FISH SILAGE FROM SIDE STREAMS OF PROCESSING FACTORIES AS RAW MATERIAL FOR AQUAFEED

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ABSTRACT

In this study, silage was produced from viscera of Atlantic Red fish using two organic acids, formic and lactic acid at two different temperature (25^0C and 45^0C) conditions. The changes in pH, hydrolysis, protein in soluble phase, amino acid compositions, and free fatty acid formation were analysed for untreated raw material and the fish silages. The temperature 45^0C was used to accelerate the rate of hydrolysis and on day 5, heat treatment was done to inactivate the digestive enzymes for the rest of the storage. Formic acid silages were stabilized at pH 3.38 (25^0C) and 3.26 (45^0C) while lactic acid silages were found to be stable at 4.09 (25^0C) and 4.12 (45^0C) respectively. There was no sign of putrefaction in the silages during 5 weeks of storage. Aqueous or soluble phase was found to increase due to hydrolysis in both formic and lactic acid silages with time and the proportion was more in 45^0C silages. The protein in soluble phase was observed to increase in all the silages than in the initial stage. However, it was proportionately more in lactic acid silages than in the formic acid silages. The essential amino acid tryptophan was found to be more in concentration in both formic acid and lactic acid silages (25^0C) than in the untreated raw material. Free fatty acid content was observed to rise in the silages at the beginning before slowing down. The increase was proportionately more in the silages of 45^0C which may be a direct influence of higher temperature.

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1 INTRODUCTION

1.1 Background

Bangladesh has a very rich and diversified fishery resources encompassing 121,110 square km of marine water area in the Bay of Bengal and 4.71 million ha of inland fresh water bodies (Chowdhury, 2014; FRSS, 2017). Inland fisheries sector of the country has two sub-sectors; inland capture and inland culture. Inland capture fisheries include rivers and estuaries, beels, floodplain, Sundarbans and Kaptai lake; on the other hand, inland culture fisheries comprise pond, seasonal waterbody, baor, shrimp/prawn farm, crab, pen and cage culture. Further, the marine fisheries sector consists of both artisanal and industrial (Trawl) fisheries (FRSS, 2017).

In 2015-16, the total production of fish was 3.87 million metric tons. The contribution of inland culture fisheries was 56% and inland capture fisheries was 27% to the total production; while the marine capture fisheries contributed only 16%. It is evident that aquaculture in Bangladesh has been increasing moderately in terms of both production and area (FRSS, 2017).

The fisheries sector of Bangladesh plays significant role in the national economy and nutritional security of the people. This dynamic sector contributes 3.65% to our national Gross Domestic Product (GDP). It has been estimated that 12 million people are directly or indirectly employed in the fisheries sector which is more than 7% of the total population (Anon, 2014). The total export earnings from fisheries was about US$ 535 thousand in 2015-16 representing 1.97% of total export value (FRSS, 2017). Traditionally, Bangladeshis are well known as fish eating people. Fish is substantially important in the daily diet of the people as 60% of the animal protein intake comes from fish (Anon, n.d.).

The fish industry of Bangladesh faces big challenge in handling the huge volume of waste or by-products generated by the landing sites, fresh fish markets, and processing factories. Fish processing wastes or discards usually include a wide range of body parts comprising viscera, liver, scales, fins, heads, frames, skin, air bladders, gonads, trimmings or side streams of fillets etc. At present, there is no centralized system for collection of these waste materials for further processing into value added products. Besides, there is lack of knowledge on the nutritional value, chemical composition and diverse use of different parts of fish processing waste. Moreover, new technology is needed to preserve the quality of these raw materials immediately after processing to obtain higher quality final products.

According to the data of Department of Fisheries, 71% of fish is used for human consumption and the rest 29% becomes waste after processing. This corresponds to an estimate of around 1.12 million metric tons of fish waste generated by Bangladesh in the year 2015-16 (Moretaza, 2016). Only a small portion of this raw material is sundried and used as animal feed, while most of it is discarded in open place or dumped into the rivers. In general, side streams or rest raw materials of fish processing are regarded as waste in our country and their indiscriminate disposal causes not only serious environmental pollution but also loss of valuable nutrients.

Side streams of fish processing is an important source of potentially rich oils, minerals, enzymes, pigments and flavours (Archer, 2001). There are diverse uses of these food products worldwide. Nutritional uses include food, pharmaceutical, agricultural, aquaculture and industrial applications. Production of chitin and chitosan, carotenoid, pigments, enzyme extraction, leather, glue, pharmaceuticals, cosmetics, fine chemicals, collagen, gelatine and pearl essence are some examples of non-nutritional uses. Fish meal and oil are most commonly

produced from fish processing wastes, however, there is also good potential for silage production which can be used as raw material for aquafeed.

Fish silage is a liquid product made from whole fish or parts of it, to which acids, enzymes or lactic acid producing bacteria are added to liquify the mass provoked by the action of enzymes naturally present in the fish (FAO, 2003). Silage or fermentation of feed ingredients from plant origin is a unique process to improve the nutritional value, reduce anti-nutritional factors and increase availability of certain vitamins (Felix & Brindo, 2008).

