Pelagic fish discards

Technical Report on opportunities for silage valorisation

Publication date: December 2015

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European Fisheries Fund: Investment
In sustainable fisheries

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Summary

This report is compiled in the framework of evaluating the potential of pelagic discard fish valorisation, as a consequence of the landing obligations of the European Commission as effective from January 1st 2015. The report describes the technical back ground for the production of fish silage from pelagic discards. In line with other activities in a search to evaluate the potential to valorise pelagic discards.

The silage process results in a stabile product, which is usable as ingredient for livestock. However, during long term storage (longer than a week) the degree of hydrolysis is increasing strongly. This results in a low abundance of polypeptides, having the effect that no bioactivity is present. The product then has a lower added value in livestock feed application, in comparison to e.g. soy protein. The business case for the marketing of such a quality silage is week as a replacer for fish meal and alternative protein sources in livestock grow on feed. The perspectives for application in starter feed are better (however not optimal), which is currently in investigation. In starter feed the ingredient availability, feed efficiency and specific amino acid composition is higher valued.

A valuable product with bioactive properties can be produced using a process of directed hydrolysis. The value of such a hydrolysate can be significantly higher than soy protein. In literature a price for such products is found, significantly higher than the price of silage. For the reason that the livestock industry is searching for anti-biotic replacers, this is a development with perspective. This is however a pathway, which should be further developed prior to commercial implementation.
There is a potential alternative by decreasing the ensilaging time (practically: feed within several days), which keeps a substantial part of the peptides intact. Potentially, also a significant bioactive property is present. At this moment knowledge is lacking to support the economic feasibility, since it is unknown what peptide profiles can be expected. The valuing of such a product with an additional health claim is still a long road ahead, since no only the product, but also the process needs to be sufficiently stable and robust. Furthermore marketing with health claims requires a specific (and costly) pre-assessment within the European legal framework.

A full summary in the framework of discard fish valorisation is reported separately in the report: “Pelagische discards: Ketenanalyse en productverkenning voor valorisatie discards en bijproducten Bevindingen en conclusies.”(in prep, 2016).

1 Scope

This report is compiled in the framework of evaluating the potential of pelagic discard fish valorisation, as a consequence of the landing obligations of the European Commission as effective from January 1st 2015. Following an inventory of the expected volumes and composition of discards in the pelagic industry. An inventory of the potential processing possibilities for pelagic discard fish was performed. In discussion with the industry a long list of potential processes was generated of which two business cases were selected to be further described (silage with extraction of bio-active peptides, and hydrolysis for bio-active peptides).

This report describes the technical back ground for the production of fish silage from pelagic discards. In line with other activities in a search to evaluate the potential to valorise pelagic discards.

2 Definition of silage

Fish silage is a brown liquefied product by the action of enzymes prepared by acidifying finely grounded whole fish or parts of fish. Fish silage is produced from fish of low commercial value, not suitable for consumption or further processing, and from by-products from fish processing. Discards fall in this category of unwanted fish on-board that before the introduction of the landing obligation could be thrown over-board in the sea. They also include fish of low quality for example those damaged during the fishing process.

During the silage production process, endogenous enzymes hydrolyse the proteins in the fish in the presence of added acids or acid-producing bacteria via carbohydrate fermentation. Depending on the process used, the final product is an acid silage or a fermented silage. The enzymes increase their activity in the acidic medium and breakdown fish proteins into smaller soluble units. Acids, in addition to creating the optimal conditions for the enzymatic activity, dissolve the bones and the cartilaginous tissue and decrease the pH which inhibits the growth of mould and spoilage bacteria, making long term storage possible. With proteins hydrolysed into free amino acids, fish silage is the most available amino acid source for protein biosynthesis in animal and fish nutrition.

The manufacturing process of fish silage needs low investment costs, uses low technology and low energy and is more economical than that of fish meal. Stabilised fish silage can be kept at room temperature without the need of cooling. Fish silage is a convenient protein-rich ingredient used locally, but its high water content makes long distance transport uneconomic. It can be produced in small isolated places where fish meal plants cannot be operated economically and from which the transport of small amounts of relatively low value fish and offal over considerable distances to the nearest factory for reduction is not economical. Since no fish meal plant exist in the Netherlands, production of fish silage can be a profitable use of landed fish discards.

3 Characteristics of pelagic species to support a value chain

The Dutch pelagic fishery is dominated at nearly 90% by herring (*Clupea harengus*), mackerel (*Scomber scombrus*), horse mackerel (*Trachurus trachurus*), blue whiting (*Micromesistius poutassou*) and boarfish (*Capros aper*). Among them, three fish species are considered oily: herring (14-18%), mackerel (11.3-21.3%), horse mackerel (8.0-12.3%) and two fish species are considered slightly lean: blue whiting (3.1-5.4%) and boarfish (4.8%). Sardine, another fatty fish is caught in small quantities and rarely kept on-board (Goudswaard, 2014). For detailed composition and seasonal fluctuations of pelagic discards, we refer to Kals et al. (2015[1]). On an annual basis, oily fish represent 72% of the total discards from pelagic fisheries landed in the Netherlands. This percentage increases between 98 and 100% of the total volume between July and December. Fatty fish are only 61% in January-March and absent (0%) in April-June (Figure 1).

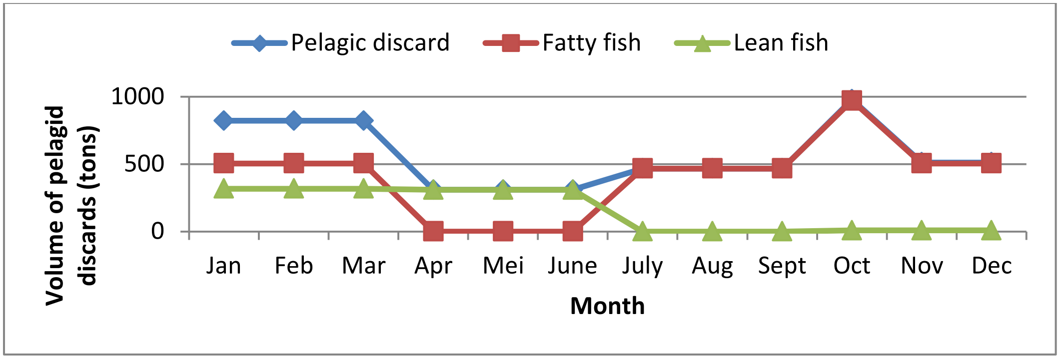

Figure 1. Estimated landings of discards from pelagic fisheries estimated for 2015 landed in the Netherlands (based on 3% discards from the total catch). (source of data: Kals et al., 2014).

A high and seasonal variation in fish composition and especially in oil content in pelagic discards make the silage product unpredictable. The variation in chemical composition, particularly of oil content, reflect natural fluctuations during growth, maturation and feeding, underlining the difficulty in predicting accurately the chemical composition of the silage from a mix of seasonally varying volumes of fatty and lean fish. The high oil content implies the necessity of an oil removal step during the fish silage processing of pelagic discards to guarantee a silage product of stable quality. Oil should be removed and refined as a secondary product to silage production.

[1] Kals J., Poelman, M., Blanco A., Goudswaard K. (2015). De samenstelling van discards in de pelagische visserij voor valorisatie doeleinden. IMARES Report C023/15.

