



## The effect of raw material combination on the nutritional composition and stability of four types of autolyzed fish silage

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### ABSTRACT

Producing fish silage for animal feed is an excellent way of valorizing underutilized fishery by-products. However, the nutritional quality of fish silage strongly depends on the freshness and composition of the raw materials. The purpose of this study was to determine the effect of raw material composition (RMC) on nutritional quality and stability of fish silage. Using different combinations of whole undersized flatfish (plaice, sole, flounder) and codfish (whiting), four fish silages were produced: a single-species (plaice) flatfish silage (F-single); a mixed flatfish silage in equal ratios (F-equal); a mixed flatfish silage in ratios similar to fish-bycatch ratios (F-bycatch); a mixed flatfish and codfish silage, also in ratios similar to fish-bycatch ratios (FC-bycatch). Raw materials were homogenized, mixed with 2.5% (v/w) formic acid and 0.2% (w/w) potassium sorbate, and stored for 91 days at ambient temperature. Dry matter (DM) increased slightly during storage in all silages, from  $228 \pm 3.7$  g/kg silage to  $256 \pm 3.6$  g/kg silage; whereas ash content slightly decreased, from  $192 \pm 15.8$  g/kg DM to  $176 \pm 13.1$  g/kg DM. Crude protein did not differ in the raw materials ( $739 \pm 18.9$  g/kg DM), but decreased at different speeds in the silages. After 91 days, protein content of F-single and F-bycatch decreased to  $621 \pm 9.5$  g/kg DM and  $634 \pm 18.8$  g/kg DM, respectively, whereas F-equal and FC-bycatch decreased to  $592 \pm 6.7$  g/kg DM and  $580 \pm 7.6$  g/kg DM, respectively. Differences in protein decrease could be caused by the higher degree of hydrolysis in F-equal ( $60.6 \pm 3.4\%$ ) and FC-bycatch ( $62.2 \pm 2.3\%$ ), compared to F-single ( $51.6 \pm 4.7\%$ ) and F-bycatch ( $52.9 \pm 1.7\%$ ), after 91 days. Extended hydrolysis leads to overall deamination, also reflected by the decrease in essential amino acids (EAA) and increase in total volatile basic nitrogen. Crude lipid decreased in F-single and F-bycatch, but remained stable in F-equal and FC-bycatch. After 91 days, there were no more differences between the silages ( $58.0 \pm 4.2$  g/kg DM). The decrease in F-single and F-bycatch could be the result of lipid oxidation, also reflected by the TBARS value and decreased polyunsaturated fatty acid (PUFA). Overall there is a significant effect of RMC on nutritional quality and stability of fish silage. A more diverse mixture of raw materials improved nutritional quality, mainly in the form of EAA and PUFA, but was also more prone to deterioration by chemical and biological processes.

**Abbreviations:** ANOVA, analysis of variance; CFU, colony forming units; DM, dry matter; DHA, docosahexaenoic acid; DoH, degree of hydrolysis; EAA, essential amino acids; EPA, eicosapentaenoic acid; FAME, fatty acid methyl ether; GC-FID, gas chromatograph-flame ionization detector; LC-MS, liquid chromatograph-mass spectrophotometer; MRM, multiple reaction monitoring; MUFA, monounsaturated fatty acid; NEAA, non-essential amino acid; PUFA, polyunsaturated fatty acid; RMC, raw material composition; SFA, saturated fatty acid; TEP, 1,1,3,3-tetraethoxypropane; TBARS, thiobarbituric acid reactive substances; TMA-N, trimethylamine nitrogen; TNBS, 2,4,6-trinitrobenzenesulfonic acid; TPC, total plate counts; TVB-N, total volatile basic nitrogen

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## 1. Introduction

The recently implemented “landing obligation” for the European fisheries (EC 1380, 2013) is accompanied by a considerable number of economic, social, and environmental implications, leaving its effectiveness up for discussion (Diamond and Beukers-Stewart, 2011; Mangi and Catchpole, 2013). One of these implications is the obligatory landing of undersized-quota-species – previously known as bycatch, and discarded at sea – which cannot be put on the market for direct human consumption (EC 1380, 2013). In order to maximally utilize these new raw materials, an alternative, preferably high value, market needs to be found. An evident option would be to produce fishmeal and fish oil. Fishmeal is mostly used as protein source in certain animal feeds, the production of which largely relies on depleting wild fish stocks as raw material, resulting in stiff competition and elevated prices (FAO, 2016). Also, this normally requires large quantities of raw material. For countries with relatively small fisheries, a fishmeal processing plant is infeasible (Mangi and Catchpole, 2013; Naylor et al., 2009; Olsen and Toppe, 2017). Consequently, much research is being conducted to find more feasible and sustainable alternatives to fishmeal, such as producing fish silage using fishery byproducts.