Feed is the most significant production input in aquaculture and constitutes more than 50% production cost in any aquaculture systems (Vassos *et al.*, 2015). Fish meal is commonly used as protein source in commercial compound feed which is expensive, and the global supplies of forage fish used for fish meal production has become static and/or diminishing (Tacon & Metian, 2008). Therefore, there is a consequent pressure on the feed manufacturers to find alternatives to fish meal so as to sustain their profitability. Significant researches have been conducted in different countries on silage and its efficacy in replacing fish meal to reduce the cost of fish farms. The technology of silage production is quite simple, not capital or labour intensive and has good storage quality (Jackson *et al.*, 1984; Edin, 1940).

Production of silage from fish wastes can offer a range of benefits to Bangladesh. It can help to minimize environmental pollution, serve as low-cost feed ingredient for fish farmers, provide extra revenues for processing industries and the fish vendors. This can also be an alternative option to preserve the nutritional quality before being used for fish meal production. However, to achieve these benefits of silage from fish wastes, we need to develop proper technology to ensure the quality of both raw materials and the end products.

1.2 Rationale

In Bangladesh, fish silage is not well known, however some research work has been done by few scientists on marine trash fish and fish offal silage using acids (Ali *et al.*, 1995; Ali *et al.*, 1994; Bhuiyan *et al.*, 1988). In fact, proper technology, skilled manpower, sound knowledge base, and awareness among people is crucial to promote the production and use of fish silage.

The aim of this project was to produce silage from fresh fish viscera using two organic acids (formic and lactic) separately and evaluate the changes in quality in different time-temperature conditions. From this study, knowledge on quality parameters in terms of crude protein, crude fat, rate of hydrolysis, amino acid composition, free fatty acid content, pH before and after production of silage will be obtained.

This knowledge will be useful to evaluate the efficiency of formic and lactic acid in silage production and also to determine the nutritional quality in end products. Based on these findings, this research can be taken forward towards feed formulation supplementing with fish silage, assess the growth performance in feeding trial, and finally can suggest for extension at farm level to reduce input costs of fish production.

1.3 Research Objectives

General objective
The general objective of this project was to produce low-cost feedstuff from fish processing waste for the aquaculture industry which in turn will help to prevent the environment pollution in Bangladesh.

Specific objectives

- To produce silage from side streams of fish processing using two organic acids
- To compare the hydrolyzation rate of the fish silages
- To evaluate the nutritional and physical quality of the untreated raw material and end products

2 LITERATURE REVIEW

2.1 Fish silage

Fish silage is a liquid product prepared with whole fish or parts of fish by using acids, enzymes or lactobacillus (Batista, 1987). In another definition, the process by which underutilized pelagic fish, fish waste and fish viscera chemically preserved by acid or bases, either with or without hydrolysis is called fish (Arason, 1994). In fact, silage has a long history in agriculture derived from the preservation of wet fodder during winter season and has been used for several decades in Scandinavian countries. Production of fish silage was developed from the concept of silage in agriculture.

In 1920s, use of sulfuric acid and hydrochloric acid mixture to preserve green fodder was first introduced by A. I. Virtanen and this acid method was adopted by Edin in the 1930s to preserve fish waste (Edin, 1940) In silage preparation, fish wastes are allowed to liquefy by the action of the enzymes naturally present in fish and the process is accelerated by digestion agents like acid (organic or inorganic) or bacteria.

The types of acids mainly used for silage production includes formic acid, acetic acid and mineral or inorganic acids. The product is generally used for animal feeding such as fish, fur animals and pigs etc. (Arason, 1994).However, organic acids more common in silage production.

2.2 Countries leading in production

The commercial production of acid fish silage is mostly done in Denmark, Norway and Poland while other European countries produce lesser amount. Fish silage has been successfully used in many Southeast Asian countries such as Indonesia, the Philippines, Malaysia, and Korea as a means of utilizing waste, by-catch and surplus fish. In Scandinavia and Poland, the annual total production of fish silage has been estimated to be about 120, 000 tons (Arason, 1994). Among the Nordic countries, the major production of fish silage is done by Norway which is about 140,000 tons/year. The raw material is obtained mainly from Salmon farms (Rustad, 2003).

2.3 Uses

Fish silage is mainly used in animal feed just like fish meal. The protein content in fish meal is 65% whereas fish silage contains only about 15% (Tatterson & Windsor, 2001). However, fish meal can be supplemented with fish silage at different levels to reduce the cost of feed. Silage seems more practical in pig farms where it can be applied directly in its liquid form. Apart from pig industry, cows and hens fed on silage gave good results such as milk and butter without taint and high egg production. Silage can be used as protein concentrate by separating the oil phase and evaporating the moisture (Rustad, 2003). In contrast to ordinary silage, use of concentrated silage (approximately 50-60% dry matter with a syrupy consistency) is advantageous as it has better nutritional value ((Arason, 1994). Further, it is also possible to mix silage or silage concentrate with other raw material to produce fish meal (Arason, Thoroddsson, & Valdimarsson, 1990).

2.4 Advantages and disadvantages

One of the main advantages of fish silage in comparison to fish meal is that it involves low capital investment and simple processing equipment (Rustad, 2003). The technology is easy, and the production unit can be planned to any size depending on the supply of raw materials (Winter & Feltham, 1983). Fish silage in fact transforms waste materials (e.g. by-catch, low value fish species, by-products like viscera, skin, head, frame, fins etc.) into resource which could otherwise create environmental pollution.