Since January 1st 2015, an European obligation of landing pelagic by-catches has been introduced. These fish which are not suitable for human consumption constitute a valuable raw material that can be used to produce low-value bulk products such as silage, fish meal and high value products such as fish oil, protein hydrolysates and others. In 2011, the total volume of discards of quota species in the Dutch fisheries sector amounted to more than 57 thousand tonnes (Buisman et al., 2013[2]), of which just under 10 thousand tonnes were in the pelagic fishery sector. This volume can vary per year depending on the quota and the total amount can decrease due to a higher selectivity during fishing operations. The volume of pelagic discard is estimated at 7 thousand tonnes in 2015 based on 3% discards of the total catch (Goudswaard, 2015[3]). Dutch pelagic fish are landed frozen mainly in IJmuiden, followed by Scheveningen, Vlissingen and incidentally Den Helder, where infrastructures for frozen storage are available. The largest quantity of fish is landed in Ijmuiden, followed by Scheveningen. The annual quantities of pelagic discards landings are estimated at 3250 tonnes for IJmuiden, 1300 tonnes for Scheveningen and 650 tonnes for Vlissingen. Fish discards from foreign owned fishing vessels (mainly Danish) landing their discards in Dutch harbours are not included in the estimations of landed volumes. Irrespective of the landing harbour, the total volume of pelagic discards landing per month varies between 300 and 980 tonnes with around 500 tonnes per month during almost 9 out of 12 months per year (Figure 1).

Demersal discards are estimated at about 47 thousand tons per year (Buisman et al., 2013) and 56 thousand tons (Goudswaard, 2015). Dutch demersal fisheries are dominated by 2 fish species which constitute more than 75% of the demersal discards (Goudswaard, 2015). These are lean fish with low fat content made up of the European plaice (Pleuronectes platessa): 20 thousand tons and the common dab (Limanda limanda): 17 thousand tons. In contrast to pelagic discards, the supply of demersal discards is bigger in volumes and is more or less equally spread over the year. Its composition is less variable and discards fish can be supplied fresh on ice. The main landing harbors for demersal discards are Den Helder (13 thousand tons), IJmuiden (9 thousand tons), Vlissingen (8 thousand tons), Stellendam (7 thousand tons), Harlingen (6 thousand tons) and Scheveningen (4 thousand tons). We refer to Goudswaard et al. (2015) for detailed data. There are also fish viscera that are removed at sea and are presently discarded. They are potentially good sources of several essential amino acids and oil (from livers).

[2] Buisman E., van Oostenbrugge H. and Beukers R. (2013). Economische effecten van een aanlandplicht voor de Nederlandse visserij. LEI rapport 2013-062.

[3] Goudswaard PC. (2014). Van discards naar bijvangst in de pelagische visserij. Imares rapport 067/15

Implications of freezing discards for further processing The effects of freezing on fish proteins are controversial. According to Mackie (1993[4]), freezing causes little damage on proteins. Freezing appears to slow the activity of enzymes (Dr. A. Meijer and colleagues in a 1977 article in "Histochemistry and Cell Biology) and the activity of the enzyme is more disturbed by the formation of ice crystals. Conflicting views exist also in literature on the effects of freezing on the protection of proteins from denaturation. This protective effect is considered to be due to the presumed dissolution of released free fatty acids in the lipids, thereby neutralizing their interaction with protein. This implies that denaturation of proteins on frozen storage depended on fatty acid production and the intervention of neutral lipid (Mackie, 1993). Freezing at around -20^{0}C offers a practical solution in pelagic fishery since it stabilises the product by stopping microbial growth and reducing autolysis and lipid oxidation. High lipid content products such as the cod liver need even colder freezing temperature (-45^{0}C) to prevent lipolysis and production of free fatty acids.

Freezing process does not seem to affect the fish silage process. According to Tatterson and Malcolm (1974[5]), no difference in the quality of silage has been shown between the use of fresh and frozen raw material. Fish silage has been successfully produced from frozen material from different fish species after thawing. The list is not exhaustive but include lean and fatty fish from different latitudes: blue whiting (*M. poutassou*) frozen at -28^{0}C for several months (Dapkevičius et al., 1998), whiting (*Gadus merlinger*) and mackerel (*Scromber scombus*) frozen at -20^{0}C for 4 days (Machin et al.,1990[6]), tropical fish silverbelly (*Leiognathus splendens*) frozen at -22^{0}C (Hall et al., 1985[7]), Indian sardine (*Sardinella longiceps*) frozen at -20^{0}C (Al-Marzooqi et al., 2010), by-products from tuna processing frozen at -18^{0}C (Mousavi et al., 2013[8]). Pelagic discards can be frozen and further processed into fish silage without affecting much the quality of the silage product.

Pelagic discards may be shredded on board

In grounded fish, polyunsaturated fatty acids (PUFA) in fish oils become highly susceptible to oxidation. If fish should be grounded on-board and further processed into fish silage, oxidation of lipids need to be prevented by the addition of antioxidants and storage in darkness and in the absence of air. Many synthetic antioxidants exist on the market, such as butylated hydroxy toluene (BHT), butylated hydroxy anisole (BHA), tertiary butyl hydro quinone (TBHQ), and propyl gallate (PG). Seldox ESG is a strong anti-oxidant used in the prevention of oxidation of salmon oil (Rurangwa et al., 2014[9]). Alternative natural antioxidants have been used and include tea, rosemary, oregano, onion. Protein hydrolysates and certain amino acids of marine origin inhibit also lipid oxidation (Shahidi and Zhong, 2007[10]). Marine algae contain a variety of ant oxidative substances, including phenolics, and can potentially be utilised as a source of natural antioxidants. Oxidised products are unpalatable or unsafe for animals (Dapkevičius,, 1998[11]).

Freezing pelagic discards on-board is possible and more indicated than producing grounded fish and further production of silage on-board. The lack of space on the fishing vessel, limited time and skills of the fishing crew does not allow the combination of fishing, sorting, freezing and silage production on-board.

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[4] Mackie I.M. (1993). The effects of freezing on flesh proteins. Food Reviews International 9 (4), 575-610.
[5] Ian N. Tatterson and Malcolm L. Windsor (1974). Fish silage. *J. Sci. Fd Agric.* 25, 369-379.
[6] Machin D.H., Panigrahi S., Bainton J. and Morris T.R. (1990). Performance of broiler chicks fed on low and high oil fish silages in relation to changes taking place in lipid and proteins components. Animal Feed Science and Technology 28, 199-223.
[7] Hall G.M., Keeble D., Ledward D.A. and Lawrie R.A. (1985). Silage from tropical fish 1. Proteolysis. Journal of Food Technology 20, 561-572.
[8] Mousavi S.L., Mohammadi G., Khodadadi M., Keysami M.A.(2013). Silage production from fish waste in cannery factories of Bushehr city using mineral acid, organic acid, and biological method. Intl J Agri Crop Sci. Vol., 6 (10), 610-616.
[9] Rurangwa E., van Vuuren A. and Poelman M. (2014). Fish silage as feed ingredient for fish and livestock. IMARES report C135/14.
[10] Shahidi F. and Zhong Y. (2007). Antioxidants from marine by-products. In Shahidi F. (Ed.): *Maximising the value of marine by-products (ISBN: 978-1-84569-013-7)*
[11] Dapkevičius M.L.E., Batista I., Nout M.J.R., Rombouts F.M. and Houben J.H. (1998). Lipids and protein changes during the ensilage blue whiting (*Micromesistius poutassou* Risso) by acid and biological methods. Food Chemistry 63 (1), 97-102.

