

DEPARTMENT OF BIOLOGICAL AND ENVIRONMENTAL SCIENCES

## DEVELOPMENT OF SUSTAINABLE "BLUE-GREEN" PRODUCTS AND HERRING AS FOOD IN PUBLIC AND SCHOOL-MEALS



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## Abstract

Herring (Clupea harengus) is a pelagic fish that is nutritious and has a low climate impact. It is however poorly utilized for food in Sweden, only fillets are used while the co-products are exported to Denmark for fishmeal production. Herring mince can be obtained from these co-products, like the backbones, but due to the high levels of hemoglobin (Hb) of the herring muscle, it is vulnerable to lipid oxidation. In order to prevent the oxidation processes which causes rancidity, antioxidants can be introduced. To meet the demand from industry for "clean labels", it is important to find natural sources of antioxidants. Also, local antioxidant sources are a way to minimize the food loop and promising sources are e.g. lingonberries and seaweed. The aim of this study was to see if the direct addition of the antioxidant material helped in preventing lipid oxidation of the herring mince during ice storage. Five antioxidative materials: lingonberry press cake, Ulva, Saccharina, blanched Saccharina, and salted Saccharina were added to the herring backbone mince in three ways; wet (untreated), dried and smoked (wet). A taste screening was done of all combinations when introduced at levels between 5-20% (w/w). In this taste test trial, 10% of antioxidant material in the herring mince was the most preferred, which was then used to perform ice storage trials. The samples were kept in darkness on ice, in order to monitor the level of lipid oxidation over time using both a chemical method (thiobarbituric acid-reactive substances, TBARS) and a sensory screening of odor. All treatments of lingonberry press-cake, blanched smoked Saccharina and smoked salted Saccharina showed promising results of inhibiting the levels of lipid oxidation in herring mince. Altogether, the project paved the way for a new type of herring product on the Swedish market, as well as for school lunches.

Keywords: herring, lipid oxidation, seaweed, lingonberry, sustainable



## Introduction

In 2010, the world population was 6.9 billion and it is estimated to hit 9.7 billion by 2050 (UN, 2022). Global agricultural production is projected to grow 1.1% yearly between 2005 to 2050, making it 60% higher in 2050 as compared to 2005, and an increased crop projection also means more arable land is required to grow said crops, but land is limited (Alexandratos & Bruinsma, 2012). At the same time, the seafood industry has also been expanding and developing rapidly over the last several decades to meet the demands of a proliferating world population. Between 1961 to 2017, global fish consumption has increased at an average yearly rate of 3.1%, thus, gaining more growth than other animal protein trades such as dairy and meat (FAO, 2020). W hile the growth of capture fisheries has been stagnating, the aquaculture industry is growing to keep up with the demand (see Fig. 1).

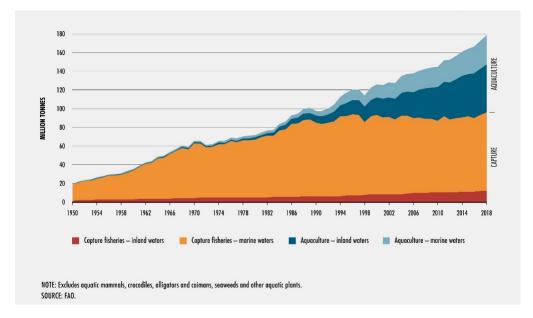


Fig. 1 World capture fisheries and aquaculture production (FAO, 2020)

Due to the rising popularity of seafood products, plenty of fish stocks are overexploited today, and the aquaculture industry has been deemed necessary to replace capture harvesting to meet fish consumption rates (FAO, 2020). According to the Food and Agriculture Organization of the United Nations (FAO) report in 2020, aquaculture production reached an all-time high in 2018, at 82.1 million tonnes in live weight and is projected to keep growing to 109 million tonnes by 2030 (FAO, 2020).

Seafood products are one of the top commodities that are traded globally and the seafood sector provides the world with 7% of all proteins, 17% of total animal protein (FAO, 2020; Sustainable-Fisheries, 2022). Although the seafood industry is growing at a fast pace and contributing to the growing need of protein sources for human consumption, there is a need to look at the bigger picture than just churning food out to supply the global market. This issue is indicated by the United Nation's (UN) Sustainable Development Goal (SDG) 12 – "ensure sustainable consumption and production patterns," with one of the main targets being the reduction of global food waste along its entire life cycle process, from post-harvesting, production and supply to retail and consumers by 2030 (United-Nations, n.d.). Therefore, instead of focusing on creating only large-scale (aquaculture) farms all over the world, the existing production houses should also be looked at for possible improvements and maximising

the potential of fish past their fillets, as well as other local marine bioresources. By finding innovative solutions to make existing co-products suitable for human consumption rather than redirecting it for other non-food processes or waste will bring us one step closer to fulfilling the targets of SDG 12 of scaling down food waste. Thus, a complementary route to aquaculture to meet the increasing seafood demand is to use more of the captured wild fish directly for food. By using parts also beyond the fillet, more food can be produced per unit of caught fish.

Another cause of concern in the public eye over the recent years is the concept of sustainability. When it comes to the term 'sustainability' it is often applied on a singular aspect such as the impact on the environment, and it is short-sighted. The bigger picture should be taken into consideration due to the finite resources that the Earth has – infinite growth is not possible, but rather a circular economy that promotes regenerative growth, as displayed in **Fig. 2** below (Fassio & Tecco, 2019).

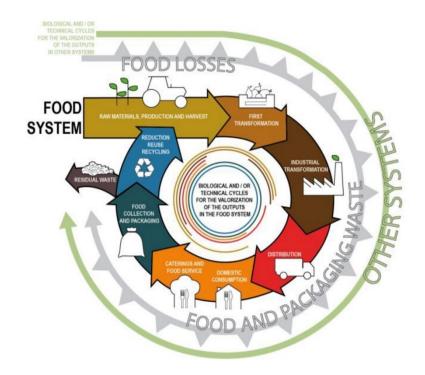


Fig. 2 Diagram of a possible circular food economy from small local loops to larger-scale loop processes (Fassio & Tecco, 2019)

By minimizing food loops, it starts a cascade effect whereby waste is reduced, existing potential of food resources are maximised and the impact on climate is lowered. This is also in line with the UN's SDG 12. Therefore, it is important to educate concepts of sustainability such as:

- (i) **reducing wastes**; by means of better transport infrastructure, teaching the importance of food and preventing waste
- (ii) **changing diets**; reducing the demand for meat proteins while encouraging intake of vegetable and marine proteins
- (iii) **increasing food production limits**; utilizing as much of a food product as possible (Godfray, 2010).

Implementing and educating on these basic principles could mean that food is not just being produced to sustain humans, but even the process of food production can be made sustainable.

To further dive into food production in the fish industry, usually after the fish have been filleted, the remaining co-products are processed into fishmeal or oil production for animal feed, and worse cases even to biogas manufacturing (Abdollahi, Olofsson, Zhang, Alminger, & Undeland, 2020; Abdollahi, Wu, & Undeland, 2021). There is an estimated global postharvest loss of 35% in the seafood value chain, which can be translated to approximately 45 million tonnes of seafood co-products. Contributing reasons are inefficient handling or transportation, whereby co-products that are still fit for consumption are instead re-directed to other processes such as animal feed, or biogas production (FAO, 2020; Sajib, 2021; Surasani, 2018).

In Sweden, the locally fished herring (*Clupea harengus*) is dominating the fisheries, but is undergoing major losses from the food chain. Firstly, 60% of the herring goes directly to feed production in Denmark, and second, out of the amount which is dedicated food, the filleting co-products such as backbones and heads are also exported to Denmark for fish meal/oil production for the fodder industry (Sajib, 2021). The loss of the herring biomass via co-product formation is around 60% (Abdollahi et al., 2020; Abdollahi et al., 2021). Every year, around 20,000 tonnes of herring are processed to food in Sweden which thus generates 12,000 tonnes of co-products (Sajib, 2021). These co-products are however high in macro- and micronutrients which spells a revenue loss for the Swedish economy as these co-products have the potential to become new products that could be produced, distributed, sold and consumed locally.

Herring is a highly nutritious fish species, partly due to the abundance of long-chain n-3 polyunsaturated fatty acids (LC n-3 PUFAs), which have been found to decrease risks of cardiovascular diseases(CVD) (Lindqvist, Langkilde, Undeland, Radendal, & Sandberg, 2007). These fatty acids however are also prone to oxidation due to their multiple double bonds (Dellarosa, Laghi, Martinsdóttir, Jónsdóttir, & Sveinsdóttir, 2015; Undeland, 1995). Lipid oxidation is a complicated process with multiple reactions but in essence, the primary lipid oxidation reaction occurs when free radicals react with the fatty acids to form lipid radicals, thereafter converting to hydroperoxides after addition of oxygen. Upon decomposition of the hydroperoxides, secondary lipid oxidation products such as malondialdehyde (MDA) are formed (Papastergiadis, Mubiru, Van Langenhove, & De Meulenaer, 2012). During the secondary oxidation process reduces the product's nutritional value. Therefore, in order to utilize the most of the herring, the process of lipid oxidation has to be combated and the best method to do so is to introduce antioxidants (Surasani, 2018; Wu, Ghirmai, & Undeland, 2020).

Antioxidants are able to stabilize free radicals, which mean that they are able to restrict lipid oxidation and thereby help extend the freshness of the product (Minatel et al., 2017; Pereira, Valentão, Pereira, & Andrade, 2009). Synthetic antioxidants such as butylated hydroxytolulene (BHT) and ethylene-diaminetetraacetic acid (EDTA) are traditionally used to prevent lipid oxidation in food but were subsequently found to have negative health risks and toxicity effects when consumed above 0.125 mg/kg (Honold, Jacobsen, Jónsdóttir, Kristinsson, & Hermund, 2015; Sun et al., 2021; Wang et al., 2010; Wang, Li, Yuan, Lin, & Pavase, 2017). Thus, the interest in using so-called "clean" ingredients such as natural antioxidants started to grow and the concept "clean" label" generally means that a food product contains natural antioxidants as additives instead of the synthetic versions (Hu & Jacobsen, 2016; Wang et al., 2010).

In Sweden, raw materials such as lingonberry and seaweed contain compounds with antioxidative properties such as polyphenols that capture free radicals caused by oxidation processes. Swedish lingonberries are industrially juiced, and the remaining press-cakes are still rich in nutrients, but are commonly discarded due to short shelf life and the lack of demand (Abdollahi et al., 2020). Seaweed have often been touted to be a future food, as they provide a

low climate impact, and have beneficial nutritive properties. Species such as *Saccharina latissima* and *Ulva fenestrata* can be found along the West Coast of Sweden, both wild and cultivated (Olsson, Toth, & Albers, 2020), which makes them ideal candidates to be studied as antioxidative ingredients.

## Aims and Objectives

The aim of this project was to create a new sustainable product from herring backbone mince that is stabilized towards lipid oxidation by addition of natural 'blue-green' antioxidants. A secondary aim was to introduce this concept to the public meal sector and local schools. The antioxidative materials selected were lingonberry press-cake, sea lettuce (*Ulva fenestrata*) and sugar kelp (*Saccharina latissimi*) with or without subjection to drying, salting or smoking. The hypotheses were that additions of these raw materials at the right level would increase the oxidative stability and shelf life of the product.

#### Specific objectives were:

- 1. To develop the flavours of the most successful combinations of herring mince and antioxidant raw materials using sensory screening and test-cooking
- 2. To find out if addition of the selected antioxidant-containing raw materials helped in preventing lipid oxidation of the herring mince during ice storage as measured both by chemical analysis (TBA-reactive substances, TBARS) and sensory screening of rancid odour.
- 3. Investigate product guidelines and food requirements for introducing new products to the general public and school, with focus on a new herring product

## Background

#### Herring (Clupea harengus)

Herring (*Clupea harengus*) is a type of pelagic fish that can be found in the Baltic Sea, Northeastern and North-western Atlantic Oceans (Whitehead, 1985), as well as the North Sea in Skagerrak and Kattegat (ICES, 2016). In 2019, 41 000 tonnes of herring was landed in Sweden, making up 68% of the total Swedish fish catch (EUMOFA, 2019). It is a highly migratory species, traveling between spawning seasons, feeding and wintering (Clausen, Bekkevold, Hatfield, & Mosegaard, 2007; ICES, 2016; Whitehead, 1985). According to the International Council for the Exploration of the Sea (ICES), stock health for herring in the North Sea and Baltic Sea is relatively good, followed by reports of satisfactory management practices of the fisheries in these areas, which ensures the viability of future herring stock (ICES, 2016; Sustainable Fisheries Partnership). The impact of herring fishing is also fairly minimal, in terms of the by-catch and discard rate in the North Sea (ICES, 2016; Lindqvist et al., 2007).

Herring is classified as a fatty fish, containing high levels of LC n-3 PUFAs which has been established to provide a range of beneficial health effects for humans, especially in relation to cardiovascular diseases (CVDs) (Lindqvist et al., 2007; Undeland, 1995). Additionally, a study done on both the nutritional composition and climate impact of seafood in Europe reported that in Sweden, pelagic fish such as herring, sprat and mackerel are higher in nutrient density in comparison to other protein sources such as meat, and have minimal impact on the environment. Hallström et al. (2019) analysed several nutrient density scores and selected the method which considered specific parameters per 100 g of material, such as the inclusion or

exclusion of nutrients, weighting of the nutrients in relation to abundance or deficiency between recommended levels, in order to draw the score of nutrient density. Hence, the authors of the paper encouraged the consumption of these fish (herring, sprat, and mackerel) in Sweden (Hallström et al., 2019).

However, due to the small size and bony nature of herring, they have only been prized for their fillets as factory filleting machines have made the fish to be of some value in Sweden (Damerau et al., 2020). The remains of the herring after the filleting process are exported to Denmark to be processed into fish meal and oil to be used in animal feed (Sajib, 2021). Since the herring is filleted by a machine, a substantial amount of the nutrient-rich muscle still remains on the herring backbones (Abdollahi et al., 2021; Wu, Abdollahi, & Undeland, 2021). As such, there is a possibility to utilise this muscle with another machine known as a meat-bone separator (**Fig. 3**). It is a simple mechanical process and cost-efficient way of removing the remaining flesh from the herring backbone. In a recent study, the yield of mince from herring backbones was >80% (Abdollahi et al., 2021). Disadvantages can be that small bones, or other particles like skin may also pass through the machine with the rest of the herring meat mince (Nolsøe & Undeland, 2009).



