardine and bogue hydrolysates presented the highest reducing power. Finally, a total of six antioxidant peptides were theoretically identified within the structure of myosin and actin proteins from sardine and small-spotted catshark. The potential antioxidant activity exhibited by the hydrolysates suggests that it is feasible to obtain added-value products such as natural antioxidants from these discarded species. All rights reserved, Elsevier.

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Authors: García Moreno, P. J. (Intern), Batista, I. (Ekstern), Pires, C. (Ekstern), Bandarra, N. M. (Ekstern), Espejo-Carpio, F. J. (Ekstern), Guadix, A. (Ekstern), Guadix, E. M. (Ekstern)
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Publication date: 2014
Main Research Area: Technical/natural sciences
Influence of Casein-Phospholipid Combinations as Emulsifier on the Physical and Oxidative Stability of Fish Oil-in-Water Emulsions

The objective of this study was to investigate the influence of casein (0.3% w/w) and phospholipid (0.5% w/w) emulsifier combinations on the physical and oxidative stability of 10% fish oil-in-water emulsions at pH 7. For that purpose, three phospholipids were evaluated, namely, lecithin (LC), phosphatidylcholine (PC), and phosphatidylethanolamine (PE). The emulsion stabilized with LC showed the best physical stability having the most negative zeta potential and the lowest mean droplet size. In addition, this emulsion was also the least oxidized in terms of peroxide value and concentration of the volatile oxidation product 1-penten-3-ol. This finding is not explained by the antioxidant activity of LC because it showed similar DPPH scavenging activity and lower metal chelating activity than the other phospholipids. Therefore, these results suggested that other factors such as the combination of casein and lecithin, which could result in a favorable structure and thickness of the interfacial layer, prevented lipid oxidation in this emulsion.
Optimisation of oil extraction from sardine (Sardina pilchardus) by hydraulic pressing

The aim of this study was to evaluate the influence of the processing conditions (pretreatment temperature: 5-55 degrees C, pressure: 60-120 bar and number of pressing stages: 1-3) on the yield and quality (free fatty acids, peroxide value, p-anisidine and Rancimat induction period) of the oil extracted from whole sardine by hydraulic pressing. Experimental factors were investigated by a designed experiment and optimised by response surface methodology. A maximum yield of oil, 12.47%, was obtained at 55 degrees C, 60 bar and two pressing stages. Regarding oil quality, it was found minimum values for acidity (0.25% oleic at 55 degrees C, 60 bar and one pressing stage) and for peroxide value (0.29 meq kg(-1) oil at 5 degrees C, 60 bar and one pressing stage). Hence, the opposite effect of pretreatment temperature and number of pressing stages on the yield of oil and on its oxidation parameters suggested applying the weighted-sum method as multiobjective optimisation technique.
Optimization of biodiesel production from waste fish oil

The present study deals with the production of biodiesel using waste fish oil. The research assesses the effect of the transesterification parameters on the biodiesel yield and its properties, including temperature (40–60 °C), molar ratio methanol to oil (3:1–9:1) and reaction time (30–90 min). The experimental results were fitted to complete quadratic models and optimized by response surface methodology. All the biodiesel samples presented a FAME content higher than 93 wt.% with a maximum, 95.39 wt.%, at 60 °C, 9:1 of methanol to oil ratio and 90 min. On the other hand, a maximum biodiesel yield was found at the same methanol to oil ratio and reaction time conditions but at lower temperature, 40 °C, which reduced the saponification of triglycerides by the alkaline catalyst employed. Adequate values of kinematic viscosity (measured at 30 °C) were obtained, with a minimum of 6.30 mm²/s obtained at 60 °C, 5.15:1 of methanol to oil ratio and 55.52 min. However, the oxidative stability of the biodiesels produced must be further improved by adding antioxidants because low values of IP, below 2.22 h, were obtained. Finally, satisfactory values of completion of melt onset temperature, ranging from 3.31 °C to 3.83 °C, were measured.

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Discarded species in the west Mediterranean sea as sources of omega-3 PUFA