Fish silage is considered as a low investment, low cost, and easy to produce fishmeal substitute (Arason, 1994; de Arruda et al., 2007; Olsen et al., 2014; Olsen and Toppe, 2017). Fish silage implies the liquefaction and stabilization of minced whole fish or fish offal by the addition of inorganic and/or organic acids. Fish silage is often produced using formic acid and preferably kept between pH 3.5 and 4.5. A pH below 4.5 prevents microbial growth, while keeping the pH above 3.5 eliminates the need to neutralize the product before further use. The low pH induces liquefaction and creates an ideal environment for autolysis by endogenous enzymes, resulting in a mixture of proteins, short peptides, and free amino acids, among other nutritional components such as fatty acids and minerals (Arason, 1994; de Arruda et al., 2007; Gildberg, 1994; Olsen and Toppe, 2017). Several experiments have shown that acid-preserved fish silage can completely or partially replace fishmeal in feed for fish (Espe et al., 1992; Goosen et al., 2016; Hardy et al., 1983; Jackson et al., 1984b; Mach et al., 2010; Majumdar et al., 2014; Ramasubburayan et al., 2013), shrimp (Ali and Sahu, 2002; Gallardo et al., 2012), broilers (Santana-Delgado et al., 2008; Vizcarra-Magaña et al., 1999), and pigs (Batterham et al., 1983; Nørgaard et al., 2012), without negatively affecting performance parameters.

An issue concerning the production of fish silage is the possibility of having excessive variation in the end product. Product composition and nutritional quality are mostly determined by the composition and freshness of the raw materials (Arason, 1994; Espe and Lied, 1999; Haaland et al., 1990; Heras et al., 1994; Mach and Nortvedt, 2009; Nørgaard et al., 2015; Vidotti et al., 2003). Additionally, processing parameters such as, acid choice, pH, additional additives, temperature and storage time, can influence the final composition and nutritional quality of the fish silage (Arason, 1994), though these parameters are mostly controlled during production. The raw material composition (RMC) and freshness are harder to control since they strongly depend on the fisheries' catch composition, processing method, and handling time. Ideally, a consistent supply of fresh raw materials with a low variety is preferred for fish silage production. However, this is unattainable for mixed-fisheries, as the catch consists of a fluctuating variety of fishes.

Literature focusing on the production and consistency of fish silage with a varying raw material input is limited. In the following study, the nutritional composition and stability of four types of fish silage were investigated, each produced using a different mixture of demersal undersized-quota-species. The goal is to determine the effect of RMC on fish silage nutritional quality and its shelf-life, considering its use as fishmeal substitute in animal feed.

## 2. Materials and methods

### 2.1. Raw materials

Undersized specimens of four fish species were collected from several fishing trips in the Southern North Sea at the beginning of July 2015. The species selection was based on the ‘ILVO discard atlas’ (Vanellander et al., 2014), which provides an approximation of demersal fish-bycatch by the Belgian fleet. Plaice (*Pleuronectes platessa*), sole (*Solea solea*), and flounder (*Platichthys flesus*) were chosen to represent flatfish, which comprise approximately three-quarters of the fish-bycatch. The remaining one-quarter of the fish-bycatch consists mostly of Gadiform fish (codfish); therefore, whiting (*Merlangius merlangus*) was chosen to represent this fraction. Once ashore, the fish were stored at  $-20\text{ }^{\circ}\text{C}$  for a maximum of two months prior to processing.

### 2.2. Fish silage production

Different species combinations were used to produce three independent replicates of four types of fish silage: a single-species fish silage (F-single) consisting purely of plaice ( $n = 3$ ); a flatfish silage (F-equal) consisting of plaice, sole, and flounder in equal ratios ( $n = 3$ ); a flatfish silage (F-bycatch) consisting of plaice, sole, and flounder in ratios similar to the fish-bycatch ratios ( $n = 3$ ); a flatfish and codfish (FC-bycatch) silage consisting of plaice, sole, flounder, and whiting, also in ratios similar to the fish-bycatch ratios ( $n = 3$ ). For each replicate, 2 kg of each frozen raw material combination (Table 1) was homogenized using a meat mincer (Seydelmann<sup>®</sup> MD 114) with an 8 mm die and stored in 3 l polypropylene buckets.

To produce fish silage, 2.5% (v/w) formic acid (85%) was added to each raw material replicate. Furthermore, 0.2% (w/w) potassium sorbate was added as a fungicide, whereas no antioxidants were added due to the use of low-fat fish species. Each replicate was mixed thoroughly, covered with a lid, and stored at ambient temperature ( $17.2 \pm 1.1\text{ }^{\circ}\text{C}$ ) for three months. During the first two weeks, the silages were mixed manually on a daily basis, thereafter mixing occurred once a week. pH was monitored frequently (Metrohm<sup>®</sup> 913 pH meter). Samples of the raw material mixtures were taken prior to acid addition (day 0). Successively, silage

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