4 Flow scheme fish silage production (incl. oil separation and drying option)

Three production methods of fish silage exist and are based on the addition of acids, enzymes or lactic acid producing bacteria (ex. *Lactobacillus* sp.). A source of carbohydrate (ex. molasses) is added if microbiological methods are used. The acid method is the most common and different mineral acids (hydrochloric, sulphuric) and organic acids (formic, acetic, propionic) and their mixtures are used. Fish silage is made by mixing 2-3.5% acids into the minced raw material and storing at ambient temperatures until endogenous enzymes (pepsins and other acid proteases) have dissolved the fish tissue. It is important to mix thoroughly so that all the fish comes into contact with acid, because pockets of untreated material will putrefy. The process for complete protein liquefaction can take up to 1 week and is dependent on the ambient temperature, the pH, the quality and the freshness of the starting material. Organic acids are more expensive than inorganic acids but have better anti-microbial properties than inorganic acids. When using mineral acids the silage should reach a pH of 2. With the use of organic acids, preservation is achieved at a higher pH and therefore the silage does not require neutralization prior to use in animal feeds like in the case of mineral acids. A well-preserved fish silage has a pH of 3-4, the optimum pH for fish pepsins. The different steps for silage production process and oil removal are illustrated in Figure 2.

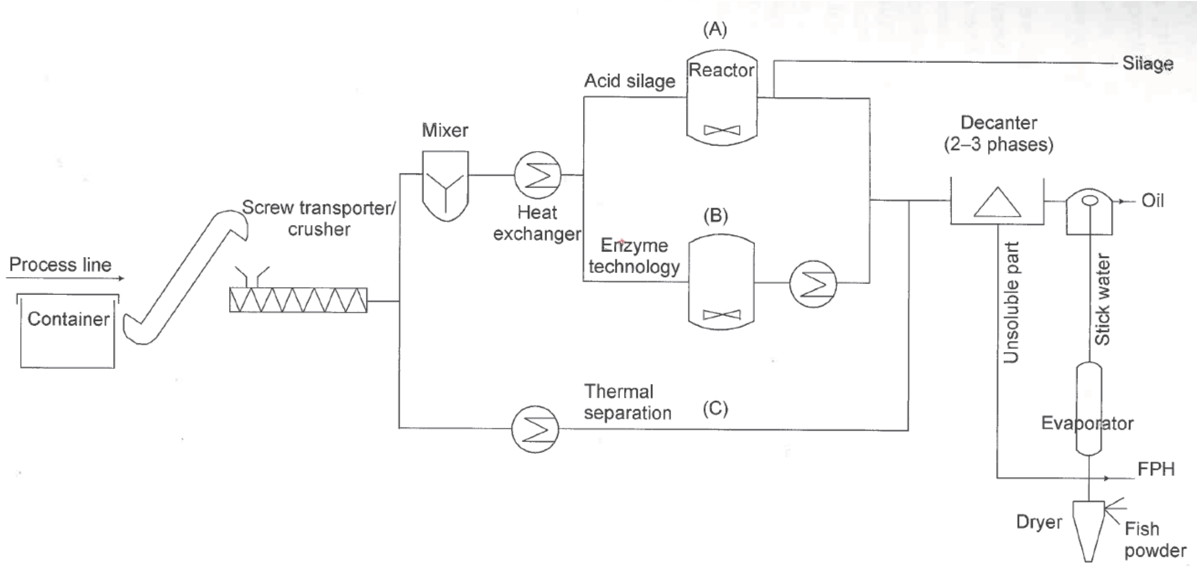

Figure 2. Silage production with addition of acid (A). Controlled hydrolysis of proteins by a reaction catalysed by added enzymes (B). Traditional production process of cod liver oil by thermal treatment followed by centrifugal separation (C). Bulk products from raw material are fish silage, fish oil, fish proteins hydrosylates (FPH) or fish powder. Source: Falch et al. (2007[12]).

The common procedure for fish oil extraction include heating-pressing-centrifugation (FAO, 1986[13]). The heating process liberates the oil from the fat depots of fish and condition the material to subsequent treatment. The walls of fat cells are broken down before the temperature reaches 50^{0}C making the oil free. Heating fish silage to 80-90^{0}C facilitates oil separation from solids but risks to denaturate the proteins of the silage products. In industrial processes aiming first at the production of premium oil, the raw material is heated to 70-90^{0}C (Rustad et al, 2011[14]). If fish oil is the target product, it is recommended to de-oil the fish mash before adding acids but this is only recommended if it is a fatty fish and when collecting, ensilage and drying operations are done centralized. When the oil is removed at the end of the silage process i.e. after the addition of acid, the oil recovered from fatty fish material is of bad

[12] Falch E., Sandbakk M. and Aursand M. (2007). On-board handling of marine by-products to prevent microbial spoilage, enzymatic and lipid oxidation. In Shahidi F. (Ed.): *Maximising the value of marine by-products. Pp. 47-64.*
[13] FAO, 1986. The production of fish meal and oil
[14] Rustad, T., Størro, I. and Slizyte R. (2011). Possibilities for the utilisation of marine by-products. International Journal of Food Science and Technology 46, 2001-2014.

quality. It contains high levels of free fatty acid (FFA) released during liquefaction (Reece, 1981[15]). The long incubation period results in a breakdown of the oil by a low pH optimum lipase present in some fish species. Oil recovered from fish silage is darker in colour than from fresh fish and has a reduced iodine value. Pigmentation of the oil is caused by haemin, a product of acid hydrolysis of haemoglobin. In pelagic species where the haemoglobin content of blood is high, pigmentation of oil is particularly obvious. Addition of hydrogen peroxide has been shown to inhibit oil pigmentation and to reduce the FFA content in the recovered oil.

Although heating results in the release of the oil and a major part of the water that can be removed from the solids by simple drainage, removal of more liquid is achieved by subsequent pressing and centrifugation of the solids. Currently decanters instead of pressers are most indicated to separate by centrifugation solids from liquids of very fluid material like fish silage. 2-phase decanters (clarifying decanters) are used for the continuous separation of solids from liquids with a high solid content. Separators are used to separate oil and water from the liquid fraction. Wittemore and Taylor (1976) heated herring silage to 75°C and centrifuged with a GEA Westfalia decanter to remove solid particles. The effluent from the decanter was reheated to 70°C to separate into solids, effluent and oil with a separator. The fractions other than oil were mixed to form the de-oiled herring silage. 3-phase decanters achieve high separation efficiencies of solids, oil and water in one operation.

At the end of the process of separation of oil from solids, leftover sticky water is evaporated and the silage can be dried for stable storage in powder. In the fishmeal industry, the drying temperature does not exceed 90^{0}C to maintain nutritional value of the product. Evaporation at high temperatures affects the quality of the final product since some of the vitamins and amino acids are sensitive to heat. Vitamin B12 and important amino acids, cysteine, lysine and tryptophan are lost at a temperature above 130^{0}C at any length of time. Heating also destroy bacteria, like Salmonella, that may infect the product during early process.

Application of pelagic fish silage products in the Netherlands Fish silage is used in the same way as fish meal in animal feed. Fish meal contains about 65% protein meaning that about four times as much silage (about 16% protein) is required for the same protein intake. Fish silage is a semi-liquid product which can be either concentrated into a paste-like product or dried into a fish powder after removal of oil and evaporation of water. Fish silage can be dried using wheat bran as a dehydration carrier and processed into dried silage meals. To prevent spoilage, the dried silage should have a low moisture content. However, to our best knowledge, fish silage is not yet listed as feed ingredients for livestock and fish in the Netherlands although it is in Norway. Similar processes have been reviewed by EFSA (European Food Safety Authority) on food Safety aspects (Anonymous, 2013[16]).

A silage of lean fish may be used directly in animal rations without de-oiling. Because of their high oil content, silage from pelagic discards cannot be used without de-oiling in animal feeds to prevent the "fishy taint" of animal food products (Raa and Gildberg, 1992). The fat content of Dutch pelagic discards vary significantly between fish species and seasons in the range between 3.1 and 21.3% fat. The protein content of pelagic discards does not vary much between fish species and seasons and falls in the same range between 16 and 19% for different fish species (Kals et al., 2015). Fish silage concentrate contains highly digested proteins and is a cheap alternative source of proteins to fish meals in animal feeds. It provides essential amino-acids, often lacking in vegetable protein sources.