Five discarded fish species in the Alboran Sea, namely axillary seabream (Pagellus acarne), small-spotted catshark (Scyliorhinus canicula), sardine (Sardina pilchardus), horse mackerel (Trachurus mediterraneus), and bogue (Boops boops) were evaluated as novel sources for the production of omega-3 polyunsaturated fatty acids (PUFAs). The lipid content of the five species varied significantly within the different seasons, being maximum in spring for axillary seabream, small-spotted catshark, and bogue (5.1, 2.7, 2.5%, respectively) and in summer for sardine and horse mackerel (13.6 and 6.2%, respectively). Sardine and horse mackerel presented also the maximum amount of eicosapentaenoic acid (EPA)+docosohexaenoic acid (DHA), 3000 and 1300mg/100g fish, respectively. Their oils exhibited a composition of EPA+DHA higher than 23% and they were mainly composed by triacylglycerols. Axillary seabream, small-spotted
catshark, and bogue presented a lower amount of EPA+DHA, 960, 650, and 157mg/100g fish, respectively, but their oils also exhibited a composition of EPA+DHA higher than 20%. Particularly important was the composition of DHA, 23%, of the oil extracted from small-spotted catshark. Therefore, the five discarded species studied were found to be valuable raw material for the production of fish oil presenting a high content in EPA and DHA. Practical applications: This work is in line with ongoing EU regulations avoiding fish discards. This has boosted research on the potential of these raw materials for the production of high added-value products such as omega-3 PUFAs, which are experiencing a growing commercial demand. This study provides a complete characterization of five discarded species in the Alboran Sea, with special focus on the availability of their omega-3 content throughout the year. Despite their different lipid content, all the species were good sources for the production of omega-3. The oils extracted from these species presented a maximum composition of EPA+DHA higher than 20%. Interestingly, small-spotted catshark's oil was relatively rich in DHA (up to 23%), which makes this oil an excellent functional ingredient for brain and children's health applications. The results obtained provide valuable information for food scientists interested in the production of omega-3 PUFAs from traditional and alternative fish species.
Influence of the parameters of the Rancimat test on the determination of the oxidative stability index of cod liver oil

The operational parameters of the Rancimat method (airflow rate, sample weight and temperature) were studied to determine their effects on the oxidative stability index (OSI) of cod liver oil. To this end, experimental data were firstly fitted to a complete quadratic model and an ANOVA analysis was performed, which concluded that airflow rate and temperature were significant (p < 0.05). By means of this model and using response surface methodology in order to minimize the OSI, the optimal conditions for the three parameters of the Rancimat method were found to be Q = 25 L/h, M = 6.91 g and T = 88.26 degrees C. The different trend obtained for the OSI at increasing temperatures supports that the oxidation mechanism of fish oil at the conditions studied may differ from the lipoperoxidation mechanism at room temperature. Secondly, a simplified linear model was assayed, obtaining also that the influences of airflow rate and temperature were significant (p < 0.05). Moreover, the temperature contribution resulted to have the most important effect on the OSI, obtaining a temperature coefficient of -3.29 x 10(-2). (C) 2012 Elsevier Ltd. All rights reserved.

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Lipid characterization and properties of protein hydrolysates obtained from discarded Mediterranean fish species

Background. Discards are an important fraction of the by-products produced by the fishing industry. As a consequence of their low commercial acceptance, it is necessary to provide added value to these underutilized materials. In this study the lipid fraction of three discarded fish species in the western Mediterranean Sea, namely sardine (Sardina pilchardus), mackerel (Scomber colias) and horse mackerel (Trachurus trachurus), was characterized and the angiotensin I-converting enzyme (ACE)-inhibitory and antioxidative activities of their protein hydrolysates were evaluated. Results. Processing of these biomaterials led to oils with a high content of omega-3 polyunsaturated fatty acids (PUFAs), ranging from 220.5 g kg-1 for horse mackerel to 306.0 g kg-1 for sardine. Regarding the protein fraction, most of the hydrolysates presented ACE inhibition values higher than 60%, corresponding to IC50 values varying from 345 µg protein mL-1 for mackerel to 400 µg protein mL-1 for sardine. Moreover, most of the hydrolysates exhibited acceptable antioxidative activity, namely 35-45% inhibition of 1,1-diphenyl-2-picrylhydrazyl (DPPH). Conclusion. This study suggests that the three discarded species evaluated are valuable raw materials for the production of bioactive ingredients such as omega-3 PUFAs and protein hydrolysates exhibiting antihypertensive and antioxidative activities. © 2013 Society of Chemical Industry.

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Optimization of bleaching conditions for sardine oil

This work studies the influence of the operational conditions (temperature: 90-130 degrees C, activated clay amount: 1-5 wt% and contact time: 20-60 min) on the bleaching process of degummed and neutralized sardine oil. The bleached oils were evaluated for free fatty acids, peroxide value, p-anisidine value, totox, oxidation stability (Rancimat) and color (CIELAB coordinates). Such measured variables were statistically modeled by full factorial experimental design and response surface methodology. An individual optimum value for totox of 21.38 (which minimizes oxidation products content) was obtained at 130 degrees C, 5 wt% of clay amount and 60 min. Regarding the color quality, it was found a maximum value for hue-angle (89.19 at 99.2 degrees C, 5 wt% of clay and 56.6 min) and a minimum value for chroma (81.76 at 109.4 degrees C, 5 wt% of clay and 49.4 mm). The conflicting effect of temperature and time on the location of the individual optima motivated the generation of a Pareto front (set of non inferior solutions) employing the weighted-sum multi-objective optimization technique. (C) 2013 Elsevier Ltd. All rights reserved.