On the other hand, the main disadvantage of fish silage is the high transportation cost as it is bulky containing huge amount of water (Tatterson & Windsor, 2001). Also, the profit margin of silage is low which is a disadvantage for the processor. However, fish silage can be used in dried concentrated form but drying of the product implies additional cost of energy (Kompiang, 1981; Beerli, Beerli, & Logato, 2004).

2.5 The appropriateness of fish silage

Fish silage is important as it is a versatile feedstuff, economical, easy to make, long term storage is safe and overall; it is environmentally sound way of disposal of fish waste (Winter & Feltham, 1983). Although the production and use of fish silage commercialized in Scandinavia, many efforts had also been taken in Canada mainly because of economic pressures, stringent environmental regulations and the need for protein sources (Winter & Feltham, 1983). More specifically, production of fish silage is an attractive alternative for small processors, for whom fish meal production is too costly and where the supply of raw materials is too low or infrequent. In small remote fishing ports where supply of fish waste is low in volume, fish meal plant may not be economical in such places, nevertheless fish silage can be a feasible alternative (Arason, 1994).

2.6 Side streams of fish processing

Side streams of fish processing is attributed to those parts of fish body usually not suitable for human consumption such as viscera, skin, fins, scale, frame, head, tail, liver etc. These non-edible parts of fish are generally regarded as discards, waste or fish by-products having low commercial value. In recent time, utilization of these raw materials into value added products has become a promising sector for many countries particularly Iceland. The value-pyramid in

Figure 1 showing that feed is at the bottom level while pharmaceutical products are at the top most level of value-added products from fish raw materials (Jónsson & Viðarsson , 2016).

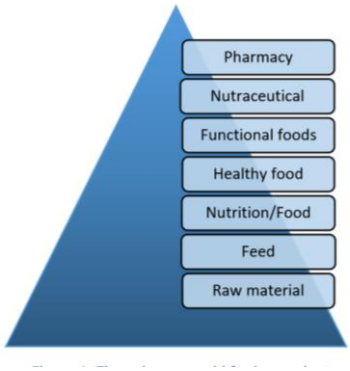


Figure 1: The value-pyramid for fish by-products (Jónsson & Viðarsson, 2016)

Iceland has achieved remarkable success in processing fish by-products and earning considerable amount of revenue from export since 2004 (Moretaza, 2016). In Figure 2, it is shown that the export value of cod products has increased since 2000 (Jónsson & Viðarsson , 2016).

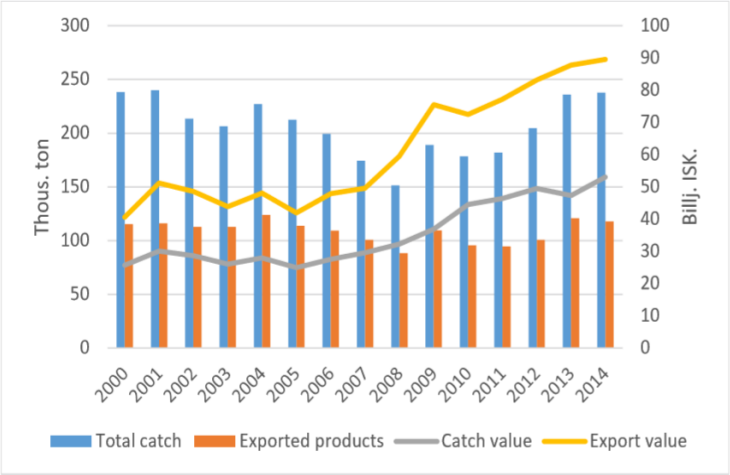


Figure 2: Total Icelandic cod catches and exported cod products in quantity and value (FOB) 2000-2014.

In Icelandic fish industry, most of the raw materials from processing cod are being utilized which include cut-offs, head, frame, skin, liver, roe and milt, skin and viscera (Figure 3). These processing waste represent 57% of the whole fish after filleting. Though it is theoretically possible to utilize 100% of the fish landed but in reality, there are difficulties in freezing and storing facilities for raw by-products (Jónsson & Viðarsson , 2016). For example, factory vessels are not provided with storage facilities for viscera and therefore the viscera of degutted fish are usually dumped into the sea at present.

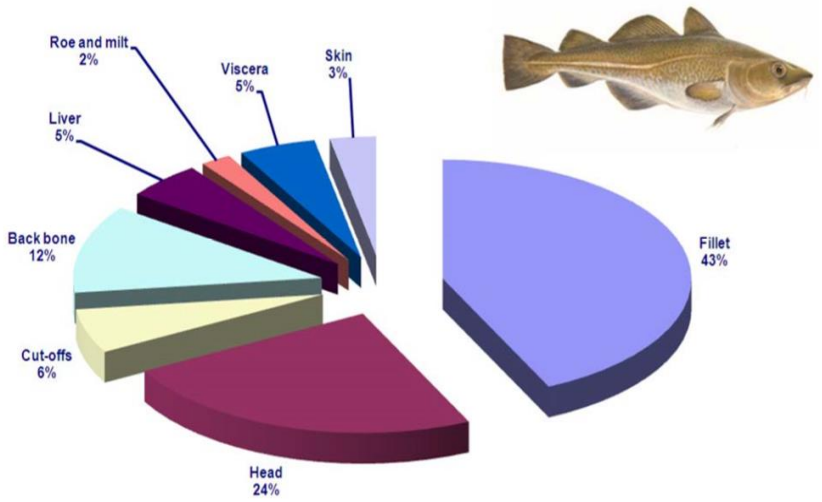


Figure 3: Ratio of the raw materials in cod when producing skinless and boneless fillets (Jónsson & Viðarsson, 2016).