The most suitable outlet for silage appears to be in pig farming, since it can be used in liquid feeding systems or mixed with carbohydrates and fed as a moist or dry mixture. Feeding trials show that pigs grow as fast on silage as on meal, and the quality and flavour of the meat is good. Fish silage is used in the Danish pig industry, and most nutritional work has been done there. Feeding trials conducted in Denmark suggested that if the oil is removed from the herring, the silage may be a much more acceptable product for supplementing the protein in diets for growing pigs (Hansen, V. Bertn. Forsøgslan. (1970), 385, 42 in: Wittemore and Taylor, 1976). In a study conducted in UK, 100 g oily silage from mackerel per kg diet dry matter resulted in best performance and feed conversion rate in pigs with FCR:1.96 and a daily live weight gain of 725 g without adverse effects on the carcass quality (Green et

[15] Reece P. (1981). Recovery of High Quality Oil from Mackerel and Sprat by the Silage Process. *J. Sci. Food Agric.* 1981, *32,* 531-538

al., 1988[16]). At high oily fish silage inclusion level (150 g per kg diet dry matter), soft yellow fat was deposited and the meat unacceptable for trade. A reduction in food intake was observed as a consequence of differences in feed palatability and associated to fat content and possible fat oxidation in diets. Using a higher concentration of de-oiled herring silage (252 g/ kg diet dry matter), Whittemore and Taylor (1976) observed that pigs fed de-oiled silage herring silage ate slightly less than pigs fed a diet containing fish meal. The amount of fish silage incorporated into a diet is constrained by the adverse effects of high levels of dietary fish oil on daily food intake and the quality of the carcass. Trials in UK have estimated the maximum permissible level of oil in pig feeds at a dry matter inclusion level of 2.5%, corresponding to a fish oil content of about 1% in the compounded feed (Tatterson, 1982[17]).

In Australia pigs fed acid silage in barley-based diets grew faster and more efficiently than control animals fed soybean meal as a protein supplement (Reilly, 1985[18]). Acid and fermented silages were highly digestible in terms of nitrogen and energy in pigs. Fish silage made from white fish offal can replace conventional white fish meal and soya bean meal in diets for bacon pigs and does not affect the pork quality (Whitternore and Taylor, 1976[19]).

In the Netherlands, pig farming and processing is concentrated in the South-Eastern part (Gelderland and North Brabant) at 150-250 km far by road from discard landing harbours, all located on the Western part of the country (Figure 3). Broiler chicken production is fairly distributed across the country with a high concentration in the centre and the Eastern part of the country (Figure 4). Fish silage is a bulk product and is best used locally or within a short distance from the point of manufacture. Transport costs from the silage production site to the use site should be considered in the calculation of the cost price of the fish silage as animal (pig and chicken) feed. In April 2012 the Dutch population of pigs and broilers was estimated at 12.2 million pigs and 44.6 million broilers (PVE_Annual survey 2012[20]). Fish silage can replace a part of the feed ingredients as a source of cheap proteins.

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[16] Green S., Wiseman J. and Cole D.J.A. (1988). Examination of stability, and its effect on the nutritive value, of fish silage in diets for growing pigs. Animal Feed Science and Technology 21, 43-56
[17] Tatterson I.N. (1982). Fish silage – Preparation, properties and uses. Animal Feed Science and Technology 7, 153-159.
[18] Broun N. and Summer J. (1985). Fish Silage. In: Reilly A. (1985). Spoilage of Tropical Fish and Product Development: Proceedings of a ...FAO Fisheries report No 317 supplement.
[19] C.T. Whitternore and A.G. Taylor (1976). Nutritive Value to the Growing Pig of Deoiled Liquefied Herring Offal Preserved with Formic Acid (Fish Silage). *J. Sci. Fd Agric.* 1976, *27,* 239-243
[20]http://www.pve.nl/wdocs/dbedrijfsnet/up1/ZamyibaJM_432682PVEpromoENG_LR_definitief.pdf

Number of animals slaughtered	Number of slaughter-houses		Number of animals slaughtered x 1,000	
	2008	2009	2008	2009
100,000 - 500,000	4	4	753	707
500,000 - 1,000,000	4	4	3,440	3,510
> 1,000,000	7	6	9,717	9,184

Size category: 100,000 - 500,000 pig slaughterings:
Son: Ballering Export
Geldrop: De Wit Slachthuis
Kerkrade: Slachthuis Kerkrade
Lith: Ebergen Vlees

Size category: 500,000 - 1,000,000 pig slaughterings:
Epe: Exportslachterij J. Gosschalk en Zn
Gorinchem: Westfort
Groenlo: Vion Food Group
Druten: Vion Food Group

Size category: > 1,000,000 pig slaughterings:
Helmond: Van Rooi Meat
Nijmegen: Hilckmann
Zevenaar: Compaxo Vlees
Apeldoorn: Vion Food Group
Helmond: Vion Food Group
Boxtel: Vion Food Group

Figure 3. Map of pork slaughter houses distribution in the Netherlands in 2009. Assumptions are that slaughter houses are located near production sites.

Number of animals slaughtered	Number of slaughter-houses		Number of animals slaughtered x 1,000	
	2008	2009	2008	2009
10,000 - 30,000	4	4	93	75
30,000 - 50,000	6	6	241	224
> 50,000	5	6	364	440

Size category: 10,000 - 30,000 ton
Panningen: Van der Linden Poultry
Ommel: Flandrex*
Leek: Gebr. Heijs
Hunsel: Mieki Hunsel B.V.

Size category: 30,000 - 50,000 ton
Doetinchem: Esbro
Haulerwijk: J. de Vries & Zn.
Goor: Pingo Poultry*
Stroe: Fa. Jan van Ee B.V.
Breukelen: C. van Miert Pluimveeverwerking
Nijkerkerveen: v.d. Bor Pluimveeslachterij

Size category: > 50,000 ton
Blokker: Vleesch Du Bois B.V.*
Dedemsvaart: Vleesch Du Bois*
Zevenhuizen: Clazing Slachterij
Putten: Storteboom Fresh
Grootegast: Storteboom Kornhorn B.V.
Nunspeet: Gecombineerde Pluimvee Slachterijen

* Part of Plukon Royale Group,
Flandrex closed 30/6 2009.

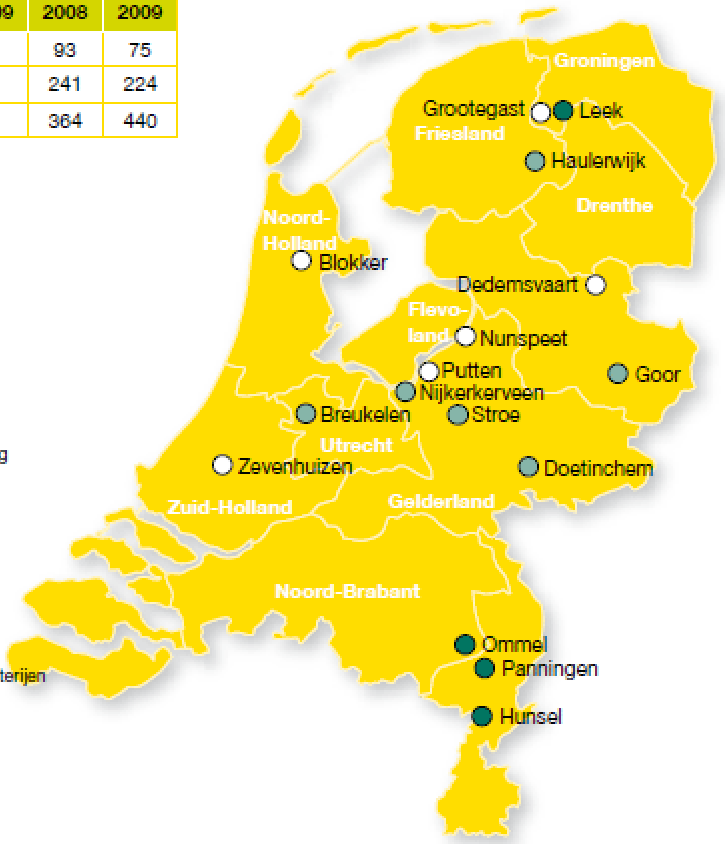

Figure 4. Map of chicken slaughter houses distribution in the Netherlands in 2009.