Fig. 3 Baader meat-bone separator machine (Photo by: Baader / https://baadering.baader.com/products/baader-600)

#### Herring Lipids

The lipid composition in fish in general can vary and it is dependent upon factors such as species, sex, feeding habits, harvested season, age and geographical location, the latter as temperatures and water salinity may also influence the lipid contents (Nielsen, Hyldig, Nielsen, & Nielsen, 2005; Undeland, 1995). When it comes to herring, the Baltic herring has a lower fat content as compared to the North sea herring, partly due to the environmental differences such as different feed (Undeland, 1995). Another study found different levels of fats in male and female herring at different stages of development where immature females had higher

levels of lipids than immature males, and in the case of the males, the lipid content was directly correlated to the gonad growth during maturation (Nielsen et al., 2005). Herring, just as mackerel and sprat show very large seasonal variations in lipid content; spring spawners are lean in the spring and fatty in the fall; fall spawners vice versa. That white fish as cod is so much leaner than e.g. herring is since they store their fats mainly in liver or intestines, while herring store their lipids in the entire body (Sajib, 2021). It has been reported that out of the total fatty acids in herring,  $\sim 43.1\%$  (in the fall) are PUFAs, mainly eicosapentaenoic acid (EPA) and docosahexaenoic acid (DHA) (Wu, Forghani, Abdollahi, & Undeland, 2022), which are nutritionally beneficial for humans (Hu & Jacobsen, 2016; Jensen, Jacobsen, & Nielsen, 2007). According to Jensen et al. (2007), herrings are lipid dense and by consuming 100 grams of North Sea herring harvested during any season would give an intake of more than 0.5g of DHA and EPA, which is the daily recommended amount.

#### Lipid Oxidation

Lipid oxidation is a complex reaction whereby PUFAs break down and causes food to become rancid i.e., lose its sensory properties, nutritional quality and shelf-life (Hu & Jacobsen, 2016; Surasani, 2018; Wetterskog & Undeland, 2004). While the high levels of PUFAs in herring are beneficial to humans, they also play an active role in rapid lipid oxidation (Hu & Jacobsen, 2016; Sampels, Asli, Vogt, & Morkore, 2010; Undeland, 1995).

Oxidation is an autocatalytic process which means that once it begins, it is a self-sustained reaction that is triggered by its own products (Undeland, 1995). In **Fig. 4** below, the oxidation pattern of unsaturated lipids is shown in chart form. Initial stages begin slowly due to the low concentration of radicals, but once it begins, it grows exponentially during the induction stages (**Fig. 4** (**A**)). In the initial reaction stages, free radicals are reacting with the bis-allylic hydrogen in PUFAs, which produces alkoxy radicals. Oxygen then quicky adds to these radicals, creating peroxy radicals which reacts with a new intact unsaturated fatty acid to form primary oxidation products: (**Fig. 4** (**B**)) odourless and tasteless hydroperoxides (Papastergiadis et al., 2012; Undeland, 1995).

When these hydroperoxides degrade, they form secondary oxidation aldehyde compounds like hexanal and malondialdehyde (MDA) that also produces odours (**Fig. 4 (C & D)**). MDA is commonly used to determine levels of oxidation in food by a reaction with thiobarbituric acid (TBA) in the 2-thiobarbituric acid-reactive substances (TBARS) analysis, which produces redviolet colours that are measured with a spectrophotometer (Papastergiadis et al., 2012; Schmedes & Hølmer, 1989). Most importantly, these different steps of the oxidation reaction do not occur sequentially but in parallel, which is also what contributes to the complexity of oxidation processes (Undeland, 1995).

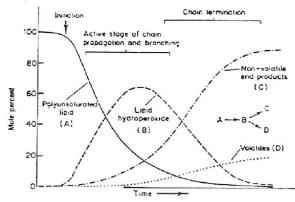


Fig. 4 Simultaneous oxidation process of unsaturated lipids (Gardner, 1983)

Fish also consists of pro-oxidants that controls the onset of lipid oxidation after death and their action can be triggered by a wide range of things from storage temperature, pH, cutting or mincing of the flesh, levels of oxygen and amount of time passed since death (Baron & Andersen, 2002; Undeland, 1995).

The main contributors to lipid oxidation in small pelagic fish as herring is haemoglobin from red blood cells and myoglobin in the muscle, which thus affect both the colour and rancidity of the fish flesh (Chaijan & Undeland, 2015; Sajib, Wu, Fristedt, & Undeland, 2021; Wu et al., 2020). As aforementioned, herring muscle from backbones is abundant in both unsaturated lipids and blood making them highly susceptible to oxidation (Hu & Jacobsen, 2016; Wu et al., 2020).

#### Sensory Qualities as a Function of Lipid Oxidation

The sensory qualities that are affected by the onset of lipid oxidation are the aroma, taste, texture and colour. For any food product, particularly seafood, these are vital factors to keep under control (Kemp, 2009). Studies have recorded fresh fish aromas as green, melon-like and "geranium-leaf-like" but as lipid oxidation progresses, those initial scents are lost and e.g. fishy, painty and metallic odours develop (Hu & Jacobsen, 2016). The 'paintiness' aroma is characterised as the scents found in oil paints or whale-oil and is a result of e.g. hexanal that occur from oxidation of PUFAs in fish and linolenic acid-rich oils (Karahadian & Lindsay, 1989).

Different meats such as chicken, beef or fish that contain haemoglobin have shown significant loss of red colour and converts to a dull brown colour when the heme proteins oxidise to their met-form, affecting the visual appearance of the meat (Sajib et al., 2021; Wetterskog & Undeland, 2004). The extent of red colour losses also occurs in correlation to the level of TBARS, indicating secondary products (carbonyls) of oxidation (Sajib et al., 2021; Wang et al., 2017; Wetterskog & Undeland, 2004).

The loss of fresh aroma coupled with the loss of reddish pink hues on the flesh not only indicate the development of lipid oxidation, it also deters the senses from wanting to consume the product. Overall, studies have shown that introduction of antioxidants is the most successful way of averting lipid oxidation (Surasani, 2018; Wu et al., 2020). Such introduction is facilitated when the raw material is in the form of a mince. Thus, while mincing of the herring *per se* has been described to promote the process of lipid oxidation, it on the other hand allows for the herring to be fully utilized, and allowing the mixing in of antioxidants that helps prevents lipid oxidation (Undeland, 1995).

#### Lingonberries (Vaccinium vitis-idaea)

Lingonberries grow abundantly in the Northern parts of the world and is also a widely recognised food in Sweden (Dróżdż, Šėžienė, Wójcik, & Pyrzyńska, 2017). These bright red berries are rich in anthocyanins; red pigments that are ascribed antioxidative, antiinflammatory and antimicrobial qualities, the former that are also involved in reducing the risk CVDs (Bujor, Ginies, Popa, & Dufour, 2018; Dróżdż et al., 2017; Khoo, Azlan, Tang, & Lim, 2017; Vilkickyte et al., 2019). The anthocyanins, also including pro-anthocyanidins, are so called polyphenols and belong to the group flavonoids, and help to retard the process of lipid oxidation (Abdollahi et al., 2020; Dróżdż et al., 2017; Kylli et al., 2011). Phenolic compounds work as antioxidants by stabilizing free radical activity or chelating metal cations which also prevents free radicals from forming (Minatel et al., 2017). There have been a few studies investigating the use of various berry extracts and juice presscakes to reduce lipid oxidation in herring. To determine the effect that berries may have on herring lipid oxidation, a study was done by marinating herring fillets with berry extracts namely elderberries, cranberries and black currants. The marinated herring was first placed on ice for 7 days and was then stored at -20 °C for 6 months. Results from this experiment concluded that these berry marinades reduced levels of protein and lipid oxidation (Sampels et al., 2010). Another investigation similar to this Master's project was done using Baltic herring mince patties which were mixed with either lingonberry (*Vaccinium vitis-idaea L.*), bilberry (*Vaccinium myrtillus L.*) or sea buckthorn (*Hippophaë rhamnoides L.*) press-cakes and the study showed positive results of the inhibition of lipid oxidation in all the herring and berry mixtures (Damerau et al., 2020). Another paper, studying the effects of lingonberry press-cake, shrimp shells and brown seaweed in minimising lipid oxidation was significantly reduced in the fish protein isolates produced with lingonberry press-cake (Abdollahi et al., 2020).

#### Macroalgae (Seaweed)

Seaweed production in Europe is still in its early stages as compared to Asia and there is great potential to expand the seaweed cultivation industry in Sweden and along certain parts of the West Coast (Hasselström et al., 2020; Hasselstrom, Visch, Grondahl, Nylund, & Pavia, 2018; Olsson, Toth, & Albers, 2020). One such study by Thomas, Ramos, and Gröndahl (2019) focused on geographical sites with good potential for cultivating seaweed along the Swedish West Coast by utilizing geographic information systems (GIS) and multi-criteria analysis (MCA) tools for assessment. They identified 13 aspects of seaweed farming that were crucial to the assessment ranging from the port positions, shipping lanes and traffic, ocean depths, marine protected areas, military and economic zones to the possibility of oil spills. These factors were put through 2 MCA techniques: Boolean and weighted linear combination and both approaches were overlaid to obtain the best suitable areas along the Swedish West Coast to grow sugar kelp, as seen in **Fig. 5** below (Thomas et al., 2019)

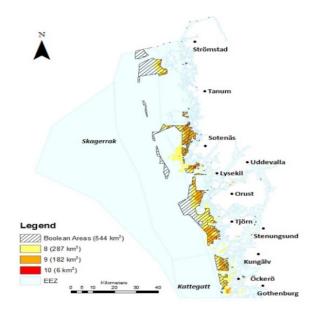


Fig. 5 Potential areas (both line-shaded and coloured portions) for macroalgae cultivation areas along the Swedish West Coast (Thomas et al., 2019)

A separate in-depth study simulating a single seaweed farm and its scalability along Western Sweden was found to be promising in terms of profitability, reversing eutrophication of the ocean and additionally, the future possibility to extract phosphorus as a resource (Hasselström et al., 2020). The multifunctionality of macroalgae to become food, bioenergy, animal feed, industrial stabilizers; ability to remediate the ocean waters and its low climate impact make it a promising biomass to produce and utilize.

#### Sea Lettuce (*Ulva fenestra*)

The Ulva species have characteristics of rapidly producing a large biomass; being able to grow well in density; exhibiting a high tolerance towards external environmental factors; containing antioxidants such as ulvan, a high protein content, minerals, PUFA and essential amino acids and vitamins (Lahaye & Robic, 2007; Olsson, Toth, Oerbekke, et al., 2020; Steinhagen et al., 2021). They are structurally composed of various polysaccharides such as cellulose, xyloglucan, starch, glucuronan and ulvan (Lahaye & Robic, 2007; Olsson, Toth, Oerbekke, et al., 2020). The water-soluble polysaccharide, ulvan, has been of interest as it has a wide range of biological and physio-chemical properties such as being antioxidative, anticoagulative, antitumor, cholesterol-reducing and containing rare sugars such as rhamnose and iduronic acid (Lahaye & Robic, 2007; Olsson, Toth, Oerbekke, et al., 2020; Steinhagen et al., 2022). Rhamnose is a highly valued compound found in Ulva and studies have shown that by adjusting external environmental factors such as having a high temperature and high irradiance could produce Ulva with 26% more rhamnose (Olsson, Toth, Oerbekke, et al., 2020). All these components along with their growing characteristics and the ability to control the ambient aspects of cultivating Ulva to produce high value compounds make it a practical and big profit potential biomass to produce.

On the other hand, the capability of being able to tweak specific cultivation factors of *Ulva* in Europe lay in its land-based tanks, basins, pond-based or shoreline cultivations (Steinhagen et al., 2022; Steinhagen et al., 2021). This diminishes the environmentally sustainable aspect of cultivating seaweed as it then requires lots of energy, additional construction materials, set-up, maintenance and costs for cultivation tanks, and more importantly, land space (Steinhagen et al., 2022). At the same time, ocean cultivation of *Ulva* exposes it to biofouling and there is still a gap in the literature with regards to preventing biofouling in *Ulva* spp. as compared to other kelp types (Bannister, Sievers, Bush, & Bloecher, 2019; Steinhagen et al., 2022). Biofouling is the build-up of unwanted microorganisms that may cause injury to the seaweed, such as breakage due to the additional weight; slower seaweed growth rate due to scarcity of nutrients and light, as well as damage to the infrastructure of the seaweed farm (Steinhagen et al., 2022). However, there is also potential in land based cultivation and a recent study demonstrated the possibility of reusing food processing waters e.g. from shrimp peeling and herring marination to increase growth rate, as well as crude protein content of *Ulva*, albeit requiring a further look into actual scalability (Stedt et al., 2022)

Nonetheless, macroalgae cultivation is a growing industry and *Ulva* is a species that can be cultivated along the Western Sweden coasts which spans about 400 kilometres long (Hasselstrom et al., 2018; Steinhagen et al., 2021; Thomas et al., 2019). The bountiful harvest and multifunctionality of *Ulva* biomass are optimal for large-scale cultivation in the Swedish waters, and could potentially be a sustainable source of marine proteins and other nutrients. This has been detailed in a new study done by Steinhagen et al. (2021), displaying the first successes of *Ulva* cultivation in an offshore farm in Scandinavia and proving the feasibility and economic viability of large-scale *Ulva* production in the ocean.

#### Sugar Kelp (Saccharina latissima)

Brown seaweed such as *Saccharina latissima*, commonly known as sugar kelp can be found along the West Coasts of Sweden (Olsson, Toth, & Albers, 2020) and have the potential to produce a high yield in a short time (Akomea-Frempong, Perry, & Skonberg, 2021). *Saccharina* is typically cultivated during the fall period and the harvest season begins at either the end of spring, or the early days of summer (Hasselstrom et al., 2018; Lüning & Mortensen, 2015).