Since fish waste has already been proven as a valuable resource, positive terminologies like side streams or rest raw materials are intentionally used by the researchers and the fishing industry instead of waste, discards or by-products.

It has been estimated that the volume of waste produced by processing plants is 50% of the processed fish. According to this, about 50% of the world whole fish or parts of it, becomes waste material corresponding to the amount of 65.2 million metric tons of fish waste (Arruda, Borghesi, & Oetterer, 2007). In the United Kingdom, 43% of the total production of fish and shell fish estimated to be used for human consumption and the rest of it is classified as waste. Major portion of the fish waste is produced in the on-shore processing sector (Archer, 2001).

In Bangladesh, 71% of the fish is consumed and 29% is wasted as was found from the research of Bangladesh Shrimp and Fish Foundation (Moretaza, 2016). There are 78 fish processing plants in operation with the average processing capacity of 2- 3.5 tons/day/unit and producing 16.3 - 16.4 MT of waste materials. There is huge prospect in converting these fish residues into asset for revenue generation of the country. But the main challenges lie in skilled manpower, proper technology to begin with, investment and infrastructure for collection and maintenance of good quality raw materials.

2.7 Production methods

There are two methods of fish silage preparation:

i. Addition of acid (organic and/or inorganic)

The product is called acid fish silage. In this method, pH is lowered off sufficiently to prevent bacterial spoilage and the liquefaction of fish tissue is made by enzymes naturally present in the raw material. When inorganic acids used for fish silage, pH is lowered to 2 or below in order to obtain a well-preserved product. The amount of acid required to get the desired pH of 2 depends on the protein and ash content in the raw material. However, it should be mentioned that neutralization or deacidification is required for such product prior to feeding to animal.

Organic acids namely formic acid, acetic acid and propionic acid are generally used for fish silage preparation. For formic acid and propionic acid, the silage becomes stable at 3.5-4.0 and 4.5 respectively. The advantage with organic acid is that neutralization of product before feeding is not required though these are more expensive than inorganic acids (Batista, 1987).

The general recommendation for the proportion of organic acid to be used is 3.5% (w/v) which is applicable for most fish or fish wastes. However, a mixture of inorganic and organic acid is preferable considering the high price of organic acids. In such cases, formic acid (85%) and sulfuric acid (50%) in different mixtures are referred by many authors (Arason, 1994; Batista, 1987).

ii. Bacterial fermentation with lactobacilli

The product is called microbial/fermented/ biological silage. A starter culture of proper lactic acid bacteria is added with a fermentable sugar to produce lactic acid which lowers the pH off and enhances the break-down of tissue protein by the enzymes in the raw materials (Batista, 1987).

2.8 Preparation of acid fish silage

There are three basic steps in preparation of fish silage:

i. Mincing or chopping of the raw materials
ii. Addition of acid and blending
iii. Storing

For fatty fish, de-oiling is necessary only if the oil content of the silage is more than about 2% of the wet weight (Raa & Gildberg, 1982). During preparation, care should be taken so that acid and minced fish/offal gets mixed thoroughly, otherwise putrefaction may occur in pockets of untreated raw material (Windsor, 1981).

For successful fish silage production, freshness of raw material is vital. Raw materials already spoiled or with bacterial breakdown is most likely to be unsuitable for silage-making, as the end product would be poor in quality, with a high bacterial content and produce unpleasant odour (Winter & Feltham, 1983). Post-handling of raw fish waste is quite advance in countries like Denmark, where chilling of fish and fish wastes are done before taken to silage production plants which obviously prevents deterioration of raw materials. It is imperative that fish waste or discards must be looked upon as valuable resources rather as trash by the fishing industry which will in turn ensure high quality fresh fish waste.

2.9 Chemical composition

It is reported that composition of fish silage and the raw materials used for its production are quite similar (Arason, 1994). However, the composition varies with species, fat content in the muscle and type of fish by-products (e.g. viscera, head, skin, bone etc.). For example, if the protein, fat, moisture and mineral content of white fish offal were found as 15%, 0.5%, 80% and 4.5% respectively then the whole fatty fish will have comparatively higher protein and fat content and correspondingly lower value for moisture and mineral content (Tatterson & Windsor, 2001). The approximate composition of some fish silage from by-products are shown in Table 1 (Arason, 1994).

Table 1: Common composition of certain fish by-products silage (Arason, 1994).

Silage	Moisture %	Protein %	Oil %	Ash %
White fish viscera	60.0	11.5	26.5	1.5
White fish offal[a]	78.9	15.0	0.5	4.2
Herring offal[b]	75.4	13.5	8.7	2.6
Lumpsucker offal (*Cyclopterus lumpus*)	86.8	7.8	3.2	1.0

[a]Without viscera

[b]With viscera

2.10 Hydrolysis and chemical changes

During storage of fish silage, series of chemical changes take place by the action of digestive enzymes proteases and lipases. This process is known as hydrolysis or liquefaction in which tissue protein and fat breaks down into smaller functional units (Arason, 1994). As liquefaction (autolysis) continues, there is an increase in the moisture or aqueous phase (Batista, 1987). At the initial stage of hydrolysis, protein molecules break down into small peptides and amino acids which is called proteolysis. However, further degradation of amino acids produces ammonia and results in losses of essential amino acids. Tryptophan is the limiting amino acid in fish silage which easily degrade at high temperature and low pH (Arason, 1994). The maximum activity of proteases occurred at 45-50^{o}C (Raa & Gildberg, 1982). The optimum pH range for digestive proteases is 2-4 and above pH 4 their activity shows a sharp decrease (Raa & Gildberg, 1976).