Assumptions are that slaughter houses are located near production sites. Source: http://www.thepoultrysite.com/articles/1755/poultry-meat-in-the-netherlands/

Broiler chickens fed on low oil content fish (whiting) silage had significantly greater weight gains than those fed on high oil content fish (mackerel) silage (Machin et al., 1990). Rapid oxidation of HUFA in fish materials during silage production and air drying of feeds reduce their palatability. Oxidised fats reduce appetite and could have adverse effects on nutritive value of foods. Chicks fed diets with concentrated de-oiled farmed salmon silage (50 and 100 g/kg diet) had a greater weight gain and a greater feed intake than those fed the control diet (no silage). High levels of added fish fats in diets caused off-odour and off-taste of chicken meat (Kjos et al., 2000[21]). Fish silage can replace up to 20% of soybean meal in broiler diets without affecting either growth or the sensory quality of broiler meat (Al-Marzooqi et al., 2010[22]). Ileal digestibility coefficients of amino-acids of fish silage are considerably higher than those of soybean meal. The lower digestibility of amino-acids from soybean meal is related to anti-nutritional factors such as trypsin inhibitors. Other animals have been fed on fish silage with good results: cow's milk and butter are without taint, and egg production from hens is high.

[21] N. P. Kjos, O. Herstad, M. Øverland, A. Skrede (2000). Effects of dietary fish silage and fish fat on growth performance and meat quality of broiler chicks. *Canadian Journal of Animal Science* 80(4): 625-632.
[22] Al-Marzooqi W., Al-Fari M.A., Kadim I.T., Mahgoub O. and Goddard J.S. (2010). The effect of feeding different levels of sardin fish silage

A fish meal factory requires a high investment and a minimum and constant volume of input fish material. The small size of Dutch pelagic discards might be more interesting for processing into silage than into fish meals. This should be determined on the basis of market and product potential, and economic feasibility. In harbours not having a fish meal plant, the silage process is particularly appropriate with a minimum investment. IJmuiden seems the most central location for the collection and further processing of Dutch discards. However the silage production should take into account the seasonal variation in total landing volumes of discards, the species composition and especially the fat content of discards to adapt its operations. To overcome the limited volume of landing discards from pelagic fisheries, the silage production in the future should also consider the valorisation of demersal discards and of by-products from fish processing (carcass, heads, skin, viscera) and non-saleable fish.

Economic prospects for fish silage production To assess the economic potential of fish silage production we have performed a rough economic assessment on fish silage production. We indicate that the revenues of this strategy highly depend on the value of the end product. Therefore, the silage production needs to be combined with additional value added sources. In this case fish oil as well as Fish Protein Hydrolysate is combined.

The market survey carried out by Buisman et al. (2013) demonstrates that a selling price of between €0.15/kg and €0.30/kg can be expected for landed by-catches. The market price for small plaice is €0.14/kg and between €0.10/kg and €0.20/kg for waste fish (industry, personal communication). The price of frozen fish on-board of pelagic trawler is €0.50/kg while the purchase for fish meal is < € 0.20/kg (Goudswaard[23]).

The technique for making fish silage is simple and cheap. Using a conservative average price of €0.20/kg for input material, the cost price is € 1.08/kg of dry silage (Table 1). Current price of fish meal on international market is ~€1.65/kg (figure 7). We assume the market price of silage is one fourth of this (based on protein levels). This results in a negative business case, unless the cost price of raw material is reduced significantly.

There is potential to increase the economic favourability by implementing separate market segments.

[23] Goudswaard, K. (2014). Van discards naar bijvangst in de pelagische visserij. Imares rapport 067/15

5 Technical potential for fish silage production

For the preparation of a fish silage case the potential of practical fish silage production, ultimately with inclusion of bioactive peptide properties, was performed. To achieve this, a cooperation between a demersal project on fish silage production was set up (Innofish, 2015). The experimental work was combined for this purpose, to avoid duplications.

Fish silage was produced using fresh sardine from the retail channels in IJmuiden. The samples were prepared by preparing fish mince from sardines. An acidification process was performed by the addition of different percentages of acids (provided by Selko B.V.) (2%, 2.5%, 3%, 3.5%). Oxidation was reduced by the inclusion of 0.5% commercially available anti-oxidant. The process is shown in figure 5.

A control sample and the silage products were analysed for:

- Moisture content (%)
- Fat content (%)
- Ash content (%)
- Malon-dialdehyde (ug/g)
- Total nitrogen content (%)
- Non-protein bound nitrogen (%)
- Water-soluble nitrogen (%)

Analysis were performed at the RIKILT-laboratory using standardised and accredited methods.

After an incubation period of 3 months the silage was analysed for the same parameters with the additional of the degree of hydrolysis, as well as potential activity of bioactive peptides.

The degree of hydrolysis was analysed to quantify the amount of protein degeneration. A bioactive screening was performed to detect the presence of bioactive properties. The following bio active properties were analysed for:

• Angiotensin Converting Enzyme 1 (ACE-1) . ACE-1 has bioactive properties on the control of blood pressure, as well as reduction of risks on hart and vessel diseases. Fish proteins contain peptides which have the ability to inhibit the function of ACE-1.

• DipeptidylPeptidase-IV (DPP4). DPP4 controls the blood sugar level, and contributes to the reduction of chance on development of diabetic symptoms. Fish proteins contain peptides, which may inhibit DPP4, and thus result in reduction of the blood sugar level.

Figure 5. The process of fish silage production from sardines.

The results of the trial are shown in figure 6 as back ground information. Malon-dialdehyde values are high, indicating significant oxidation of the oil fraction. This is most likely a result of the time between the fish grinding process and the start of the start of the silage process (30 min - 2 h), resulting in a rapid degradation of oils. This also indicates the need for a robust technology for fatty fish processing.