There has been a strong interest in Saccharina since it is rich in vitamins, nutrients and minerals such as iodine. However, although iodine is important for the thyroid gland and also exhibit anti-allergenic attributes (Akomea-Frempong et al., 2021; Yesuraj, Deepika, Ravishankar, & Ranga Rao, 2022), levels in Saccharina are so high that only small amounts can be consumed without exceeding the tolerable weekly intake (Blikra, Henjum, & Aakre, 2022). This is why Saccharina is commonly blanched before use. Furthermore, Saccharina contains phenolic compounds such as phlorotannins, which has the potential to increase oxidative stability in fish mince, as well as other foods (Abdollahi et al., 2020; Wang et al., 2017) due to their strong antioxidative properties (Susanto, Fahmi, Agustini, Rosyadi, & Wardani, 2017; T. Wang et al., 2010; Wang et al., 2017). An earlier study by Xiaojun, Xiancui, Chengxu, and Xiao (1996) isolated brown algal phlorotannins from Sargassum kjellmanianum to study their antioxidative capabilities in fish oil and found that a high molecular weight phlorotannin fraction was more effective in preventing oxidation in the fish oil as compared to 0.02% BHT. Additionally, research by Wang et al. (2010) showed promising effects of brown algal (Fucus vesiculosus) phlorotannins inhibiting lipid oxidation in cod muscle mince, particularly from phlorotannins that have high polymer weight. This antioxidative properties are likely due to the chemical structure of phlorotannins which has up to 8 phloroglucinol units (compound rings) as compared to mono- or polyphenols with less phenolic rings (Honold et al., 2015; Xiaojun et al., 1996). Phenolic compounds work as antioxidants by scavenging free radicals (Minatel et al., 2017; Pereira et al., 2009). The study concluded that while the phlorotannins had antioxidant capabilities and was indeed effective in preventing lipid oxidation, there were potentially other mechanisms in the cod mince system at play, which required further research. It should also be considered that there is a gap in the literature with regards to the full antioxidative effects of polyphenols such as phlorotannins in complex food systems, as most studies have been focused on the pharmaceutical aspect of antioxidation using simple in-vitro assays (Honold et al., 2015; Wang et al., 2017).

Seaweed has multiple potential health benefits, but at the same time, *Saccharina* also contains potentially toxic elements (PTEs) such as iodine, mercury, lead and arsenic. As stated above, the high iodine content has made sugar kelp a bit difficult to use as a food source as compared to e.g., the Porphyra species, better known as nori, which has 10 times less iodine content (Blikra, Wang, James, & Skipnes, 2021; Lüning & Mortensen, 2015). Also, the green seaweed species are low in iodine. The level of PTEs found in *Saccharina* however varies with external environmental factors such as its geographic location and salinity levels (Blikra et al., 2021; Lüning & Mortensen, 2015).

Regarding iodine - 150  $\mu$ g is the daily recommended intake levels (RDI) for a healthy thyroid and nervous system, though however, an excess of iodine can cause thyroid dysfunction disorders and a lack of it affects brain development in children (Blikra et al., 2021). The maximum daily intake level is set at 600  $\mu$ g in Europe. It has been found that simple treatments such as blanching or boiling of the *Saccharina* can reduce the amounts of arsenic and iodine, which could encourage sugar kelp consumption or utilization (Akomea-Frempong et al., 2021; Lüning & Mortensen, 2015; Nielsen et al., 2020). Lüning and Mortensen (2015) found that the length of time that the *Saccharina* was boiled also had an effect on the iodine levels, as shown in **Fig. 6** below. It can here be seen that just a short boiling time of approximately 40 seconds can reduce the iodine levels drastically, with temperature having a clear effect on reducing the iodine levels.

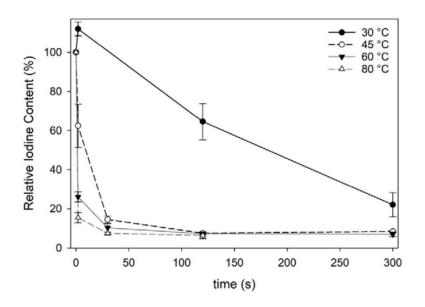


Fig. 6 Iodine content in *Saccharina* over blanching time at different temperatures in seconds (Nielsen et al., 2020)

A paper focusing on how to prolong the long-term storage of *Saccharina* studied the impacts of blanching *Saccharina* at 100°C prior to freezing and found that the high temperature increased the total phenolic content (TPC) of the blanched *Saccharina* as compared to the raw material (Akomea-Frempong et al., 2021). This is interesting as other studies have reported a decrease in TPC upon blanching vegetables and seaweeds (Khoo et al., 2017; Minatel et al., 2017; Susanto et al., 2017).

There are different aspects of blanching that could affect the sensory perception of *Saccharina*. While blanching lowers the iodine levels in *Saccharina* so that more of it could be included in the human diet, it also reduced the levels of glutamic acid that contributes to the umami flavour (Blikra et al., 2021). Another positive sensory reason for blanching *Saccharina* was that it turned the brown algae a vibrant, bright green, which may appeal to consumers according to the authors Akomea-Frempong et al. (2021) and Perry, Brodt, and Skonberg (2019), but this colour perception preference may be subjective as the surveys were done in the US, a country that is less familiar with consuming seaweed.

#### **Drying and Freezing as Preservation Methods**

Seaweed typically has a short shelf-life of 3 to 14 days and drying is one way of preserving it (Harrysson et al., 2021). Another method of preservation is freezing, which is more effective at preservingbio-components such as polyphenols, PUFAs and amino acids than heat drying, but has the disadvantage of being energy inefficient, as it firstly takes energy to freeze the seaweed, and secondly requires continuous energy for storage of the frozen product (Harrysson et al., 2021).

#### Smoking

Smoking is an old method of preservation for fish and meat (Varlet, Prost, & Serot, 2007). When the wood smoke is produced via incomplete combustion, not only does it provide a unique flavour and changes in texture, it also releases phenolics in a specific environment that gives rise to antimicrobial properties as well as antioxidative features which can prevent lipid oxidation processes in fish and meat (Albishi, Banoub, de Camargo, & Shahidi, 2019; Oz, 2020). Aldehydes are another important group of volatiles that is formed during the process of smoking and can indicate levels of oxidation, smokiness and even toxicity (Varlet et al., 2007). There are also different smoking techniques, such as cold smoking and hot smoking and a variation of cold, warm and hot smoking from 0°C - 30°C, 30°C - 50°C and 50°C - 80°C, respectively, is even recognised in Japan (Huang et al., 2019). Both cold and hot smoking have their own advantages and disadvantages. With hot smoking, some level of dehydration and cooking occurs, which results in a more visually appealing product due to the Maillard reaction, and have also showed an increase in nutritional value in hot smoked fish (Aremu, Namo, Salau, Agbo, & Ibrahim, 2013; Baten et al., 2020; Huang et al., 2019). However, heat may also cause proteins to denature and nutritional quality of food may decrease (Baten et al., 2020). When it comes to cold smoking, the food can retain moisture as there is no excess heat to dry it out, and since moisture is not lost, it allows for better smoke absorption (Huang et al., 2019). Another important aspect of smoking is the type of wood chips utilized since different woods can create different types of volatiles in the smoke and this in turn can also have an impact on the outcome of the products (Albishi et al., 2019).

#### Salting as a Preservation Method

Yet another method of preservation that is particularly effective for macroalgae due to its high moisture content, is salting (Perry et al., 2019). The process of salting begins with loss of water in the food that occurs via osmosis, whereby the water gets drawn out of the food towards areas with higher salt concentrations that in turn dissolves the salt which is then absorbed back into the food piece (Albarracín, Sánchez, Grau, & Barat, 2011). Salting works as a preservative in various ways by reducing water activity, lowering pH in fermentation processes and triggering enzymatic pathways which contribute to inhibiting microbial growth (Albarracín et al., 2011). The level of salt added to the seaweed may also affect its shelf-life and storage temperatures, which can range from room temperature to refrigerator temperature (Perry et al., 2019).

Salting was reported to not affect the antioxidant activity of unfrozen *Laminaria ochroleuca*, but when salted seaweed had been frozen it significantly decreased its antioxidative properties, in a study by Del Olmo, Picon, and Nuñez (2019). Another point to consider about salt is that it is also both a pro-oxidant and an antioxidant (Albarracín et al., 2011) thus, salted ingredients could either exacerbate or alleviate the rate of oxidation in seafood.

## **Materials and Methods**

#### (A) Product Development to Select the Right Level of Antioxidant Helper Material

A food trial-tasting was conducted with the herring mince and a few selected helper materials in order to help determine the level of antioxidant helper material to be used in the main experiment for this thesis. The dry matter content of all the helper materials were measured and calculated prior to the cooking. The helpers selected were dried *Ulva*; wet lingonberry press-cake; dried lingonberry press-cake; wet, unblanched *Saccharina* and dried, blanched *Saccharina*. Each helper was added in increments of 5%, 10% and 20% to 60 grams (dw) of herring mince and mixed with 1.33 grams (1/8 teaspoon) of salt. After blending, each mixture was divided into four parts, rolled into balls and pan-fried with rapeseed oil. One portion of herring mince mixed with salt and no added helpers was also cooked in the same manner and included in the tasting as a control.

The herring-fish balls were randomly arranged and numbered on a plate for three panellists to have a blind tasting (Appendix A). They were asked to judge the herring-fish balls based on the taste, looks and texture. The collated survey results were considered for the pre-(ice storage) trial.

### (B) Ice Storage Trial to Evaluate the Ability of Helpers to Prevent Oxidation in Herring Mince

## **Experimental Design**

A selected range of combinations of herring mince with the direct addition of five different antioxidative "helper materials"; lingonberry press-cake, *Saccharina* (blanched, unblanched, salted) and *Ulva* were evaluated. The antioxidative helpers were also treated in three different ways: wet (untreated), dried and wet-smoked. To assess how these helper combinations (16 different combinations, including 1 control) could prevent oxidation of herring mince during storage, the helper-fortified herring samples plus control were subjected to an ice storage trial during which samples were retrieved on selected days and evaluated by TBARS. A sensory screening of odour was also done in parallel during the ice-storage trial to measure "rancid", "fishy" and "painty" odours on a linear scale of 0 to 10, represented in centimetres (cm). This scale is marked correspondingly to each sensory scent to be measured and followed by additional descriptive comments, if any.

#### Seaweed Controls

To rule out additional oxidation interference from the seaweeds, 10% (w/w) of each wet (untreated) seaweed was added to 0.9% NaCl solution (to mimic the herring mince) and extracted as described in the TBARS section with chloroform/methanol and analysed for TBARS on Day 0.

#### pH Adjusted Control

The addition of lingonberry press-cake to herring mince lowered the pH value from 6.52 to 6.23. During the ice storage trial, one of herring mince controls was added with 1M HCl to lower the pH from 6.52 to 6.29. This was to determine if the lowered pH value, without the aid of the lingonberry press-cake, had any additional effects on preventing lipid oxidation.

## **Raw Materials and Sample Preparation**

#### Herring Mince

The herring mince came from Sweden Pelagic AB in Ellös on 30<sup>th</sup> September, 2021, whereby the fish that was landed on the same morning was factory processed. The post-filleted herring backbones (**Fig. 7**), without heads, intestines, belly flaps and tails, were collected and put into a meat-bone separator from Baader. The backbones come on a conveyor belt and are being dropped into a spinning, perforated drum. The backbones are then pressed against the drum and the flesh is drawn out through the slotted drum by high centrifugal force and the herring

mince is collected, without any bones. The machine is able to separate most of the muscle from the backbones; when **Fig. 8** and **9** are compared, it can be seen how much meat has been pulled off the backbones by the machine. Once extracted, the herring mince was vacuum-packed in plastic and transported on ice, in an ice-box, to the premises of Chalmers University of Technology (Chalmers) and stored in the freezer (-80°C) until required.



Fig. 7 Post-filleted herring backbones, heads and tails removed, from factory at Sweden Pelagic, Ellös



Fig. 8 Clean backbones after separation



**Fig. 9** Herring backbone mince (Photo by: Haizhou Wu / Chalmers University)

## **Helper Materials**

## Lingonberry Press-cake

The lingonberry press-cake was obtained from Olle Svensson Partiaffär AB on 28<sup>th</sup> October, 2021. The carton of lingonberry press-cake was transported frozen in a 10 kilograms block, and was stored in a -80°C freezer upon arrival at Chalmers. A small block of press-cake was

chipped off whenever it was required for usage and the remainder continued to be stored in the -80°C freezer.

#### Ulva

Ulva was obtained from the Tjärnö Marine Lab where they were cultivated in tanks in a greenhouse and harvested on 30<sup>th</sup> April, 2021. They were frozen at -60°C after harvest, and transported frozen to Chalmers and stored in the freezer (-80°C) until needed. Although the Ulva used in this project have been cultivated and harvested from land-based tanks, it is being considered as a helper material today as it is found locally, and have a promising future as a scaled up marine crop to be farmed in Sweden.

#### Saccharina (Blanched & Unblanched)

Both blanched and unblanched *Saccharina* were procured by Nordic Sea Farm, batch number: 2021-04-20, and transported frozen to Chalmers on 2<sup>nd</sup> December, 2021, where it was kept in the -80°C freezer until required. Since the role of taste is also important for this project, both blanched and unblanched sugar kelp are used as antioxidative helpers in this paper in order to determine any flavour loss.

#### Salted Saccharina

Salted *Saccharina* was obtained pre-brined from Kobb. It arrived in a vacuum sealed plastic container and upon opening, the salted *Saccharina* was drained to separate the seaweed from the brine. The *Saccharina* and brine were packaged separately and stored in the -80°C freezer at Chalmers until it was needed.

#### **Treatment Methods**

#### **Preparation of Helper-Fortified Herring Mince**

Samples that required thawing were thawed in a tight plastic zip-lock bag under cold running water before use.

#### Flaking of Frozen Material

The portion of seaweed needed was weighed out and put in a plastic zip-lock bag. To get wet seaweed flakes, the plastic zip-lock bag was subjected to hammering to break down the seaweed into smaller flakes, between 2 to 5 millimetres in diameter.

#### Drying and Milling

For drying, the thawed lingonberry press-cake was spread out in an even layer on three separate trays and put in the laboratory oven (model: BKE 30; Memmert GmbH., Schwabach, Germany) to dry at 40°C, for 4 hours. Each tray of dried press-cake was then weighed into batches of approximately 18 to 20 grams and processed in a spice blender (model: Style Collection SG21U; CuisinArt, Stamford, United States of America). Each batch was grinded for 30 seconds to obtain a fine powder.

To dry the different seaweeds, *Ulva* and *Saccharina* (blanched, unblanched or salted), they were first thawed and then spread out evenly on 3 oven trays lined with baking paper. They were dried in the laboratory oven (model: BKE 30; Memmert GmbH., Schwabach, Germany) at 40°C, for 6 hours (*Ulva*) or 7 hours (all *Saccharina* types). The dried seaweed was then put in a spice blender (model: Style Collection SG21U; CuisinArt, Stamford, United States of America) and ground for 30 seconds to obtain a powder.

#### Wet-Smoking

Only wet helper materials were smoked. Although fish and meat are the food items that are traditionally smoked, the helper materials in this project are smoked instead of the herring, due

to the fact that the fish used in this project is in minced form. Hot smoking was also selected as the method as it had the advantage of raising the product's nutritional value and smoking also creates another layer of flavour that may be an interesting addition to this project's final herring product. The thawed helper materials were placed on an aluminium foil tray and smoked in a smokebox (model: #1230; Fladen, Varberg, Sweden) for 5 minutes with Swedish apple wood chips from Lilla Rökeriet.