The level of protein hydrolysis can be measured from the ratio of protein/nitrogen solubilized in the aqueous phase expressed as percentage of total nitrogen. The degree of hydrolysis can also be measured from the non-protein nitrogen (NPN) content present in the aqueous phase. The NPN value increases with storage time and varies with different parts of fish; e.g. viscera silage liquifies faster than other body parts (Jayawardena & Poulter, 1980). In Figure 4, the rates of NPN formation at 30^{0}C were shown to vary for different parts of cod in silage prepared with formic acid (Backhoff, 1976).

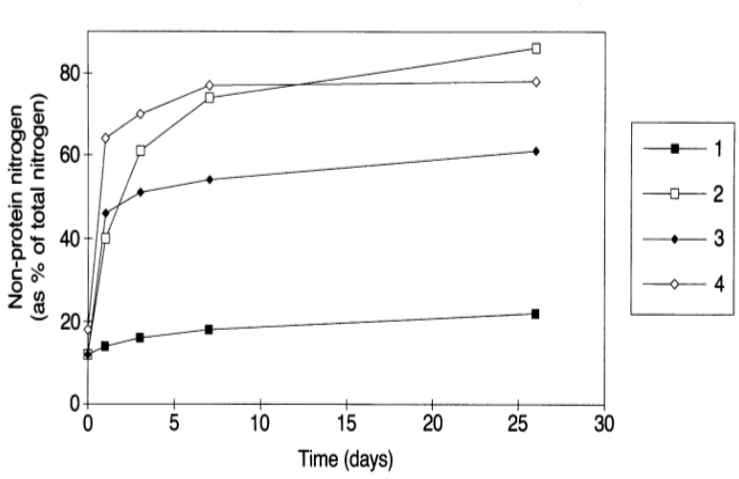


Figure 4: Formation of non-protein nitrogen in silages produced from different parts of cod at pH 3.9 and 300C. (4) silage from viscera; (3) silage from skin; (2) silage from head; (1) silage from flesh (Backhoff, 1976).

Hydrolysis is a temperature dependent process and development of soluble nitrogen is more rapid during the first few days of storage (Tatterson & Windsor, 2001). To determine the freshness raw material and the increase of NH_3-N (from degradation of amino acids) in silage total volatile nitrogen (TVN) is a useful measure (Arason, 1994).

During storage of fish silage, triglycerides in fish oil break down to free fatty acids by the enzyme lipases and this process is known as lipolysis. The formation of free fatty acids is temperature dependent which is shown in Figure 5 (Tatterson, 1976). High level of unsaturated free fatty acid has the risk of being oxidized which is known as rancidity. As a result of oxidation, hydroperoxides are produced initially which readily degraded through a free radical chain to give secondary stable products (Tatterson & Windsor, 2001).

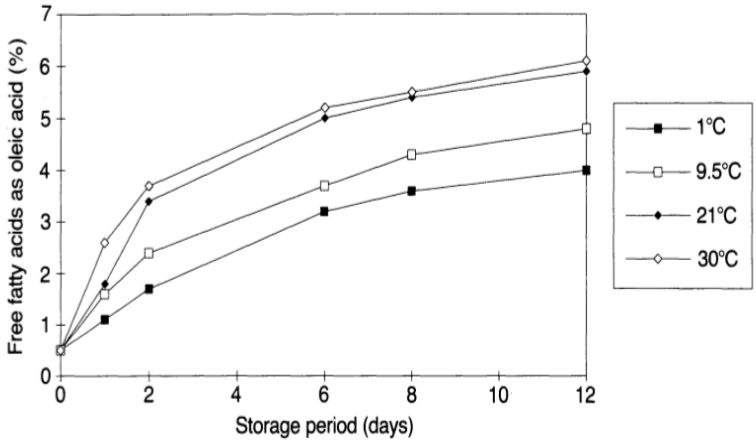


Figure 5: Free fatty acid content of extracted oil during storage of sprat silage (Tatterson, 1976).

Fat oxidation is nutritionally undesirable in fish silage and can be slowed down by the use anti-oxidant such as ethoxyquin (Arason, 1994). Oxidation of fat is measured by the peroxide values (PV) and thiobarbutaric acid reactive substances (TBARS). Oxidized fat in animal feed causes loss of appetite, inhibit growth and even death when the peroxide value increased above 100 ml/kg diet (Tatterson & Windsor, 2001).

2.11 Heat treatment to stop hydrolysis

Termination of hydrolysis in silage production is essential to obtain stable end products. This is usually done by heating the silage from 85^0C for 2 minutes and then cooled down to room temperature. The main purpose of heat treatment or pasteurization is to stop the activity of digestive enzymes from further breaking down of protein and to produce peptides of suitable length. Pasteurization also prevents lipolysis of triglycerides in storage. This process is more applicable when large proportion of the tissue components are dissolved although heating can used at any level of degradation (Arason, 1994).