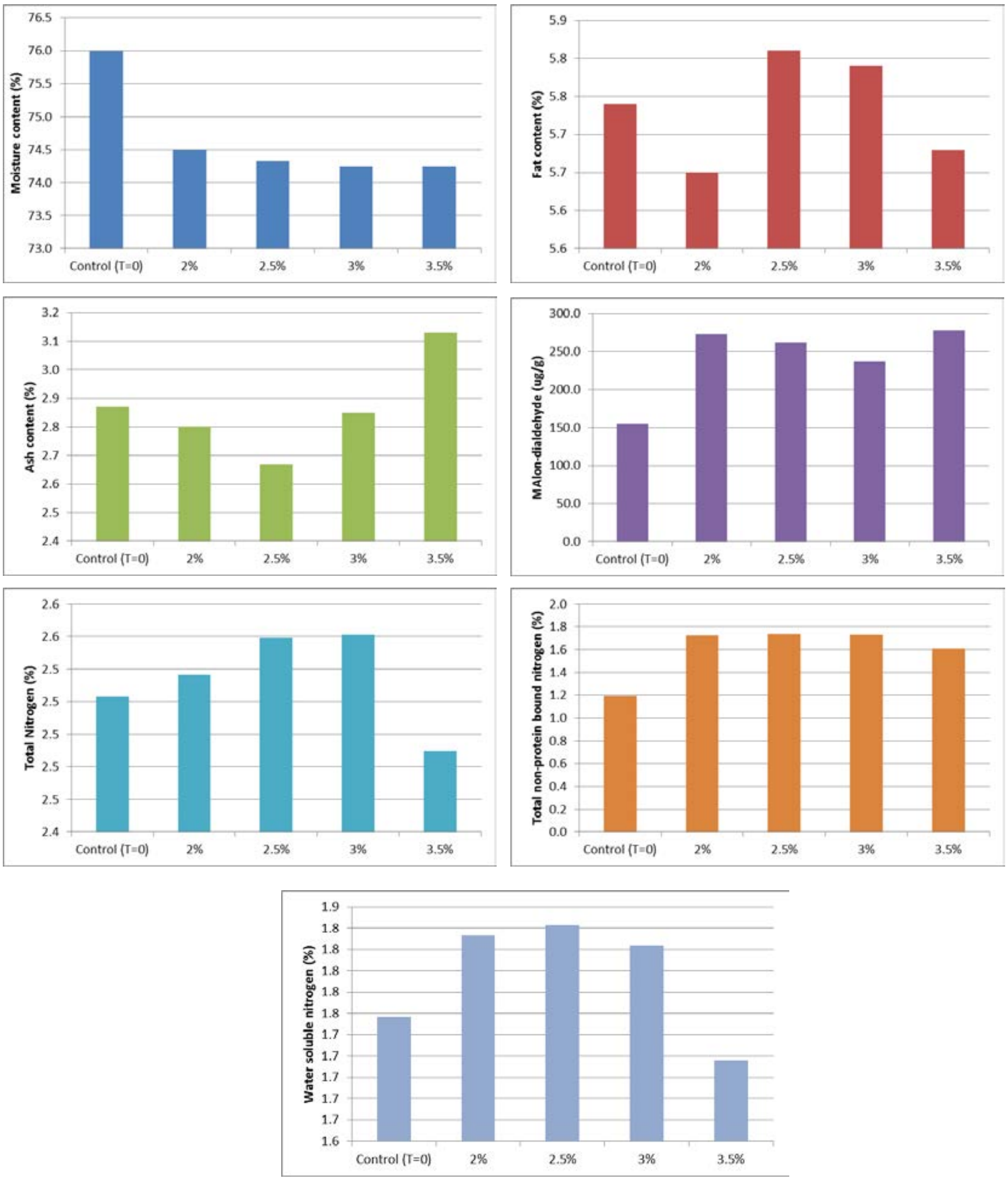

Figure 6. Results of the pelagic fish silage production trial. Control (C) is t=0, raw material. 2%, 2.5%, 3% and 3.5% furnic acid concentration, including 0.5% antioxidants, at 3 months hydrolysis time. Measured parameters were moisture content (%), ash content (%), fat content (%), total nitrogen (%), water soluble nitrogen (%) and non-protein bound nitrogen (%) and malon-dialdehyde (µg/g). Malon-dialdehyde shows high values, indicating a rapid oxidation of the oil fraction.

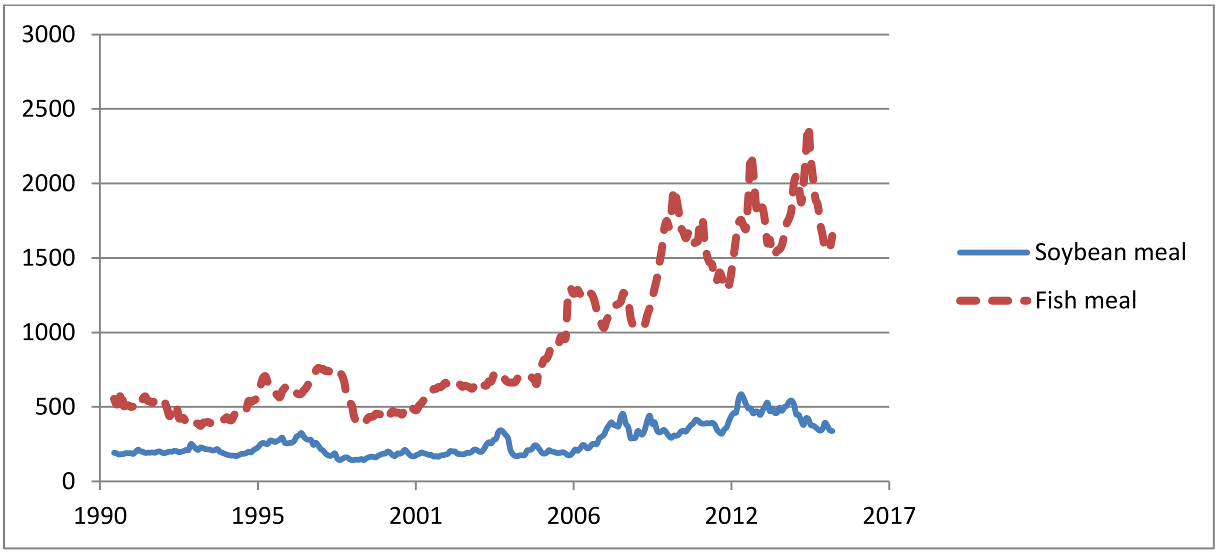

Figure 7. Price development of fishmeal and soymeal from 1990 to 2015 in US$ per tonne. Specific: Fishmeal Peru Fish meal/pellets 65% protein resp. Soybean Meal, Chicago Soybean, Minimum 48 percent protein. (data: www.indexmundi.com)

Fish silage seems to be interesting as an application in high value juvenile animal feeds. This trial is currently in investigation by Innofish and the pork industry. Standard application of silage is animal feed, as a wet fraction (direct application or mixed feed) or as a dried fraction. The product is not attractive for human consumption. A substantial part of by products from fish processing in Norway is being processed as fish silage.

In different published feed trials the product was demonstrated to have a higher nutritional value than soymeal, however this was slightly lower compared to fish meal (Ologhobo, 1988, Balios, 2003, El-Hakim et al., 2007). In the Netherlands currently there is a trial on feed efficiency and feasibility of fish silage as an ingredient for juvenile animal (pork) production.
One of the conclusion is that silage for animal feed is not feasible if the product is considered as alternative for soymeal. A feasible business case is developed only when the raw material becomes available in cheaper forms (such as by-products from large scale fish processing and in developing countries) or if an added value product is produced.

The ensilaging process may also be performed on board of a vessel, however for practical reasons application on shore is more desirable (freezing does not have an influence on the product quality). One of the disadvantages of this process is the oxidation of the oil fraction, therefore prior to the processing chain antioxidants need to be added or the oil fraction needs to be technically separated from the protein fraction.

The silage process requires relatively low capital investments, simple technology and a has a low energy demand. Another benefit is that this solution is well applicable for varying fish species throughout the year. For the feasibility in the value chain, there is a need to separate the oil and protein fraction. This separation process may be combined with the potential separation of interesting fish parts, such as skins, and white meat for further added value and increased margins.