## **Ice Storage Trial**

The ice storage trial was done to determine the level of lipid oxidation of the herring and helper combinations over time, in days. The dry matter content of the herring mince and helper materials were measured and calculated prior to the ice storage trial (Appendix C). Herring mince and the required helpers for the trial were first thawed (see Raw Materials Sample Preparation) and then put into a beaker for manual mixing on ice for 1 minute.

For every 100 g (dw) of herring mince, 10% of the antioxidative helper material was added. 200 ppm streptomycin was also added to all the samples to inhibit any growth of bacteria. An additional beaker of only herring mince was included as control, with only the addition of 200 ppm streptomycin. Approximately 30g of sample was carefully added into 250 mL Erlenmeyer flasks (e-flasks) to avoid tainting the wall of the flasks and flattened to an even layer of about 4-5 mm, in triplicates. The e-flasks were capped, wrapped in aluminium foil so as to avoid oxidation caused by light, and labelled with a three-digit randomized number code. The wrapped flasks were then kept on ice in ice-boxes and stored in a cold room (4°C) until required for sensory analysis (see Sensory Evaluation) and/or sampling. To obtain samples, approximately 1.0-1.2 g of sample was carefully retrieved from the e-flask and avoiding the flask walls as much as possible. Excess sample was discarded each time as no sample was to be put back into the e-flask, and the obtained samples were wrapped in aluminium foil, labelled, put in plastic zip-lock bags and stored in the freezer (-80°C) for future experiments. The trial was deemed completed when the samples were found to be bacterially spoiled, a time-frame of 14 to 18 days.

Due to the number of variations of the helpers and their different treatments, the experiment was split up into three separate trials. For each of the five helpers (lingonberry press-cake, *Ulva*, blanched sugar kelp, unblanched sugar kelp and salted sugar kelp), there were three variations: wet, dried and smoked. Ice Storage Trial 1 (IST1) was carried out with lingonberry press-cake and *Ulva*; Ice Storage Trial 2 (IST2) was executed with both blanched and unblanched sugar kelp and Ice Storage Trial 3 (IST3) was done with salted sugar kelp.

#### Sensory Screening of Rancid Odour

One trained panellist conducted a sensory screening throughout each of the three ice storage trials. Prior to evaluating the scent of the samples, the e-flasks were removed from ice-boxes in the cold storage room and put on ice and left at room temperature for at least 30 minutes. The participant then uncapped each number coded e-flask before smelling it, recorded the three-digit code of the flask and scored the samples on odours of rancidity, fishiness and paintiness on an unstructured scale of 0-10 (cm) and included further comments, if any.

The screening was administered daily for the first week, not including weekends, and when possible, every other weekday during the subsequent weeks i.e., Monday, Wednesday and Friday.

#### **Colour Measurement**

For each ice storage trial, approximately 2 to 3 grams of sample from the control and each of the combined herring and 10% of helper material were placed in petri-dishes and stored in the same conditions as the e-flasks from the ice storage trial. Due to the range of sizes of the helper materials from powder form to small flakes, some of the combinations were not able to be completely homogenised to use a colorimeter to record the colours. As such, to measure the colours, these petri-dishes were photographed close to daily to keep record of colour changes.

#### Thiobarbituric Acid-Reactive Substances (TBARS)

In order to determine the level of oxidation in the samples, the selected samples were put through the TBARS analysis following the method detailed by Sajib et al. (2021). The samples selected for TBARS measurement were removed from -80°C storage and put in new plastic zip-lock bags and thawed under cold, running water. The TBARS analysis was done in 2 phases: extraction and reaction.

#### **Extraction** Phase

One g of sample was measured and put in a 50-mL tube. Five mL of ice-cold chloroform:methanol (2:1) containing 0.05% (w/v) BHT was added to the test tube and subjected to homogenization with the polytron (Ultra Turrax, IKA Werks, Intermed Labassco) for 15 seconds at speed 13. Another 5 mL of the same chloroform:methanol with 0.05% BHT solution was added into the tube and homogenized for another 15 seconds. 3.08 mL of ice-cold 0.5% NaCl was then added and vortexed for 30 seconds. The samples were then centrifuged for 6 minutes (2000g) at 4°C and the upper methanol:water phase was quantitatively removed into new 4 mL glass tubes and stored at -80°C until analyses.

The samples were kept on ice throughout the whole extraction process.

#### TBA Reaction Phase

During the reaction phase, the extracted upper phases stored in the glass tubes were thawed under cold, running water. 2 mL of the extracted phase was added with 2 mL of TBA reagent into falcon tubes and boiled for 30 minutes until the pink hues developed. The tubes were then cooled under cold, running tap water. When cooled, they were vortexed shortly, degassed and centrifuged for 3 minutes (2000g). The absorbance was measured at 532 nm and the TBARS values (refer to *Lipid Oxidation* section) were obtained from a prepared standard curve of 1,1,3,3-tetraethoxypropane (TEP).

# (C) Investigation of Strategy Within the Gothenburg Municipality Regarding School Lunches

To understand the guidelines of producing a sustainable food product to the general public and school, the Gothenburg City Council was contacted and the environmental representatives were interviewed over e-mail and research was done on the state public policy documents. A range of public-school representatives were also contacted regarding the purchasing of food for public schools. Further food industry related sustainability initiatives that were either connected to the Gothenburg City Council, or not, were looked up on-line. The Swedish Food Agency (*Livsmedelsverket*) were also contacted regarding the pre-requisites for the production and release of such a new herring food product.

## Results

#### (A) Product Development to Select the Right Level of Antioxidant Helper Material

The trial-tasting survey results were collated (Appendix A) and the overall consensus was that 10% of helper material added to the herring mince had the best balance in terms of flavour, texture and looks (with the exception of No. 04: dried, blanched *Saccharina*). These results from the trial-tasting were then considered for the pre-(ice-storage) trial. The level of helper addition was 10% (w/w) based on the survey results.

A total of four samples were used for the pre-trial; two lingonberry press-cake combinations, one of which was pH adjusted; unblanched, wet *Saccharina* and a control with only herring mince. When pH was measured, the lingonberry press-cake and herring mince combination was of significant difference to only the herring mince (see **Table 1** below) and as such, an additional lingonberry press-cake sample with an adjusted pH (1M NaOH added) was included in the pre-trial to see if the pH level made a difference in terms of preventing lipid oxidation.

Sample	рН
Control, only herring	6.52
Herring and dried lingonberry press-cake	6.23
Herring and dried lingonberry press-cake, pH adjusted	6.47
Herring and wet, unblanched Saccharina	6.48

**Table 1.** pH measurement of herring mixtures

Selected samples from the pre-trial were analysed with TBARS and the results (Appendix B) showed that both the lingonberry press-cake samples (regular and pH adjusted) showed lower levels of TBARS on Day 0 as compared to the control. Both lingonberry press-cake samples also showed the same results, showing no difference with regards to the adjusted pH sample affecting lipid oxidation effects. The unblanched, wet *Saccharina* sample showed slightly higher levels of TBARS than the control.

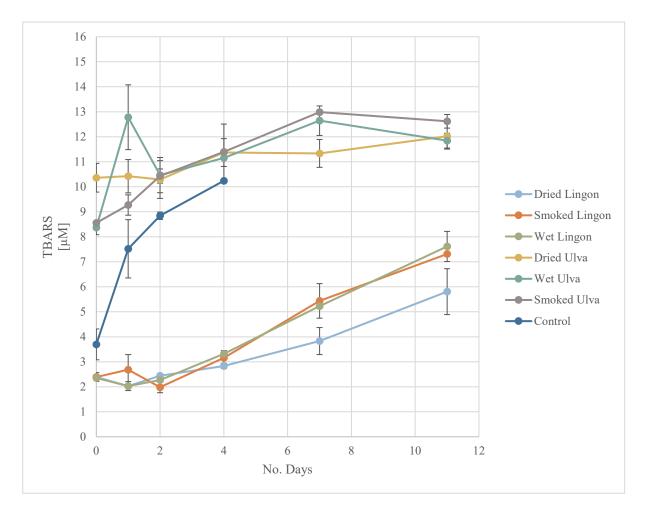
### (B) Ice Storage Trial to Evaluate the Ability of Helpers to Prevent Oxidation in Herring Mince

#### **TBARS Development During Ice Storage**

#### Ice Storage Trial 1

In **Fig. 10** below, all variations of lingonberry press-cake show lower levels of TBARS from Day 0 as compared to the control whereas all variations of *Ulva* show elevated levels of TBARS. The dried lingonberry press-cake had the lowest end point of TBARS out of all the

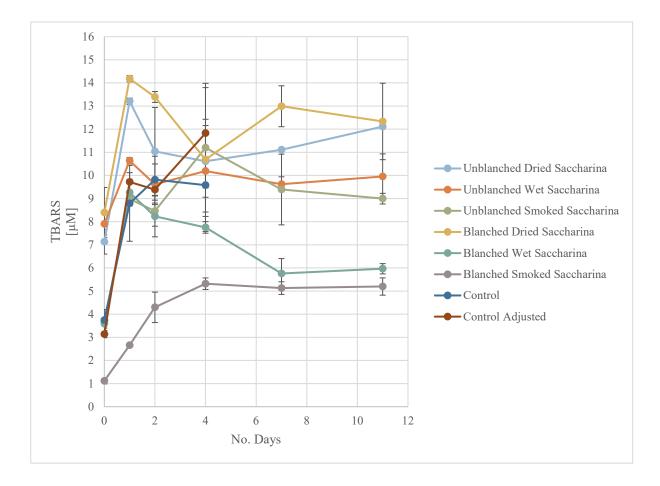
samples in IST1 on Day 11, while the smoked *Ulva* samples had the highest. The wet *Ulva* sample peaked on Day 1, decreased and gradually increased again and all other samples showed the same trend of gradually increasing over time.



**Fig. 10** Effect of Lingonberry Press-cake and *Ulva* on TBARS development in herring mince during Ice Storage Trial 1, data represents mean ± standard deviation (n=2)

#### Ice Storage Trial 2

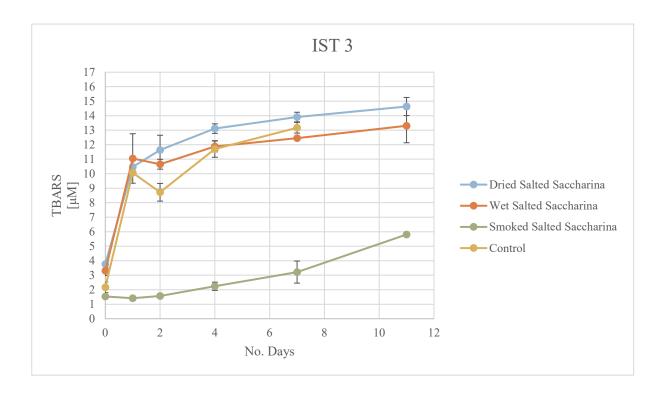
Of the unblanched *Saccharina* series, both the wet and dried *Saccharina* gave rise to a higher concentration of TBARS than the controls on Day 0 but the unblanched smoked *Saccharina* had about the same levels as the control (**Fig. 11**). As for the blanched *Saccharina* variations, the dried one gave rise to the highest concentration of TBARS from Day 0 and overall, through the trial, ending with the highest on Day 11 as well. Blanched wet *Saccharina* sample started off around the same concentrations as the control on Day 0 but had a lower level of TBARS as compared to the control over the subsequent days. Blanched smoked *Saccharina* sample had decreased levels of TBARS as compared to the rest of the samples from Day 0 to 11 and finished with the lowest concentration on the last day of the trial.



**Fig. 11** Effect of Unblanched and Blanched *Saccharina* on TBARS development in herring mince during Ice Storage Trial 2, data represents mean ± standard deviation (n=2)

#### Ice Storage Trial 3

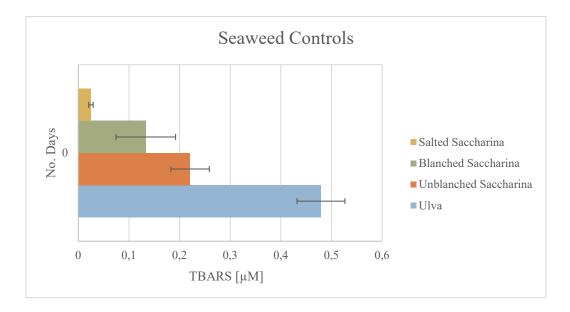
In IST3, the dried salted *Saccharina* sample showed the highest concentrations of TBARS on Day 0, then increased further and ended with the overall highest levels (**Fig. 12**). Although the wet salted *Saccharina* showed a peak on Day 1, higher than all the other samples, it decreased again on Day 2 before gradually increasing over time. Finally, the smoked salted *Saccharina* behaved similarly to the blanched smoked *Saccharina* of Ice storage trial 2 (**Fig. 11**), i.e., lower TBARS than the control from Day 0 and remaining at lower concentrations throughout the trial.

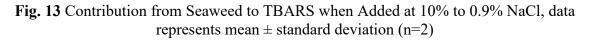


**Fig. 12** Effect of Salted *Saccharina* on TBARS development in herring mince during Ice Storage Trial 3, data represents mean ± standard deviation (n=2)

#### Contribution from Seaweed Alone to TBARS Data

Given the high 0-time levels of TBARS in seaweed-containing herring mince, seaweed controls were also run to check for interference with the assay. Of all the seaweeds used, *Ulva* displayed the highest TBARS concentration, followed by the unblanched *Saccharina*, blanched *Saccharina* and salted *Saccharina*, respectively (**Fig. 13**).





#### **Sensory Screening**

The results from the sensory screening were collated in standard deviation mean values and presented in plotted charts. Rancid odour (**Figs. 14 to 16**) was used as a primary result, and is shown below, while the results for the fishy odour and paintiness is found in Appendix D.

#### Rancidity

It can be observed that in all three trials, the control sample of herring mince peaked on Day 1 or 2 (**Figs. 14 to 16**), and they mostly also peaked higher than the other helper and herring combined mixtures. However, there was one exception; the one containing dried salted *Saccharina* from IST3, which peaked higher than the control on Day 2 as seen in **Fig. 16**. These early peak points correlate to the susceptibility of the pure herring mince oxidising by itself. Overall, the panellist found it hard to detect rancid odour given the large background odour provided by the helpers, e.g., seaweed. Therefore, only the largest and clearest changes are described below.