2.12 Phase separation

In storage, silage is separated into mainly three phases – a surface layer of lipid-protein emulsion, a middle layer of aqueous soluble phase and a bottom layer of small sediment of heavy and insoluble fragments. There will be no separation of layers if silage is in well-mixed condition (Arason, 1994). The oil from upper surface can be separated by the separation

technique applied in fish meal factory. The amount of oil recovery from silage by centrifugation increase with time of incubation. The experiment done on mackerel silage containing 12.5% oil showed that 6.5% was recovered immediately after acidification without heating whereas more oil i.e. 8.2% was recovered after 12 days incubation or storage. Further, 1.8% of oil was possible to recover from the same silage if it was heated to 70^0C (Reece, 1981).

2.13 Storage

In general, fish silage is considered as a stable product and keeps well in extended period of storage without becoming spoiled or rancid (Tatterson & Windsor, 2001). It is reported that silage can be stored for at least 1.5 to 2 years, but addition of antioxidant is essential to prevent oxidation of fat (Raa & Gildberg, 1982). However, in experiments by Hardy et al. (Hardy, Shearer, & Spinelli, 1984), length of storage was found to have directly affect the nutritional value of fish silage since continuous liquefaction process results in lesser amount of dry matter in silage.

2.14 Quality of fish silage

Quality of silage depends on the freshness of the raw materials at the first place. Though, the term quality for fish silage is difficult to define. pH, protein, fat, dry matter and mineral content are commonly regarded as quality indicating parameters. However, there are ranges of other parameters which have been evaluated to determine the quality of fish silage such as TMA, TMAO and TVN (Arason, 1994). TMA is the degraded product of TMAO formed by bacterial decomposition of TMAO. TMA is basically used to determine the freshness of raw materials (Pedersen, 1987) while TVN is tested to measure NH_3 as an indicator of freshness of materials. TVN value more than 50g/ 100g is not suitable for high quality silage (Arason, 1994).

It is essential to ensure well mixing of silage before sample is taken, otherwise the sample will consist of only one out of the three basic layers; oil, aqueous and bottom solid (Arason, 1994) and give wrong information on the quality aspects. Inconsistent quality is one of the main reasons for slow growth of silage industry. It should be kept in mind that silage process cannot improve quality of already spoiled raw materials. Therefore, product declaration for the user is required to get standard information on protein, fat ash and dry matter. The value of mineral in particular is an indicator of the type of raw material used for silage (Jangaard, 1991).

2.15 Nutritional value

The protein and amino acid composition of fish silage is almost similar to that of fish meal made from same type of raw materials. Due to autolysis, protein in silage becomes solubilized and broken down into high level of free amino acids and therefore, fish silage is considered as a good source of essential amino acids (Winter & Feltham, 1983). Loss of amino acid tryptophan and sometimes histidine during storage of acid silage is common, however, most other amino acids were found to remain stable.

Silage consisting fish viscera tends to have lower mineral contents than the silage made from whole fish or heads and frames. In general, fish silage provides good amount of essential minerals such as calcium and phosphorous in readily available form. Vitamin E and A can be destroyed by oxidation of oil in silage. This can be minimized by using anti-oxidants or vitamin additives in diet supplemented with fish silage (Winter & Feltham, 1983).

2.16 Use in aquafeed

Fish silage contains high quality crude protein which can supplement the protein requirement of monogastric (single stomached) animals such as pigs, poultry, fur-bearing animals and fish. In Norway, up to 60% fish silage along with 40% binders is used as pelleted fish feeds. Use of antioxidants is necessary for high oil fish silage containing oil up to 25% (Winter & Feltham, 1983).

Fish silage in fish feed showed varying success in different researches depending on the fish species, type of acid used for ensilation and the method of processing. Silage based moist pellet provided excellent performance in the growth of salmonid fish in Norway (Batista, 1987). Nutritional value of fish silage was found to be affected by prolonged storage. Therefore, heat treatment of fish silage is recommended to stop enzymatic activity prior to drying for diet formulation (Hardy, Shearer, & Spinelli, 1984).

3 MATERIALS AND METHOD

3.1 Experimental design

Red fish (*Sebastes marinus)* viscera was used in this study for production of silage using two organic acids; formic acid and lactic acid. Two different temperatures 25^0C and 45^0C were used for each acid treatment. Here, 25^0C was considered as ambient temperature condition in Bangladesh, while elevated temperature 45^0C was used to accelerate the enzymatic activity. On day 5, the temperature of 45^0C treatment for both lactic and formic acid were raised to 80^0C for 1 minute to inactivate the enzyme and then cooled down to 25^0C for the rest of the experimental period. This was done to obtain a more stable product of silage.

The raw material was kept for 5 days in untreated condition to analyse the degradation in quality relevant to the present handling practice in Bangladesh and then discarded. The duration of the whole experiment was 5 weeks and sampling were done on day-0, day-1, day-5 and week-5 respectively. The structure of the analyses done in this study are shown in Appendix 1.

3.2 Collection of raw materials

Fresh viscera of Red fish (*Sebastes marinus*) were collected from the fish processing company HB Grandi, Reykjavik from Baader gutting machine. Besides, viscera were obtained by degutting some big size Red fish manually to increase the amount of raw material. The raw material consisted mostly of viscera, small parts of fin, gonad and liver. A plastic container of 50 kg was used to collect the raw material and then brought to the Lab of MATIS. The collection process is shown in Appendix 2.