6 Bridge to added value: bioactive molecules in fish silage

As concluded earlier a higher added value is required to increase the economic potential of the silage production route. The growing price difference between fish meal and soymeal (Figure 7) shows significant opportunities. The potential to produce bio-active peptides in silage is explored in our study. The production of silage is a hydrolysis process, however this is on a rough basis. The following two production chains are investigated:

1. Extraction of bio active functional fractions for feed applications from silage, existing of three steps;
    I. Ensilaging as a simplified first processing step
    II. Extraction of bioactive molecules/fractions from silage in a dedicated separation process
    III. Residue of the extraction can still be applied as animal feed silage
2. Animal feed with added value: production of fish silage including bioactive properties/components

The principle is tested for by screening the bioactive properties in produced fish silage. Therefore the produced fish silage in the production demonstration have been tested for bioactive properties (chapter 7). In addition to performed demersal fisheries trials, a trial on pelagic discards was added. Fish silage was produced with different time intervals (mainly for stability testing of protein and fat), the 3 months interval was tested for presence of bioactive properties. Different acid concentrations were tested. The degree of hydrolysis was measured (results in Annex 2). All samples revealed a high degree of hydrolysis, in addition the bioactive properties could not be demonstrated due to the low abundance of activity.
It could be concluded that there is no significant presence of bioactive peptides in longer term stored fish silage products. Therefore bioactive peptides could not be extracted, or no added value could be given to the silage products (e.g. Roadmap to a health claim, or early extraction of bioactive peptides).

Retrospectively, this conclusion could be clarified. In literature (a.o. Haard e.a., 1985) it was demonstrated that the hydrolysis process continues during long term storage (*Figure* 8). Some days after the hydrolysis bioactive peptides are present, however the extraction requires a well-designed technical approach. Therefore this option is not recognized as practically feasible unless neutralisors etc are used in the silage process.

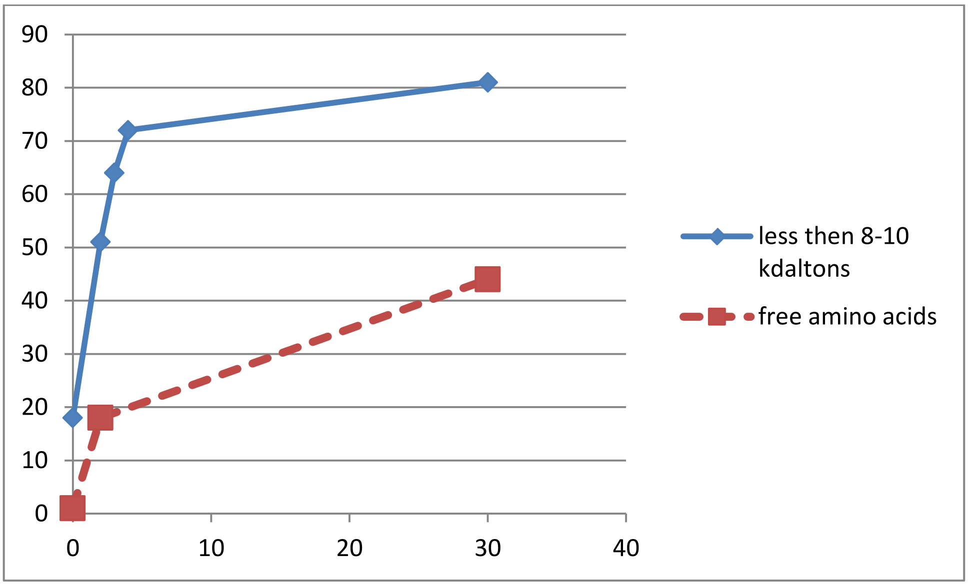

Figure 8. Protein degradation in fish silage: percentage nitrogen components smaller than 8 à 10 kdaltonnes (solid line)and percentage of nitrogen in free amino acids (dotted line). Horizontal axis is the hydrolysis time (days), the vertical axis represents the Percentage of nitrogen compounds Source: Haard e.a. (1985).

Remaining questions:

The following knowledge is lacking before fish silage can be applied on large scale:

- *Nutritional composition and nutrient digestibility, (pathogenic) microbial composition*
- *Content in common pollutants (Hg, PCB,..) in relation to fish species and fishing grounds,*
- *Zoo technical performances of animals fed fish silage containing diets in Dutch conditions*
- *Product quality of animal fed silage based feeds*
- *Economics of silage production and markets (silage use in animal feeds and others)*
- *Added value routes for fish silage (bioactive properties*
- *Develop technological production of fish silage and bioactive peptides*
- *Test for application in juvenile ruminant animal and fish feed*

7 An example of a fish silage value chain

Based on information available information, the following value chain can be proposed for fish silage production and use. In a value chain based on wet silage, silage production can be done at the users 'site' end or nearby frozen storage. Transport is less an issue. In a value chain based on dry silage, production may be most feasible at the frozen storage site to reduce transportation costs of water.

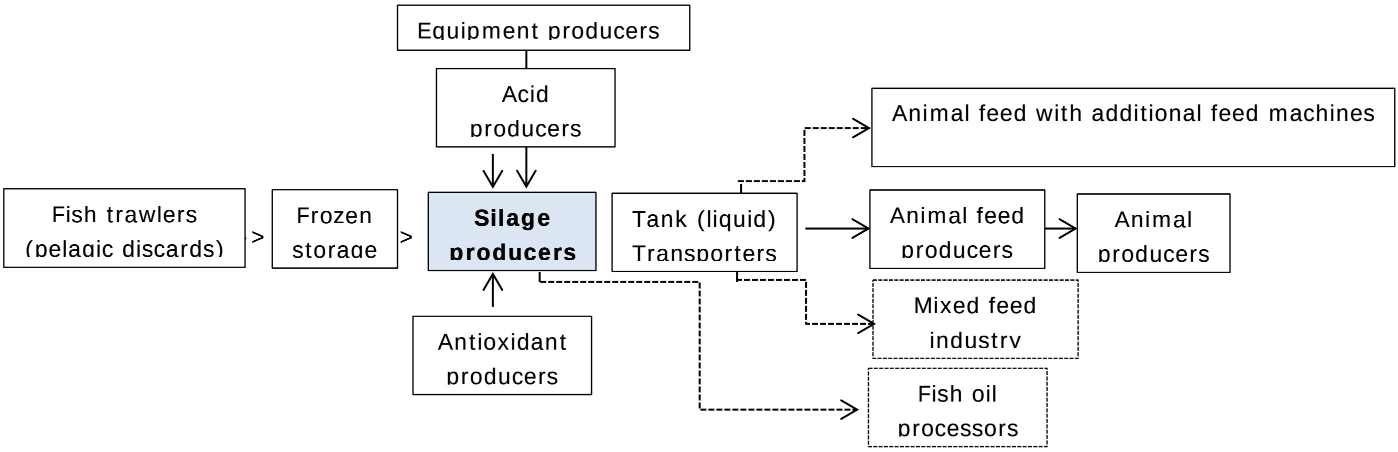

Figure 9. Value chain for fish silage production and use. A) Based on wet silage production

8 Conclusions

The hydrolysis process results in a stabile product, which is usable as ingredient for livestock. However, during long term storage (longer than a week) the degree of hydrolysis is increasing strongly. This results in a low abundance of polypeptides, having the effect that no bioactivity is present. The product then has a lower added value in live stock feed application, in comparison to e.g. soy protein. The business case for the marketing of such a quality silage is week as a replacer for fish meal and alternative protein sources in livestock grow on feed. The perspectives for application in starter feed are better (however not optimal), which is currently in investigation. In starter feed the ingredient availability, feed efficiency and specific amino acid composition is higher valued.

A valuable product with bioactive properties can be produced using a process of directed hydrolysis. The value of such a hydrolysate can be significantly higher than soy protein. In literature a price for such products is found, significantly higher than the price of silage. For the reason that the livestock industry is searching for anti-biotic replacers, this is a development with perspective. This is however a pathway, which should be further developed prior to commercial implementation.
There is a potential alternative by decreasing the ensilaging time (practically: feed within several days), which keeps a substantial part of the peptides intact. Potentially, also a significant bioactive property is present. At this moment knowledge is lacking to support the economic feasibility, since it is unknown what peptide profiles can be expected. The valuing of such a product with an additional health claim is still a long road ahead, since no only the product, but also the process needs to be sufficiently stable and robust. Furthermore marketing with health claims requires a specific (and costly) pre-assessment within the European legal framework.