In **Fig. 14** below, the sample with smoked *Ulva* showed a steadily increasing level of rancidity over time from Day 1. The herring mince control increased on Day 1 and gradually declined over the next few days. On Day 4, a small level of rancidity was detected in the wet *Ulva* sample, but declined again the next day. The remaining samples with all the lingonberry helpers, and those with dried *Ulva* showed no signs of rancidity over the first week of the sensory evaluation.

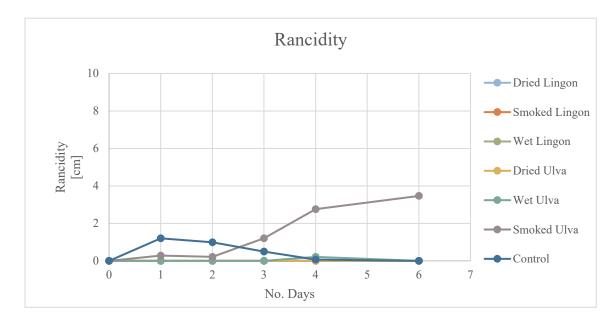


Fig. 14 Sensory assessment of rancidity in IST1

In IST2 as displayed in **Fig. 15** below, both the herring mince control and adjusted herring control peaked on Day 1 and declined on Day 2. Rancidity was picked up again by the control from Day 3, 4 while it decreased for the adjusted control and picked up again on Day 7 to the same levels as the control. Sample with unblanched dried *Saccharina* displayed low levels of rancidity from Day 0 to 7 but increased on Day 9, in contrast to all the other samples that dipped on Day 9, and declined again on Day 11.

Sample with unblanched wet *Saccharina* peaked on Day 4 but had lost all rancid odour on Day 7. Sample with blanched dried *Saccharina* peaked on Day 7 and was undetected on Day 9. Blanched wet *Saccharina* peaked on Day 2 and no rancidity was picked up from Day 3 till Day 9, after which some levels of rancidity were picked up on Day 11. Both the unblanched and blanched smoked *Saccharina* samples showed no signs of rancidity over the entire trial.

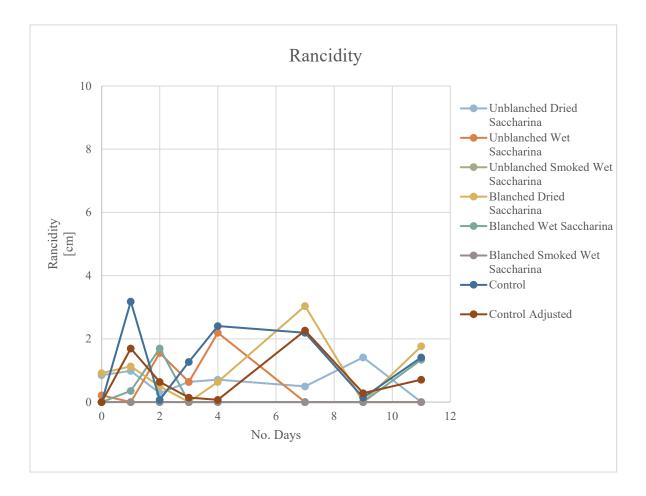


Fig. 15 Sensory assessment of rancidity in IST2

In **Fig. 16**, it can be seen that the rancidity level of dried salted *Saccharina* peaked on Day 2 along with the control herring. The dried salted *Saccharina* sample declined subsequently while the control showed no rancidity on Day 3 but was picked up again on Day 4. The wet salted *Saccharina* sample showed low levels of rancidity throughout the trial. Similar to the smoked *Saccharina* samples in IST2 (**Fig. 15**), the smoked salted *Saccharina* in IST3 also displayed undetected levels of rancidity up till Day 11 in **Fig. 16**.

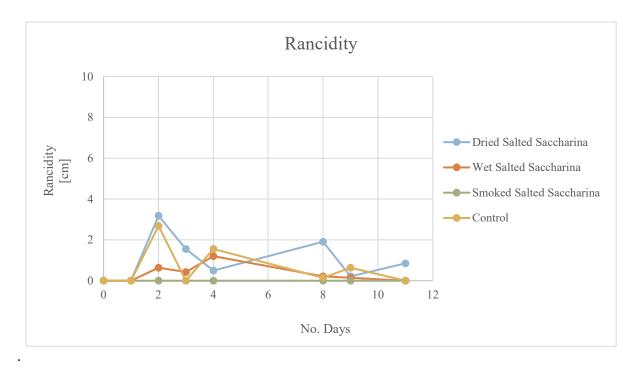


Fig. 16 Sensory assessment of rancidity in IST3

## **Visual Appearance**

Due to the volume of photos, only some of the sample combinations were selected to be displayed in this result section and the complete set of photo-comparisons can be found in Appendices E, F and G. A common occurrence that can be noted in most of the photo-sets across all the ice storage trials was the significant loss of red-pink hues of the herring mince after Day 0. This is a well-known sign of met-Hb-formation, and has in earlier trials been found to correlate closely with development of lipid oxidation.

#### Control

The control containing pure herring mince was light pink on Day 0 but quickly lost its pink colour from Day 1 (**Fig. 17**). As shown in the pictures below, there was significant loss of pink colour by Day 3. The pH-adjusted control which had a pH of 6.29 instead of 6.52 as in the regular control displayed the same colour results (Ice Storage Trial 2, see Appendix F).

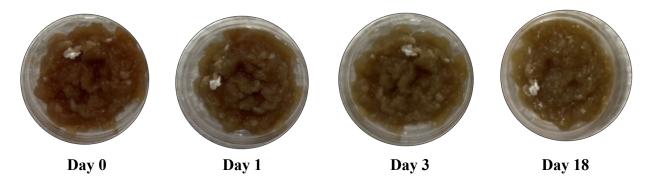


Fig. 17 Control in Petri dishes: Day 0, 1, 3 and 18

#### Wet Lingonberry Press-cake Flakes with Herring

The wet lingonberry press-cake flakes and herring combination turned out to have a vibrant reddish-pink colour that maintained its appearance up until Day 7, when it started to lose some of its vibrancy, but still remained reddish-pink (**Fig. 17**). The dried and smoked lingonberry press-cake additions showed similar results (Appendix E). The absence of redness loss despite a small development of TBARS over time (see **Fig. 14**) could be due to a larger impact from the lingonberry anthocyanins than the met-Hb-formation in herring.

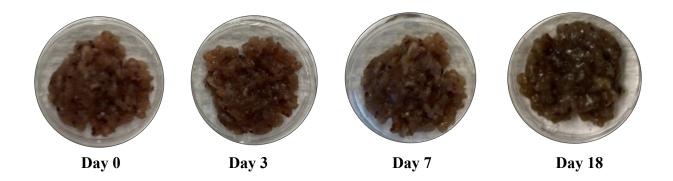


Fig. 17 Wet lingonberry press-cake flakes combination: Day 0, 3, 7 and 18

#### Dried Ulva Powder with Herring

Dried *Ulva* powder mixed with herring mince produced a green sample with cool bluish tones but it started to lose these cool hues from Day 7 and eventually became a warm yellow-green.

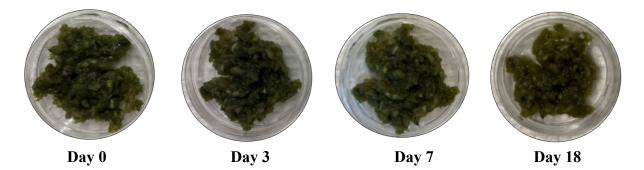


Fig. 18 Dried Ulva powder mixed with herring mince: Day 0, 3, 7 and 18

#### Wet-Smoked Ulva Flakes with Herring

The smoked Ulva flakes combined with herring retained their individual characteristics since the Ulva flakes were between 2 – 5mm and it was not completely homogenised with the herring mince (**Fig. 19**). The loss of redness in the herring mince could be noted from Day 1 and onwards and became a grey-brown shade that remained until Day 18. The smoked Ulva flakes did not change colour visibly. The wet Ulva flakes with herring showed a comparable result and those photos can be seen in Appendix E.

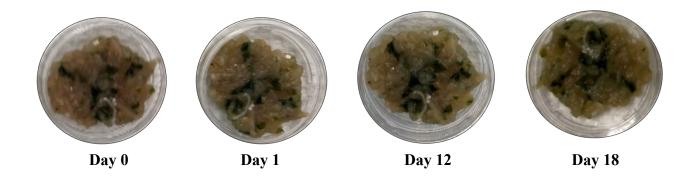


Fig. 19 Smoked, wet Ulva flakes with herring mince: Day 0, 1, 12 and 18

#### Unblanched Dried Saccharina with Herring

A slight appearance of redness could be seen on the herring mince mixed with unblanched dried *Saccharina* powder but was lost the following day (**Fig. 20**). The unblanched dried *Saccharina* powder had a dark, olive-green colour that rendered the herring mince a darker, greenish shade when combined together. The dark olive-green shade remained constant up till Day 14.

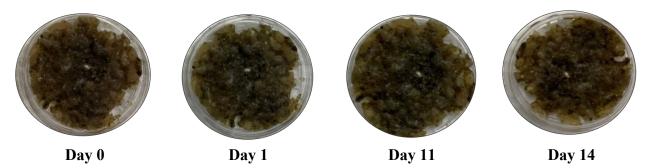


Fig. 20 Herring mince with unblanched dried Saccharina: Day 0, 1, 11 and 14

#### Blanched Smoked Wet Saccharina with Herring

Although the redness of the herring mince was low on Day 0 in the blanched smoked *Saccharina* combination, it can still be seen that the herring mince lost the slight pink tone on Day 1 (**Fig. 21**). The herring mince continued to fade from brown to a greyish shade as seen in Day 8 and Day 14. The blanched smoked *Saccharina* flakes maintained their vivid greenness from Day 0 to 14. This colour trend was also reflected in the herring mince samples combined with unblanched; wet and smoked *Saccharina* and blanched; dried and wet *Saccharina* (Appendix F).

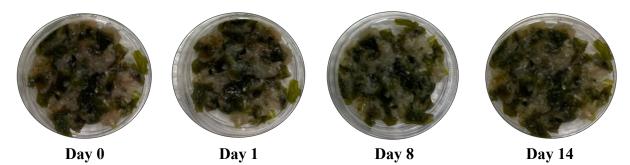
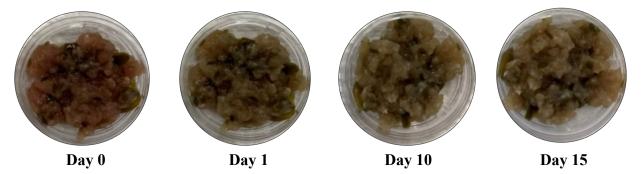
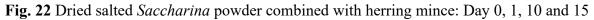


Fig. 21 Herring mince with blanched, smoked wet Saccharina: Day 0, 1, 8 and 14

#### Wet Salted Saccharina with Herring

The wet salted *Saccharina* flakes were approximately 2-5 mm in size and after mixing into the herring mince, were still distinct (**Fig. 22**). The colour pattern for the wet salted *Saccharina* combination was similar to the dried salted *Saccharina* sample (Appendix G), whereby red tones from the herring mince on Day 0 was lost on Day 1 while the dark umber colour of the salted *Saccharina* flakes kept the same shade from Day 1 to 15.





#### Smoked Saccharina with Herring

The wet-smoked salted *Saccharina* combination was the only sample that retained redness of the herring mince past Day 1 (Fig. 23). The redness started to fade on the herring mince on Day 7 and although the mince gradually lost redness towards the end of trial, it did not turn completely grey-brown like all the other herring mince combinations before. At the end of the trial on Day 15, the smoked *Saccharina* sample still retained some pinkiness and showed signs of turning brown on some areas. The smoked salted *Saccharina* flakes remained a dark umber colour for the whole trial.

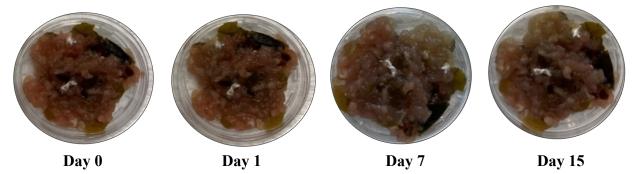


Fig. 23 Herring mince mixed with wet-smoked salted Saccharina flakes: Day 0, 1, 7 and 15

# (C) Investigation of Strategy Within the Gothenburg Municipality Regarding School Lunches

#### Sustainability Agenda of the City of Gothenburg (Göteborgs Stad)

The state of Gothenburg in Sweden has released an "Environment and Climate Programme for the City of Gothenburg 2021–2030" whereby they aim to make Gothenburg an environmentally sustainable city by 2030 in line with the Paris Agenda, United Nation's (UN) Sustainable Development Goals (SDG), Agenda 2030 and European Union (EU) directives, such as the European's Commission Green Deal (2019/909, 2019; Bergqvist, 2014). The three aspects of sustainability; environmental, social and economic are emphasized in the program to be interlinked and integral to the global SDGs (*Environment and Climate Programme for the City of Gothenburg 2021–2030*, 2021).

It can also be observed that it is of the state's interest to introduce climate friendly meals as they report to serve about 20 million meals to the various public sectors, from schools to care homes and city events, annually (Göteborg Stad, 2022). With the growing concerns regarding global climate change and to meet the city's green goal in 2030, their ability to serve up sustainable food would likely make an impact, especially with the volume of meals that they provide each year. These sustainable meals have been termed "miljömåltid," which roughly translate to "environmental meal." Not only do they aim to serve environmentally friendly food, they also encourage the public to purchase and eat ethically produced meals that are seasonal, and to minimise food wastage (Bergqvist, 2014). The schools in Gothenburg are strongly encouraged to follow these guidelines of *miljömåltid*, for serving up school meals.

When interviewed over e-mail regarding the serving of such meals, the environmental state representatives (ESR) responded that the *miljömåltider* comes as a guideline for meal-planning, rather than a rule for public schools. At the same time, all the school kitchens are required to

use this guideline to ensure that the meals are climate friendly and another important factor is the use of organic produce, which they have set to be 80% in 2030. Lastly, for the buying of fish and seafood products, they are required to be ASC, MSC or KRAV labelled.

When it comes to purchasing food for the schools, according to the ESR, the school kitchens buy their foods from large wholesale companies. In order for a new product to be available to school kitchens, it has to also be available publicly through these large distribution centers. The same response was obtained from one of the local school representatives (Annedalsskolans) interviewed over email, school food within the City of Gothenburg has to be ordered directly from a purchaser, food is ordered based of a pre-planned menu they only use the freezer in a small quantity and fish in any form is served at least once or twice a week.