3.3 Preparation of raw materials

In the lab, the raw material was minced immediately with an electric mincer (VCB-62, Hellde). Four treatments of silage were planned for lactic acid and formic acid in two different temperatures 25^0C and 45^0C (LA 25, LA 45, FA 25, FA 45) and the raw material (RM) was kept in untreated condition. A total of 15 plastic containers of approximately 3-liter capacity (3 container for each 4 treatments and 3 for untreated raw material) were prepared and labelled for the experiment. Thus, each of the treatments had three replicates (i.e. LA 25-1, LA 25-2,

LA 25-3). The minced raw materials were weighed to 2.5 kg and put into each plastic container. 3 samples of 100 gm from each replicate (i.e. LA 25-1A, LA 25-1B and LA 25-1C) were planned to take on every sampling day (Appendix 3). The preparation steps are shown in the flow diagram of Figure 6.

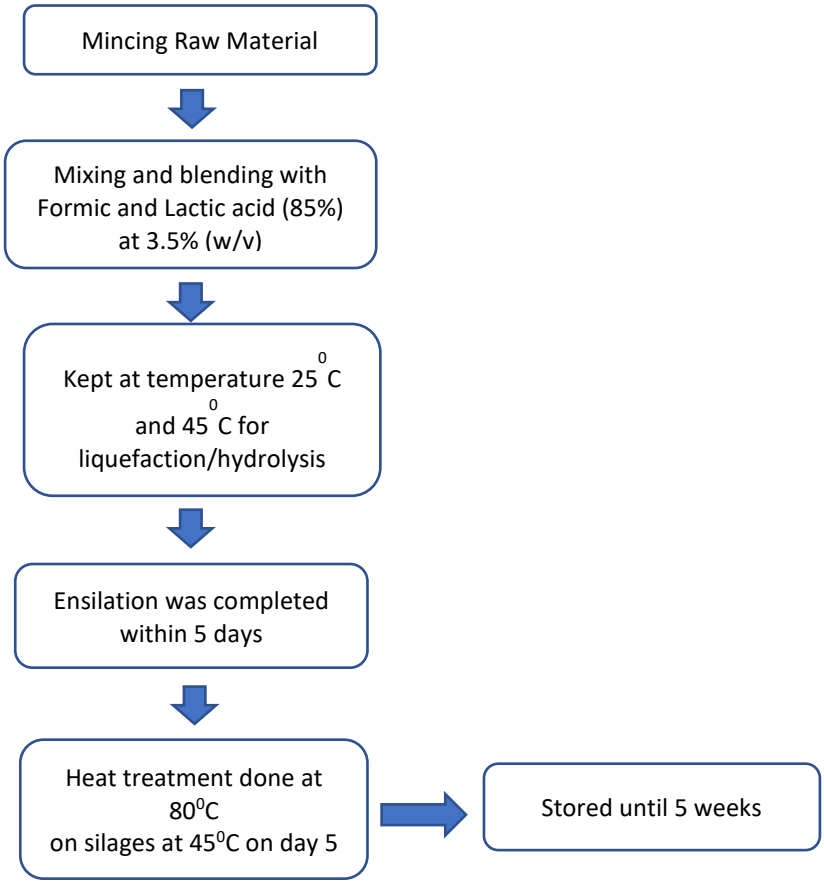


Figure 6: Preparation of Fish silages

3.4 Acid treatment

Formic acid (85%) and lactic acid (85%) were added to the minced raw material in each plastic container at the rate of 3.5% (w/v). After addition of acid, the containers were shaken vigorously so that the acid gets well-mixed and no pocket of untreated material is created. The measurements used for acid treatment was guided by previous works on fish silage production (Arason S. , 1994; Batista, 1987; FAO, 2003). The containers with untreated raw materials were kept in room temperature of 25^0C.

Temperature loggers were used to keep record of the temperatures 25^0C (ambient), 45^0C (elevated) and 80^0C (pasteurization).

3.5 Sampling procedure

After prepared with acid, one treatment for each acid was kept in ambient temperature i.e. 25^0C and the other in 45^0C in a water bath with heater and temperature logger. The sampling dates were decided as follows: day- 0, 1, 5 and week-5. On day-5, two treatments of 45^0C were heated to 80^0C for 1 minute to stop the enzymatic activity then cooled down to 25^0C and kept

in storage. Last sampling for untreated raw material was done on day-5 and then discontinued. For measurement and analysis, samples from replicate-1 were used while samples from the replicate 2 and 3 were kept in -80^{0}C as back up sample.

3.6 Visual observation

Appearance, odour and signs of putrefaction (or growth of moulds) of the silage samples and untreated raw materials were observed on each sampling day and recorded. Photographs of the samples were taken for record.

3.7 pH determination

The pH value of the silage samples and the raw material was measured and recorded on each sampling day by a digital pH meter. The probe of the pH meter was calibrated with buffer solutions of pH 4, 7 and 11 before taking measurement. The pH value was measured in three replicates along with temperature. The sample was stirred well before measuring pH.