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Authors: García Moreno, P. J. (Intern), Guadix, A. (Ekstern), Gomez-Robledo, L. (Ekstern), Melgosa, M. (Ekstern), Guadix, E. M. (Ekstern)
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Recent patents on the upgrading of fish by-products

The fish and aquaculture activities produce an important volume of by-products due to discards and processing on board and in land. These residues (fillet cuts, bones, heads, viscera and tails) require to be processed in order to not create a significant environmental problem and to maintain the economical viability of the fisheries sector. The present paper reviews a list of recent patents on fish by-products applications, specifically on the products with the highest added value, such as fish protein hydrolysates and fish oil. Initially, an assessment is presented of the processes and techniques employed to obtain fish protein hydrolysates, which are classified by their utilization: ingredients for food, products for animal feeding, bioactive compounds, fertilizers, cosmetics and nutrient media for bacterial growth in specific industrial processes. Secondly, the different methods to extract the oil from the fish, to refine the fish oil avoiding undesirable taste and odour and to improve the stability of the mentioned oil, are evaluated. Finally, procedures for the microencapsulation of the oil obtained and its incorporation into food compositions have been surveyed. © 2010 Bentham Science Publishers Ltd.

General information
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Projects:

**Protein valorization through informatics, hydrolysis, and separation**

WP leader on the industrialization part of proteins from seaweed

National Food Institute
Research Group for Bioactives – Analysis and Application
Research Group for Gut Microbiology and Immunology

Period: 01/09/2017 → 31/08/2021
Number of participants: 4
Acronym: PROVIDE
Project participant:
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Jacobsen, Charlotte (Intern)
Hansen, Egon Bech (Intern)
García Moreno, Pedro Jesús (Intern)

**Experimental project in physics and nanotechnology: Cryo SEM Characterization of Food NMS Containing PUFA**

Center for Electron Nanoscopy

DTU Danchip
National Food Institute
Research Group for Bioactives – Analysis and Application

Research Group for Nano-Bio Science
Period: 09/09/2016 → 01/12/2016
Number of participants: 5
Acronym: 33525
Project participant:
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Supervisor:
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Chronakis, Ioannis S. (Intern)

Main Supervisor:
Development of omega-3 nanodelivery systems using electrospinning processing

Functional foods containing omega-3 lipids, which have approved health claims by EFSA, have resulted in one of the fastest-growing food product categories in Europe. However, to successfully develop foods enriched with omega-3 PUFA, lipid oxidation of these highly unsaturated fatty acids must be prevented in order to avoid both the loss of nutritional value and the formation of unpleasant off-flavors. Omega-3 PUFA can be added to foods as neat oils or as a “delivery system” such as microencapsulated oil powders and oil-in-water emulsions. Nevertheless, delivery of omega-3 lipids in the form of emulsions reduces the oxidative stability of omega-3 PUFA in some products. Furthermore, microencapsulates are less suitable for liquid or semi-liquid foods than emulsified omega-3 oils due to handling/mixing issues. Therefore, the development of alternative omega-3 PUFA delivery systems, which are easy to disperse and which will lead to improved oxidative stability of omega-3 enriched food products, is urgently required. One of the more promising delivery systems can be functional nanomicrostructures obtained by electrospinning technology, which is possible to up-scale. In light of the above, the aim of this research project is to develop advanced omega-3 delivery systems such as electrospun nano-microstructures. To this end, the specific objectives are:

1) Development of physically and oxidatively stable nano-microstructures with omega-3 PUFA and natural antioxidants using electrospinning processing.
2) Production of food enriched with the nano-microstructures having appropriate structural-functional properties and being oxidatively stable.

The success of the research proposed will lead to an important advance in the protection of omega-3 PUFA against oxidation when incorporated into food. Thus, the knowledge generated by this study has the potential to being exploited by companies devoted to the production of functional
foods containing omega-3 lipids.

National Food Institute
Research Group for Bioactives – Analysis and Application
Research Group for Nano-Bio Science
Division of Industrial Food Research
Period: 24/08/2015 → 24/08/2017
Number of participants: 3
Acronym: ELECTRONANOMEGA
Project participant:
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Supervisor:
Chronakis, Ioannis S. (Intern)
Main Supervisor:
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Relations
Related projects:
Experimental project in physics and nanotechnology: Cryo SEM Characterization of Food NMS Containing PUFA
Biological Sample Preparation for Electron Microscopy
Publications:
Development of carbohydrate-based nano-microstructures loaded with fish oil by using electrohydrodynamic processing
Oxidative stability of pullulan nanofibers loaded with fish oil: effect of oil content and antioxidants addition
Protein-polysaccharide Mixtures as Wall Material in Fish Oil-loaded Nano-microcapsules Obtained by Electrospraying
Oxidative stability of pullulan electrospun fibers containing fish oil
Oxidative stability of electrospun nanofibers loaded with fish oil
Production of omega-3 nanodelivery systems by emulsion electrospinning
Encapsulation of fish oil in nanofibers by emulsion electrospinning: Physical characterization and oxidative stability
Oxidative Stability of Nano-Microstructures containing fish oil
Project