#### **Other Sustainability Initiatives**

In a separate search to seek for other sustainable food initiatives, the Gothenburg Climate Partnership (GCP), also a part of the Gothenburg City Council under the Business Region department came up. However, when reached out to, the GCP representative responded that they were not involved with the food industry.

Sweden Food Arena, another government supported initiative out of the government's Food Strategy, describes the foundation to be "a national arena where food industry stakeholders collaborate for an innovative, sustainable, and competitive food sector (Sweden Food Arena, 2022)." When contacted regarding how they work within the association to connect the various industries, there was no response.

#### Swedish Food Agency (Livsmedelsverket)

The Swedish Food Agency authorities was contacted to also find out more about how a food product such as this herring product would be produced and sold to the public. The response was an automated reply with suggestions to three web-links regarding starting of a food business, e-trade and information and labelling. On the webpage on starting a food business, the local municipality authorities should first be contacted for inspection (Livsmedelsverket, 2021). However, the webpage was regarding starting up a café, restaurant or importing prepacked food. A further look into the *Livsmedelsverket* website showed information about quality control in various food sectors, but no further information regarding producing a new food product.

## Discussion

#### (A) Product Development

#### Challenges of herring fish product

Herring is typically brined or pickled and consumed in that fashion nowadays in Sweden (Undeland, 1995). There is a lack of consumer demand as fresh herring is not as commonly consumed compared to the brined product, while salmon and cod were the most consumed fish in Sweden (Pihlajamäki, Asikainen, Ignatius, Haapasaari, & Tuomisto, 2019).

To better understand the product, research was also done on recipes involving herring mince. The Nordic Cookbook, which extensively covers traditional, local foods from the entire Nordic region (Nilsson, 2015) and *Svensk Husmanskost* (roughly translated to as traditional Swedish homecooked meals), a Swedish cookbook comprised of collected old Swedish recipes adapted to modern cooking methods (Wretman, 1967) were used. They both featured herring recipes that used salted herring with advice of soaking and rinsing the fish for hours or overnight to remove the excess salt. Furthermore, the minced herring patty recipes called for minced meat to be added into the herring patty, which reduces the volume of herring needed or used even on a small scale for home cooking. This sheds some light on the low demand of fresh herring since even salted fillets are used in recipes.

The introduction of herring mince as a low-impact and sustainable product may perhaps change the perception of consuming herring again, especially if marketed right. There are already positive correlations of perceived health benefits of eating fish and seafood long-term on the human body (Olsen, 2004). But at the same time, consumer behaviour is difficult to understand and in a survey that was done by Skallerud, Armbrecht, and Tuu (2021) on the intentions of the Swedish public to consume sustainable fish resulted in what they described as a boomerang effect. The authors found that there was a general positive attitude towards the intention of consuming fish, but not specifically towards sustainable fish. As for the boomerang effect, the more aware the consumers were regarding the environment, the less likely they were to actually consume fish and the study concluded that it was also likely due to the perception in Sweden that consuming fish is not environmentally sustainable (Skallerud et al., 2021). Based on this survey, it is then imperative to shift perspectives by educating the public on the types of sustainably produced fish available in Sweden and why it should be consumed more.

#### Seaweed and consumers attitudes

There is also the matter of perception regarding seaweed in Western cuisine. Seaweeds are widely consumed in Asia, accepted as a whole on how it looks, tastes and is part of the nutritional diet, but it is still met with a mixed reception in the Western world of food, whereby it has been used more so as gelling agents and emulsions that do not give any typical characteristics of seaweed in terms of flavours and visuals (Wendin & Undeland, 2020; Yesuraj et al., 2022). Another study in Canada revealed that survey participants preferred seaweed to be paired with savoury flavours such as fish or grains, and even hidden in seasonings or other food products (Moss & McSweeney, 2021). Both end of the views that either seaweed is still too novel, or that seaweed is slowly integrating into the kitchens and cookbooks of Europe and North America have been mentioned (Perry et al., 2019; Yesuraj et al., 2022). However, a study in Sweden did show a positive trend towards consuming seaweed, particularly in snacks, due to the awareness of seaweed being nutrient dense, and also of the need to be environmentally sustainable (Wendin & Undeland, 2020). This may be helpful and bring about positive attitudes if seaweed is used as an addition to the herring mince product.

#### Finalised Product for this Project

The herring product in this project was firstly test-cooked before the initial taste-trial in order to find out the level of salt that needed to be added to the herring mince. During the taste trial, it was noted that the cooked herring balls fell apart easily, and during the taste trial, only herring mince, antioxidative helper and salt were used. As such, the further test-cooking trials was to find out the best level of potato starch to add to the herring without the taste being affected.

In the later stages of test-cooking, aromatics such as garlic and onion were also used. It was found that the best way to integrate these aromatics was to grate them, as diced garlic and onion affected the texture and could also come off as a strong taste.

The final product for this project is featured on the cover page of the thesis and the recipe can be found in Appendix H.

# (B) Ice Storage Trial to Evaluate the Ability of Antioxidant-Containing Helpers to Prevent Oxidation in Herring Mince

#### Sensory and TBARS Analysis

While it is a subjective assessment, sensory evaluation of rancid odour has in many earlier trials with fish mince been proportional to the TBARS results (Sanchez-Alonso, Solas, & Borderias, 2007; Wetterskog & Undeland, 2004). In this case, due to the high background odor and number of similar samples during each of the trials, the panelist remarked that it was sometimes difficult to distinguish the odors. Despite of the difficulties, the sensory results were generally in line when compared with the development of TBARS. It should also be stressed that normally the sensory panel at Chalmers has  $\geq 3$  persons, which but due to the pandemic and other reasons, there have not been enough trained persons.

#### **Controls**

It can be seen that the levels of rancid aroma for the controls (including the pH-adjusted ones) decreased over time from the sensory assessment in **Figs. 14 - 16**, the levels of fishiness generally increased in relation with the TBARS (Appendix D). As the herring mince control contains no helper materials to stabilise the oxidation reactions, the levels of oxidation are expected to increase and the secondary oxidation processes that causes the aldehydes which contribute to the "rancid" and "fishy" aroma are also released, contributing to the increase of rancid and fishiness scent detected, as determined in the sensory evaluation of IST1 (Papastergiadis et al., 2012). In other studies of mechanically separated herring backbone mince, the rancid odour has been more pronounced, but here it was relatively weak, possibly due to a relatively strong intensity of fishy odour. That rancid odour in some cases peaked, and then decreased is however in line also with other studies, and is due to the high reactivity of aldehydes towards proteins and phospholipids, generating Schiff's bases.

#### Lingonberry Press-cake

In both the dried and wet lingonberry press-cake samples, no rancid odor was detected for the first week of the trial. However, when compared to the level of TBARS in **Fig. 10**, the concentration of TBARS gradually increased, showing that the oxidation process is gradually ongoing, but obviously generating aldehydes with high odor thresholds, or aldehydes being trapped by proteins or lipids of the fish matrix. However, the TBARS concentration reached was at a lower level as compared to the TBARS of the control sample (**Fig. 10**), showing some reduction in levels of oxidation, possibly aided by the antioxidative components of the wet and dried lingonberry press-cake additions. Likely molecules of the lingonberry press cake that could possess antioxidative activity are flavanols, anthocyanins and proanthocyanidins (Abdollahi et al., 2020).

#### Ulva

All three of the *Ulva* variations started on Day 0 with nearly double levels of TBARS as compared to the herring mince control (**Fig. 10**). While the TBARS assay on the seaweed controls showed some levels of TBARS formation (**Fig. 13**), with *Ulva* having the most levels of TBARS of all the seaweeds, it could not explain that *Ulva*-containing herring mince samples to have double as high in oxidation levels as the control on Day 0. The high levels of TBARS for the *Ulva*-containing samples could potentially be caused by human error such as the slow handling of the components as it was the first ice storage trial to be performed in this experiment, and furthermore, a large volume of samples to be handled at once. During this time, additional oxidation could have been induced due to light, temperature and air exposure.

Interestingly, although the wet *Ulva* combination did not give rancid scents as compared to the smoked *Ulva* sample during the sensory evaluations (**Fig. 14**), the levels of TBARS were very similar for both the wet and smoked *Ulva*-containing samples. However, there was a singular spike on Day 1 for the wet *Ulva* sample (**Fig. 10**) and the lack of spike in the TBARS of the smoked (wet) *Ulva* sample could be due to the additional phenolics from the apple wood smoke that provided more oxidative stability (Albishi et al., 2019).

#### Unblanched and Blanched Saccharina

The unblanched dried and wet *Saccharina* combinations showed elevated amount of TBARS concentration, nearly double that of the control, on Day 0 in IST2 (**Fig. 11**). This was a similar outcome with the *Ulva* combinations from IST1 (**Fig. 10**). The different treatments of blanched *Saccharina* samples showed a range of results in terms of the concentration of TBARS (**Fig. 11**). Blanched dried *Saccharina-containing samples* showed the highest levels of TBARS, with similar levels to those of the unblanched, wet and dried *Saccharina* samples (**Fig. 11**).

Both of the smoked variations from the unblanched and blanched *Saccharina* series showed lower TBARS than the control on Day 0 (**Fig. 11**). This was likely due to the additional phenolic compounds released by the hot-smoking of the *Saccharina* (Baten et al., 2020). It should however be noted that earlier findings on smoke-derived antioxidants are based on smoking of fish as there is a gap in the research regarding the components in smoked seaweed. The blanched smoked *Saccharina* sample had the lowest values of TBARS throughout IST2 and also did not give off any signs of rancidity during the entire sensory evaluation (**Fig. 15**). As aforementioned, additional phenolic compounds from the smoking process could have helped with retarding oxidation. Moreover, the flavouring from the smoke may have suppressed any rancidity aromas even at the end of the trial.

#### Salted Saccharina

All of the salted *Saccharina* combinations started with relatively low values of TBARS, particularly the smoked salted *Saccharina* variation (**Fig. 12**). Both the wet and dried salted *Saccharina* showed a sharp increase in TBARS concentration on Day 1 and continued to increase over the trial. The dried salted *Saccharina* ended on Day 11 with the highest values of TBARS out of all the three trials.

Despite the high concentrations of TBARS for the dried and wet salted *Saccharina* samples, rather low levels of rancidity were recorded during the sensory screening (**Fig. 16**). There is insufficient scientific knowledge regarding salted seaweed at this point and thus, it is difficult to draw any conclusions regarding the lack of any strong, unwanted aromas from the salted sugar kelp samples.

Smoked salted *Saccharina*-containing samples gave the lowest values of TBARS in IST3, in terms of reduced concentrations of MDA detected over the trial period. This was a similar result to both the unblanched and blanched smoked *Saccharina* in IST2, with the smoked kelp variation having the lowest values of TBARS out of their respective groups. The additional phenolics introduced by the apple wood smoke from the kelp may have contributed to the antioxidative effects on the herring mince.

Salt could either be an antioxidant or prooxidant (Albarracín et al., 2011), however, based on the results of both the wet and dried salted *Saccharina* combinations, the salted *Saccharina* did not seem to have any effects on the TBARS. It can be seen that both the wet and dried salted *Saccharina* showed nearly matching results of TBARS concentration to the control (**Fig. 12**).

Lastly, the smoked salted *Saccharina* combination was the only seaweed sample that maintained the redness, apart from the lingonberry press-cake variations containing proanthocyanins, of the herring mince over Day 1 and more (**Fig. 23**). It is therefore difficult to conclude what effects the addition of salt had.

Seaweeds contain antioxidants such as phlorotannins and ulvan, which could potentially contribute to warding off oxidation when added to herring mince. However, they also consist of PUFAs such as EPA, similar to herring, which are associated with increasing cardiovascular health but are also responsible for oxidation reactions that causes rancidity (Sánchez-Machado, López-Cervantes, López-Hernández, & Paseiro-Losada, 2004; Steinhagen et al., 2022). These same oxidation processes may further induce oxidation of pigments and vitamins in seaweed (Harrysson et al., 2021). In addition, seaweed is rich in trace elements such as iron (Kraan, 2013), and the addition of seaweed to foods as an antioxidant may thus induce co-oxidation of the mince at the same time as it provides antioxidants. The net effect from adding seaweeds to foods, not lest seafood, on lipid oxidation is not yet understood.

#### Limitations

#### **Smoking Process**

As the smoked *Saccharina* samples have displayed the best results with regards to the oxidative stability of the herring mince, it may be essential to improve the smoking processes, or even be able to control certain parameters of the smoking process. In this project, a simple smokebox was used, which uses twin small open flames to heat up the metal box, which ignites the wood chips and smokes the selected material within the box (**Fig. 24** below). As the grill rack had approximately 1.5-cm wide grills, it was unable to hold the small pieces of seaweed or lingonberry so a tray made of aluminium foil was used instead.

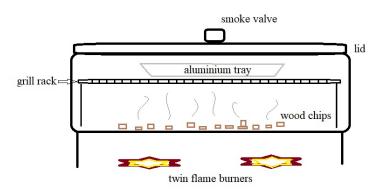


Fig. 24 Cross-section a smokebox

Due to the simple design, there is minimal control over the burners which were two, small open flames fuelled by methylated spirit. Although the same amount of apple wood chips was used for the same amount of helper materials, there was no control over the temperature, nor the amount of smoke produced in the smoke box. As the entire smokebox was quite small, the materials had to be smoked on separate occasions, so even though the materials were all smoked for 5 minutes each, they may have been smoked or warmed up to different temperatures.

#### Sensory Screening

With regards to the sensory screenings during the ice storage trials, lesser numbers of samples or perhaps a better mix of samples, such as not having two saccharina helper materials in the same trial, should be used during each trial to reduce the risk of odour saturation for the panellist assessing the aromas.

#### **TBARS** Analysis

As for the TBARS analysis, there have been previous research that challenge the precision of the results from this test due to the complex nature of food. The Maillard-browning reactions, along with protein degradation also contribute to development of red-violet colours that are measured to determine TBARS levels and as such, may cause an inaccurate, elevated reading (Papastergiadis et al., 2012). According to the authors evaluating TBARS in different types of food, the TBARS test is accurate with certain food products such as unprocessed meat and fish or vegetable oils but show signs of additional interference from food processing (apart from the secondary oxidation products ) in food items such as processed meat, fish, cheese and nuts (Papastergiadis et al., 2012).