The mentioned business cases are only feasible if the oil fraction, which can be separated in the process, is marketed separately. This can be done by the production of half fabricates (raw material processing industry) or purified oils (either simple purification, or added value processing). The business case for oils from the pelagic industry has been developed some years ago by Aidos (2002).

All processes and business cases which are mentioned should be considered in the light of a changing market. Raw materials which are derived from the pelagic and demersal sector, dedicated fisheries and by products of fish processing all compete for a market share, price and quality. A constant quality, supply and reliability are leading factors determining the market. This needs to be taken into account when developing a business case in a competitive raw material market.

9 Quality Assurance

IMARES utilises an ISO 9001:2008 certified quality management system (certificate number: 187378-2015-AQ-NLD-RvA). This certificate is valid until 15 September 2018. The organisation has been certified since 27 February 2001. The certification was issued by DNV Certification B.V. Furthermore, the chemical laboratory of the Fish Division has NEN-EN-ISO/IEC 17025:2005 accreditation for test laboratories with number L097. This accreditation is valid until 1th of April 2017 and was first issued on 27 March 1997. Accreditation was granted by the Council for Accreditation. The scope can be found at the website of the Council for Accreditation (www.rva.nl).

On the basis of this accreditation, the quality characteristic Q is awarded to results of components which are incorporated in the scope, provided they comply with all quality requirements, as described in the applied Internal Standard Working procedure (ISW) of the relevant accredited test method.

The quality of the test methods is ensured in various ways. The accuracy of the analysis is regularly assessed by participation in inter-laboratory performance studies including those organized by QUASIMEME. If no inter-laboratory study is available, a second-level control is performed. In addition, a first-level control is performed for each series of measurements.
In addition to the line controls the following general quality controls are carried out:

- Blank research.
- Recovery.
- Internal standard
- Injection standard.
- Sensitivity.

The above controls are described in IMARES ISW 2.10.2.105.

If the quality cannot be guaranteed, appropriate measures are taken.

Justification

Report C197/15
Project Number: 4301503101

The scientific quality of this report has been peer reviewed by a colleague scientist and the head of the department of IMARES.

Approved: Jan Broeze
Senior Scientist, Sustainable Processing & Process Optimisation Wageningen UR Food & Biobased Research

Signature:

Date: 13 th of January 2016

Approved: Dr. ir. Nathalie Steins
Interim MT Member

Signature:

Date: 13th of January 2016

Annex 1, Economic assessment fish silage

Discards into animal feed

Fish discard production

	Year I
Pelagic discards	**3500**
Total production in kg	3500000
Acid (in % of kg)	3.50%
Total acid per year (in kg)	122500
Cost acid per litre	1.2
Total cost acid per year	147000
Antioxidant (In % of volume)	0.5%
Total Antioxidant per yr (In ltrs)	17500
Cost Antioxidant per litre	2
Total cost Anti-oxidant per year	**35000**
Production per year (in tonnes)	
Fish discard	3500
Acid	122.5
Anti-oxidant	17.5
Total weight un-dried	3640
Drying percentage	80%
Loss of moisture	2800
Total dry-weight silage	**567**
Total fat content	**7.5%**
Total fat content production	**273**
Costs Production per year (euro)	
Fish discard	700000
Acid	147000
Anti-oxidant	35000
Total costs raw material	**882000**
Energy	9900
Packaging	11000
Total production costs	**902900**
Total production cost per ton	**1592**
Repair & maintenance	30000
Units of labour	1
Salaries & wages	60000
Annual Equipment cost	10000

Sales of animal feed/fish silage	231053
Sales of oily fraction	546000
Net result	**-225848**
Inclusion FPH	**1.5%**
Volume of FPH (ton)	**9**
Sales FPH	**85050**
Net result	**-140797.5**

Price fish discards per tonnes (euro)	200
Cost Acid per litre (euro)	1.20
Cost Price Anti-oxidant (euro per litre)	2.00
Fish oil price (euro per ton)	2000
FPH price (euro/ton)	10000
Cost price Unit of Labour (euro-year)	60000

Another source of information (Ramirez, 2007) results in comparable estimates for the operational and investment cost. The cost are furthermore dominated by labour costs.

Annex 2. Resultaten analyse bioactiviteit van vis-silage

Uitgangsmateriaal: silage van olierijke vis (aangeleverd door IMARES), met 2.5, 3.0 resp. 3.5% zuur (ondergrens ingegeven door minimale dosering voor stabiel product). Zowel 'afgedraaide' (gecentrifugeerd) als origineel silage-materiaal (inclusief de olie-fractie) zijn beoordeeld.

Beoordeeld zijn:

- ACE-1: Angiotensin Converting Enzyme (Factor in regulatie van bloeddruk; ACE-remmers hebben naast bloeddrukdaling meerdere positieve gezondheidseffecten)
- DPP4: DiPeptidylPeptidase 4 (Breekt Glucagon-Like Peptide-1 (GLP-1) af; GLP1 zorgt ervoor dat insuline vrijkomt; remming afbraak geeft langer insuline-effect)

Als referentie zijn de waarden genoemd voor een eiwithydrolysaat – "FBR standaard" in de tabel hieronder – dat momenteel in een humane trial wordt getest voor o.a. bloeddrukdaling (details over uitgangsmateriaal zijn vertrouwelijk en kunnen hier niet worden genoemd).

In de analyses is bepaald hoeveel van de stof nodig is om een bepaalde mate van bioactiviteit waar te nemen (IC50 slaat op 50% remming). Hoe lager de benodigde dosering (waarde in de tabel), hoe hoger de werking.
In de laatste kolom van de tabel is de 'degree of hydrolysis' genoemd: een maat voor de afbraak van eiwitten.

Vissoort	**percentage zuur**	**voorbehandeling**	**ACE-I IC50 (mg/mL)**	**DPP4 IC50 (mg/mL)**	**DH%**
Schar	2.5	verse vis	>0.5	>2.5	42.2
	3.0		>0.5	>2.5	65.6
	3.5		>0.5	>2.5	58.8
	2.5	ingevroren	>0.5	>2.5	57.4
	3.0		0.381	>2.5	66.0
	3.5		>0.5	>2.5	60.8
Sardines	2.5	verse vis; olie vooraf afgescheiden	>0.5	>2.5	52.8
	3.0		>0.5	>2.5	52.4
	3.5		0.496	>2.5	37.6
	2.5	vers; olie niet vooraf afgescheiden	>0.5	>2.5	47.2
	3.0		>0.5	>2.5	56.7
	3.5		0.440	>2.5	35.7
FBR standaard 1			0.058	1.7	21.5
FBR standaard 2			0.077	1.7	NA

De degree of hydrolysis (DH) is voor alle geteste vissilage-producten erg hoog. Dat wil zeggen dat er zeer veel vrije aminozuren aanwezig zullen zijn. Voor een ACE en DPP4 remmend hydrolysaat is deze hydrolysegraad te sterk.

Annex 3. Potential partners

There is potential to share market efforts with currently operating companies, which are:

Biowaste AS	Norway
Scanbio marine Group AS	Norway
Biomarinus	New Zeeland

The companies involved in the Netherlands are:

- Rainbow Agro
- Selko (acids and anti-oxidants)
- Smicon (equipment)
- GE Westfalia (equipment)
- The fiscalist
- Kwaliflex (Pork production)