In another paper about the modification of the TBARS assay for plant tissue extracts (Du & Bramlage, 1992), it was noted that since the TBARS method is mainly developed for use on animal products, the authors of the paper decided to use plant materials for the TBARS analysis to assess for additional interference that may arise out of the plants instead of the oxidation products. Plant tissues contain sugars, particularly sucrose, as well as amino acids and polyhydroxy compounds that may respond in the TBA assay and as such, these compound groups were measured (Du & Bramlage, 1992). The TBARS method was then modified to accommodate these plant extracts by including an additional standard curve of sucrose, to correct any sugar interference, as well as an additional absorbance reading at 440 nm, apart from the basic readings at 532 nm and 600 nm. The results from this modified TBARS method showed no interference from starch and glutamate, while there was still some interference from lactose, glucose and pectin while sucrose and fructose showed significant levels of interference in the TBARS analysis (Du & Bramlage, 1992). The helper materials used in this project were plant materials (lingonberries and seaweed). Lingonberries contain mostly fructose, glucose and low amounts of sucrose (Vilkickyte et al., 2019); Ulva consists of polysaccharides with glucose (Olsson, Toth, Oerbekke, et al., 2020) and Saccharina comprises fucoidan, typically found in brown algae, and monosaccharides such as glucose (Bruhn et al., 2017). These are all sugars that could explain interference in the TBARS detection according to the study mentioned above.

# (C) Investigation of Strategy Within the Gothenburg Municipality Regarding School Lunches

#### City of Gothenburg's Sustainability Initiatives

Although the city of Gothenburg has the goal of serving climate friendly meals of a large volume, there are no state initiatives that support the food industry on a commercial level. When asked about such initiatives, the ESR responded that there were currently only small initiatives focusing on urban farming for small-scale producers. However, it was on their agenda to raise the political ambitions regarding a better sustainable food system in 2023.

It can be seen that although the state of Gothenburg has been developing the idea of *miljömåltider*, more support is perhaps required for the various food production sectors, and in this case - the fish and seafood sector. With the lack of food-related initiatives and with the public schools ordering from middle-managed large-scale buyers, there seems to be a

disconnection between the state's agenda of *miljömåltider* and the local food industries. Although the school and public meals are becoming increasingly environmentally-conscious, there is still a rift between the local (sea)food sources to the table.

### Conclusion

There are a few hurdles to jump over before the production process of this herring product can properly begin. As there is a current lack of state-funded initiatives towards fish and seafood production, but strong interest in generating a large number of climate-friendly meals, there may very well be an opportunity for Sweden Pelagic AB, the company that is providing the herring mince, to pitch the idea to the Gothenburg City Council and seek some form of support to continue developing the product, as this product represents what they are looking for.

Overall, the most promising values from all the trials were the blanched smoked *Saccharina* and smoked salted *Saccharina*, as well as the lingonberry variations. The phenolic components of the apple wood smoke transformed to the smoked seaweed may have played a role in the increased oxidative stability of the herring mince. However, due to the scarcity of papers regarding smoked seaweed, more research is required in this area in order to prove so. For the lingonberry variations, despite the fact that they are post-juiced press-cakes that are utilized in this project, they still show encouraging results in terms of preventing lipid oxidation, but more scientific research is also needed on the composition and usage of post-juiced press-cakes. Other than that, this project was designed to have a direct addition of antioxidative helper materials to the herring mince to determine levels of lipid oxidation so that it could be a process that could be easily scaled up once some of these knowledge gaps are filled in.

As this project is in its early stages of a potentially bigger project, this is only the first step in the sustainable development of "blue-green" products by using co-products as much as possible to make a smaller food loop, as well as fully maximising the use of the nutritious herring for human consumption and reducing environmental impact. The development of this nutritious and sustainable herring product could potentially contribute to the state of Gothenburg's city goals of serving an environmentally friendly meal, if successfully scaled up.

### **Future Perspectives**

Since this project is still in early stages in the big picture, there are many aspects of the experiments that could be further developed in the future.

The experiments for the ice-storage trial were initially planned to comprise of 5%, 10% and 20% of antioxidative materials combined with herring mince. However, with the number of variations of the samples, it proved to be far too many for the time allocated to this thesis. Since only 10% of antioxidative helper materials to herring mince was used in this project, more trials can be repeated with different increments of helper materials to better identify how much lipid oxidation could be prevented by each helper and/or with various levels of helper material.

There were some aspects in the methodology that could also be improved. For example, there was a lack of food-grade equipment to get the seaweed flakes grinded into a finer powder for better homogenisation and while the method of hammering the flakes when frozen and brittle worked on a small scale, it may be difficult with a larger amount. Being able to get the seaweed pieces into a finer powder also increases its surface area, which may provide better antioxidative capacity when mixed with the herring mince. Furthermore, the finer powder may

increase homogenisation with the herring mince, in order to measure colour with a colorimeter than comparing it with the naked eye.

As for smoking of the helper materials and as previously mentioned in the discussion, controlled techniques of smoking may be required for a more precise result. There may also be a future in further developing the herring mince by smoking the fish, or mince itself, as research on other types of smoked, fatty fish have proved to maintain a long shelf-life.

In regards to the various stakeholders involved, there still seems to be a lot of red-tape to overcome from the local state and government as the sustainable food agenda is still just being discussed. More interest, transparency, communication, urgency as well as research, is required between the various food industries to be able to fully maximise and utilize their existing products and co-products. This project is hopefully a step towards this direction and the beginning of many more to come.

#### Acknowledgements

For even making this wonderful project possible, a big thank you to both Snuttan and Ingrid. Thank you to Dan and Martin from Pelagic Seafood Sweden and Sweden Pelagic AB for providing the delicious herring mince. There is so much potential in this product, which I really hope to see out in the stores one day (and would be happy to help continue developing).

For the seaweed components, thank you Mar, it has been fun smelling seaweed with you and I had a *wheely* good time at the Science Festival 2022.

Thank you Bovie, Semhar, Jingnan, Haizhou and Xueqing for all the help and invaluable advice with the lab-work.

Last but not least, thank you James, for providing help and support with the writing of this thesis.

### Appendix A



Fig. A1 Different combinations and increments of antioxidative materials and herring mince

The herring-fish balls were randomly arranged and numbered on a plate for three panellists to have a blind tasting. They were asked to judge the herring-fish balls based on the taste, looks and texture.



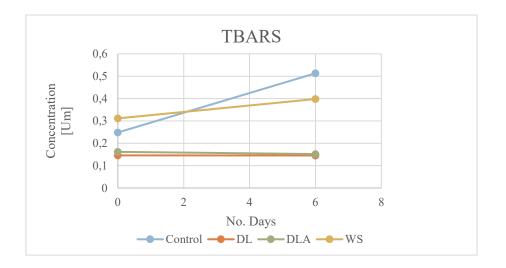
Pic. A2 Cooked herring-fish balls for blind tasting

The comments were collected and summarized after the tasting:

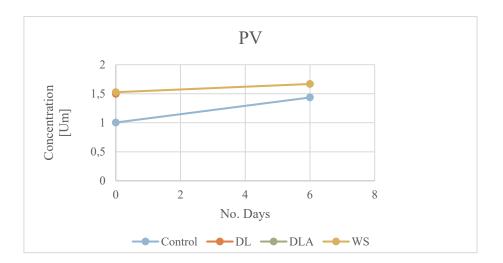
No.	Helper	Comments
06	Ulva 5%	very "seaweedy" but still nice, green, nori, interesting colour, salty, umami, no difference from 1, strong ulva taste
01	<i>Ulva</i> 10%	umami, green, grey, nori, spinach
13	Ulva 20%	too much ulva, nice texture, good, seaweed umami, too green/dark, hard, not appetising, no taste of herring, borderline too much
03	Wet Lingon 5%	good balance, tasty fish ball, very fishy, nothing ? Control
10	Wet Lingon 10%	a lot of lingon, off taste but still good, crumbly, sour, too much lingon, looks brown/red
16	Wet Lingon 20%	strong helper taste, very salty, crumbly, dry, too much lingon, weird mush texture
12	Dried Lingon 5%	god, nice consistency, metallic, no difference from 7, meatball texture
07	Dried Lingon 10%	strong lingon aftertaste, not good, chemical flavour, good consistency, looks appetising, interesting
02	Dried Lingon 20%	fruity, grynig, dry, acidic, little lingon berry flavour, a lot of lingon berry
08	Wet, Unblanched Saccharina 5%	nice, little green, not too much, slimy, chewy, good balance
05	Wet, Unblanched <i>Saccharina</i> 10%	good flavour but slimy, good ratio, nori, not slimy, god!
15	Wet, Unblanched Saccharina 20%	too "seaweedy", wet and loose texture, too much seaweed, slimy, nice, a lot of helper but mild taste
14	Dried, Blanched Saccharina 5%	nice, good balance, nice texture, seaweed visible but not unpleasant, no difference from 6, good balance
11	Dried, Blanched Saccharina 10%	too "oceany", doesn't look appealing, same as 4, körvel?, strange taste, not so good
04	Dried, Blanched Saccharina 20%	Ej god, dark, slimy yet dry, chewy, too much seaweed, nice texture
09	Control	control? Nice texture, tasty, salty, plain

### **Appendix B**

To measure the level of lipid oxidation in the pre-trial, samples from Day 0 and Day 6 were put through TBARS (Fig. B1) and PV analysis (Fig. B2).

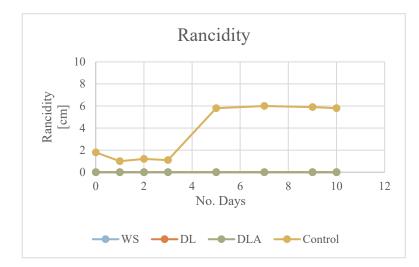


**Fig. B1** TBARS Analysis (WS = Wet, unblanched *Saccharina*; DL = Dried Lingon; DLA = Dried Lingon Adjusted pH)



**Fig. B2** PV Analysis (WS = Wet, unblanched *Saccharina*; DL = Dried Lingon; DLA = Dried Lingon Adjusted pH)

A sensory screening analysis was also recorded for the duration of the ice-storage pre-trial and graphed (See Fig. B3-B5).



**Fig. B3** Rancidity Chart (WS = Wet, unblanched *Saccharina*; DL = Dried Lingon; DLA = Dried Lingon Adjusted pH)

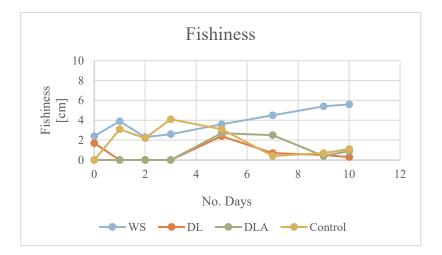
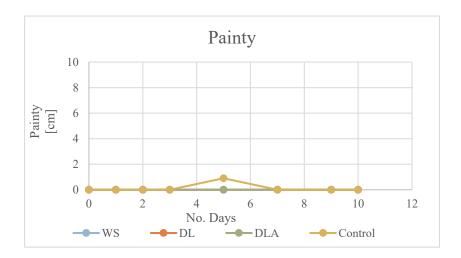


Fig. B4 Fishiness Chart (WS = Wet, unblanched *Saccharina*; DL = Dried Lingon; DLA = Dried Lingon Adjusted pH)



**Fig. B5** Painty Chart (WS = Wet, unblanched *Saccharina*; DL = Dried Lingon; DLA = Dried Lingon Adjusted pH)

## Appendix C

Material	Dry Matter Content	Mean	%		
Herring	28.06	26.72	0.27		
	25.65			Material Dr	y Weight corr. to Herring
	26.47			Mass	Helper per 100 g Herring
Lingon	56.94	57.54	0.57	2.67	4.64
Wet	58.27				
	57.43				
Lingon	65.08	67.29	0.673	2.67	3.97
Wet	68.24				
Smoked	68.56				
Lingon	94.37	94.25	0.94	2.67	2.83
Dried	94.07				
Powder	94.32				
Saccharina	5.07	5.38	0.05	2.67	49.68
Blanched, Wet	5.48				
Flaked	5.59				
Saccharina	2.56	2.59	0.027	2.67	102.93
Blanched, Wet	2.29				
Smoked	2.94				
Saccharina	91.49	91.26	0.91	2.67	2.93
Blanched,	01.01				
Dried	91.21				
Powder	91.1	12 50	0.14	2 (7	10.((
Saccharina Unblanched,	14.02	13.59	0.14	2.67	19.66
Wet	13.25				
Flakes	13.51				
Saccharina	14.41	13.93	0.14	2.67	19.18
Unblanched,	11	10170	0.11	,	
Wet	13.6				
Smoked	13.8				
Saccharina	92.87	92.96	0.92	2.67	2.87
Unblanched,					
Dried	93.1				
Ground	92.92	22.05	0.00	2 (7	11.62
Saccharina	22.42	22.97	0.23	2.67	11.63
Salted, Wet	23.57				
Flakes	22.92	22.00	0.00	2.67	10.10
Saccharina	21.81	22.08	0.22	2.67	12.10
Salted, Wet	21.55				
Smoked	22.88	07.07	0.07	2.67	2.07
Saccharina	87.01	87.37	0.87	2.67	3.06
Salted, Dried	87.52				
Powder	87.59				

Ulva Wet	20.9 20.48	20.82	0.21	2.67	12.84
Flakes	21.08				
Ulva	24.2	28.24	0.28	2.67	9.47
Wet	31.29				
Smoked	29.22				
Ulva	93.87	93.61	0.93	2.67	2.86
Dried	93.43				
Powder	93.52				

Table C1. Calculations of Dry Matter Content

### **Appendix D**

Sensory screening results for fishiness and paintiness values in all the ice storage trials.

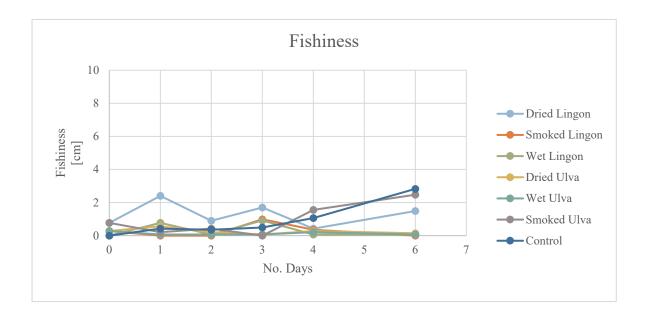


Fig. D1 Sensory assessment of fishiness in IST1

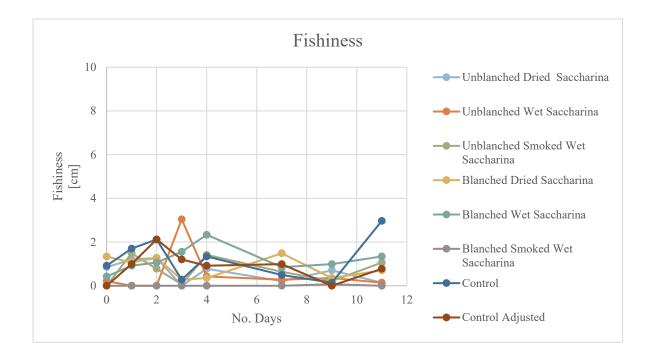


Fig. D2 Sensory assessment of fishiness in IST2

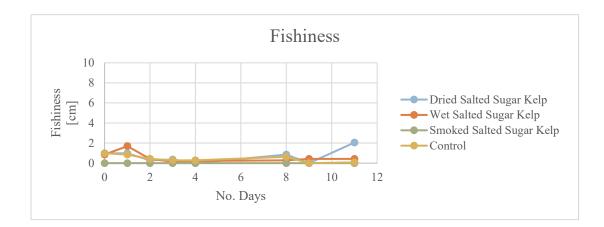


Fig. D3 Sensory assessment of fishiness in IST3

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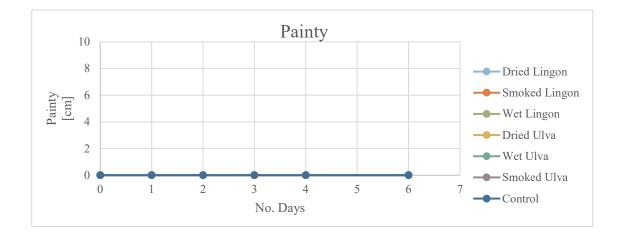


Fig. D4 Sensory assessment of paintiness in IST1

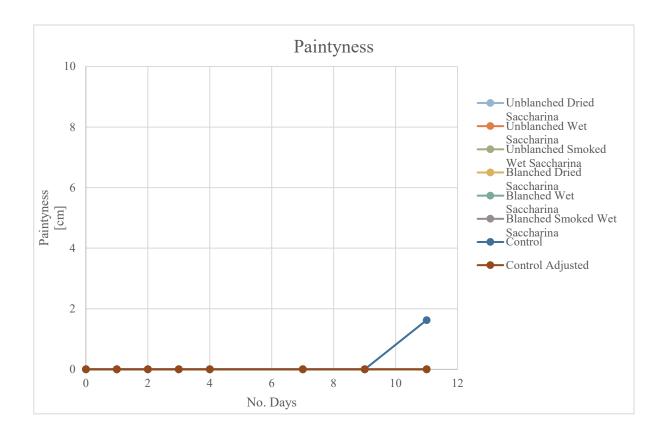


Fig. D5 Sensory assessment of paintiness in IST2

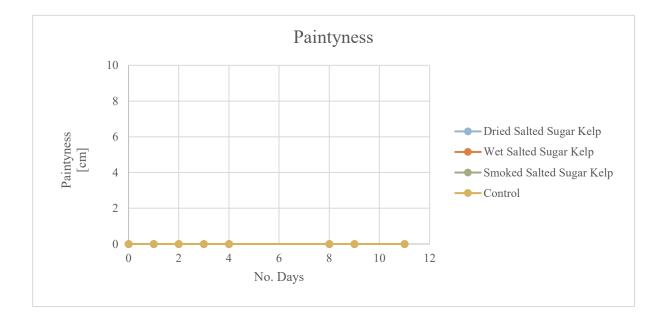
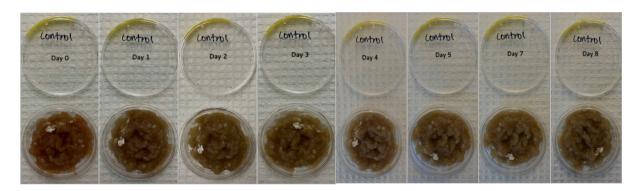


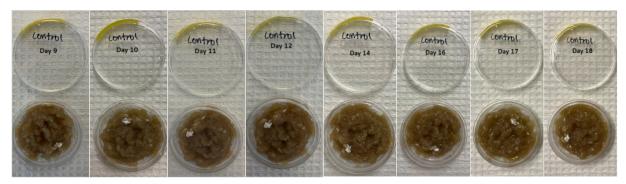
Fig. D6 Sensory assessment of paintiness in IST3

### Appendix E

#### Ice Storage Trial 1

The petri-dishes of the samples were photographed from Day 0 and almost daily until Day 18. *Control – Herring Mince* 

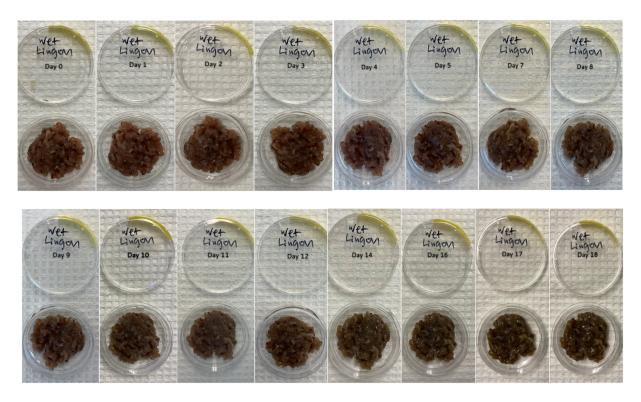




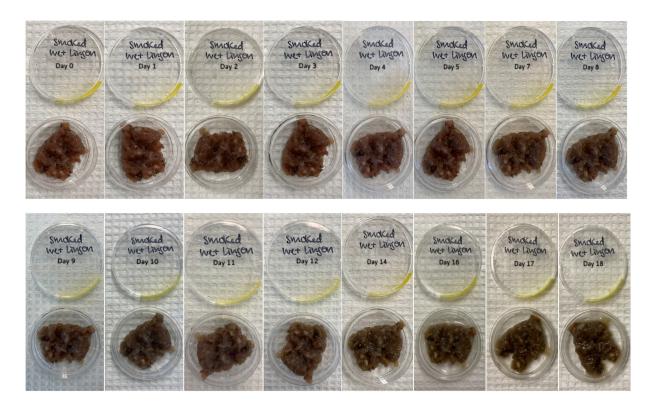
DL - Dried Lingonberry Press-cake Powder with Herring



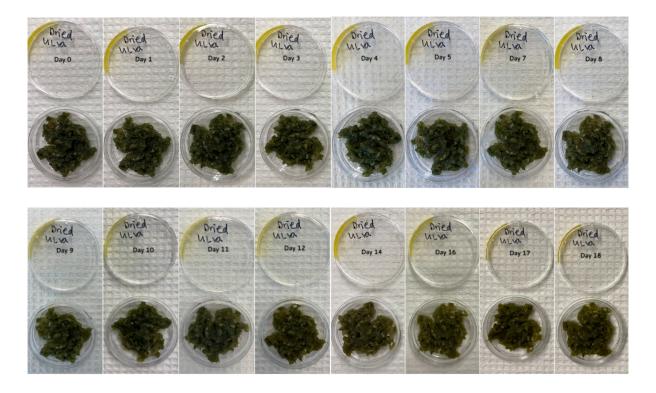
#### WL - Wet Lingonberry Press-cake with Herring



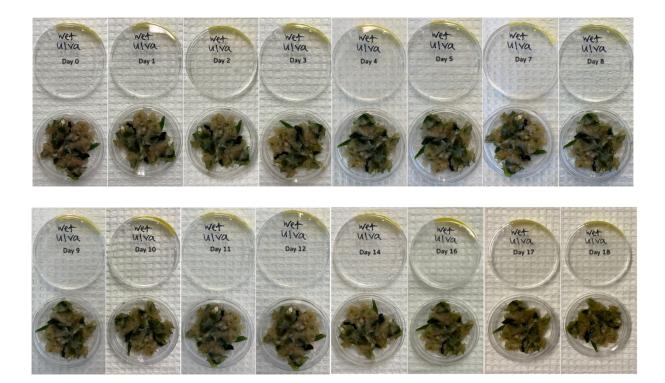
SL – Smoked Wet Lingonberry Press-cake with Herring



#### DU – Dried Ulva Powder with Herring



WU – Wet Ulva Flakes with Herring



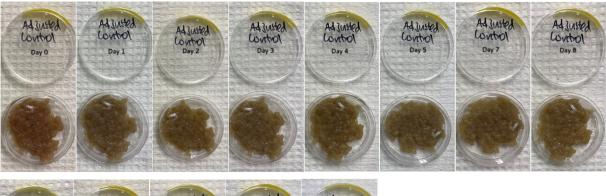
#### SU – Smoked Wet Ulva Flakes with Herring

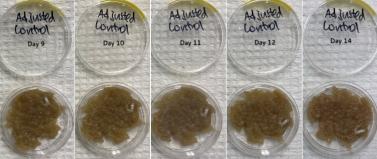


### Appendix F

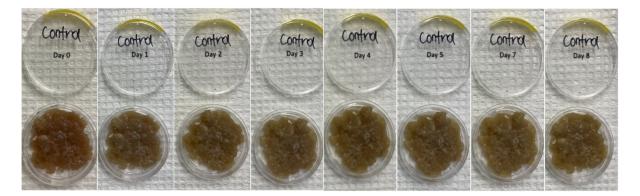
#### Ice Storage Trial 2

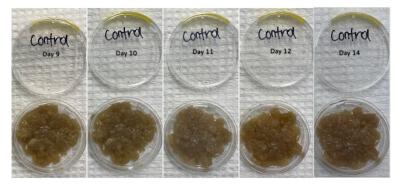
The petri-dishes of the samples were photographed from Day 0 and almost daily until Day 14. *Control Adjusted – Herring Mince with Added HCl to Lower pH* 





Control – Herring Mince

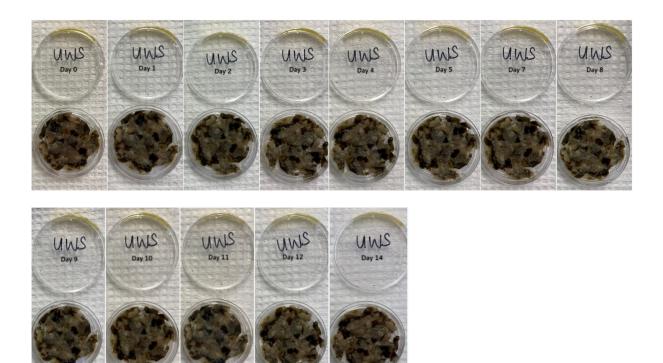




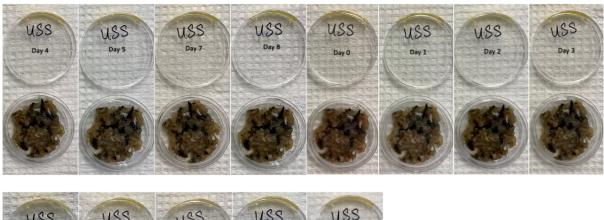




UWS – Unblanched Wet Saccharina with Herring

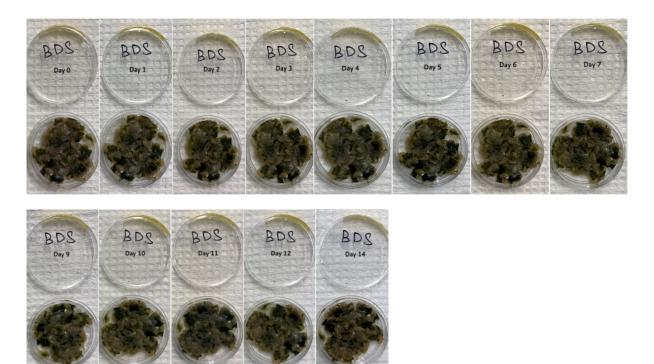


#### USS – Unblanched Smoked Wet Saccharina with Herring

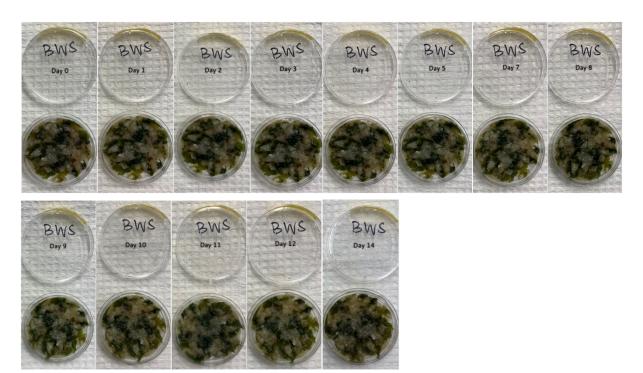




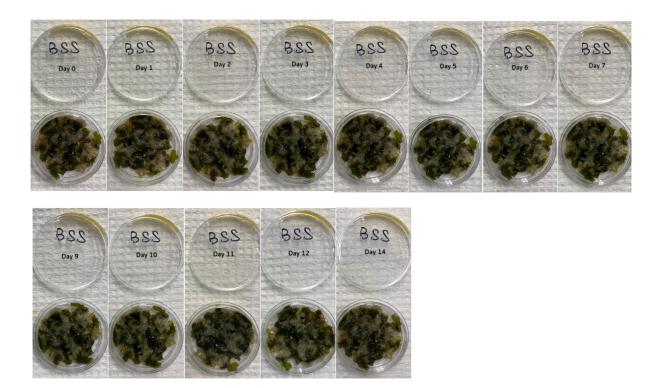
**BDS** – Blanched Dried Saccharina with Herring



#### BWS – Blanched Wet Saccharina with Herring



BSS – Blanched Smoked Wet Saccharina with Herring



### Appendix G

#### Ice Storage Trial 3

The petri-dishes of the samples were photographed from Day 0 and almost daily until Day 15.

#### **Control – Herring Mince**



DSSK – Dried Salted Sugar Kelp with Herring

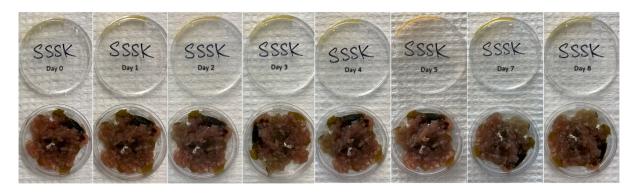




#### WSSK – Wet Salted Sugar Kelp with Herring



SSSK – Smoked Salted Sugar Kelp with Herring





### Appendix H



#### Recipe

- Makes 4 herring balls
- 75 g herring mince
- 9 g smoked salted Saccharina
- 1/8 teaspoon salt
- 1 teaspoon potato starch
- <sup>1</sup>/<sub>4</sub> teaspoon onion, grated
- 1/8 teaspoon garlic, grated

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