3.8 Dry matter and moisture content

Dry matter content (DM) was measured indirectly from moisture content (M) measurement (ISO, 1993). Approximately 2-3 g of homogenized sample was weighed (± 0.0001g) and placed in a small porcelain bowl. The porcelain bowl of sample was left to dry for 4 ± 0.1 hours in the oven at 103 ± 2℃. The bowl was removed from the oven and allowed to cool to ambient temperature in a desiccator for about 30 minutes. The dry matter and moisture content were calculated by the formula as follows;

$$M = \frac{m_2 - m_3}{m_2 - m_1} \times 100\ (\%)$$

Where;
M is the moisture content
m_1 is the mass of the bowl (g)
m_2 is the mass of the bowl, test portion (g)
m_3 is the mass of the bowl, dried test portion (g).

$$DM = 100 - M\ (\%)$$

3.9 Phase separation by centrifugation

For phase separation, 50 ml of sample were weighed by analytical scale and taken into a red screw cap graded test tube. The exact weight of each sample was recorded. Then the samples were centrifuged for 12 min at 2500 rpm at 4^{0}C. After centrifuge was done, the red screw cap test tubes were removed carefully, and the ratio of each phase was measured and recorded. The degree of liquefaction of silage was measured by the increase in volume of the aqueous phase after centrifugation. The picture of the corresponding sample was taken. Further centrifuge was done if the phases need to be separated more noticeably.

3.10 Mineral content

About 5 g of the test samples were previously heated in incineration dish and then put into Muffle furnace for incineration at 550^0C for at least 30 min. The samples were cooled in the desiccator and weighed to the nearest 0.001 g (ISO, 2002).

The crude ash, W expressed as a mass fraction in percent of the test sample, is equal to:

$$W = \frac{m_2 - m_0}{m_1 - m_0} \times 100\ (\%)$$

Where;
m_0 is the mass, in grams of the empty dish;
m_1 is the mass, in grams of the dish containing test portion;
m_2 is the mass in grams, of the dish and crude ash.

3.11 Protein content

Crude protein content is usually determined by Kjeldahl method (ISO, 2005). In this study, percentage of crude protein was determined by simply subtracting total lipid, minerals and moisture content from 100 for each sample.

3.12 Protein content in soluble phase

Protein content solubilized in the aqueous phase was determined by Kjeldahl method (ISO, 2005). After phase separation, the aqueous phase in the raw material (day-0) and silage samples (week-5) were taken out carefully by white tube and put into separate clean test tube of 15 ml. Centrifuge was repeated 2/3 times to get the aqueous phase as clear as possible from any solid particles.

For protein estimation, about 5g of homogenized sample was digested in sulfuric acid in the presence of $CuSO_4$ as a catalyst at approximately 370°C. Thereafter, the sample was placed in a distillation unit, 2400 kjeltec Auto Sampler System. The digested sample was made alkaline by the use of NaOH solution and the nitrogen was distilled off as NH_3. The ammonia was distilled into boric acid solution and the amount of ammonia nitrogen in this solution was quantified by titration with H_2SO_4. The nitrogen content was then multiplied by 6.25 to obtain % crude protein.

3.13 Amino acid compositions

The essential amino acid compositions including tryptophan of raw material (day-0) and two silage samples LA 25 and FA 25 (week-5) were analysed from Eurofins WEJ Contaminants GmbH, Hamburg, Germany. The methods used were as follows: tryptophan (EU 152/2009, IC-UV), cystein +cystine, methionine (oxidative; EU 152/2009 (F), ISO 13903:2005, IC-UV) and alanine, aspartic acid, arginine (acid hydrolysis; EU 152/2009 (F), ISO 13903:2005, AMSUR, IC-UV).

3.14 Total lipid extraction

Total lipid was extracted from samples with methanol/chloroform/0.88% KCl (at 1/1/0.5, v/v/v) according to method by Bligh and Dyer (Bligh & Dyer, 1959). The lipid content was determined gravimetrically, and the results was expressed as gram lipid/100g sample.

The lipid was extracted with a solution of chloroform: methanol: Potassium chloride according to Bligh & Dyer (1959) with adaptations from fish materials. 25 g of sample was weighed into a 250/500 mL centrifuge bottles. 25 mL of chloroform and 50 mL of methanol were added and homogenize for 2 min. Again 25 mL of chloroform was added and continued mixing for 1 min. Finally, 25 mL 0.88% KCl were added and mixed for 1 min. After homogenization, the samples were centrifuged for 20 min at 2500 rpm at 4 °C. The lower chloroform phase containing the fat was absorbed/extracted using plastic pipettes. The chloroform phase was then filtrated via disodium sulphate ($Na_2S_2O_4$) on a glass microfiber filter (Whatman GH/C) under suction. The suction flask content was then poured into a 50ml volumetric flask. Every trace of the upper phase (methanol) was removed and the 50ml volumetric flask was filled with chloroform to the mark. The fat extract was then poured into a 50 ml red screw cap tube and labelled.

For determination of fat, 0.25 ml of fat extract (sample) was taken into a glass tube with micropipette and weight was recorded (weight of the empty tube was recorded before). Then the chloroform was evaporated from the sample by using nitrogen jet at 55^0C for 10-12 min. After cooling down, the weight was recorded again. Then % of total fat was calculated as follows:

$$F_1 = \frac{G_2 - G_1}{0.25} \times 50$$

$$F(\%) = \frac{F_1}{S} \times 100$$

Where;
G_2 = weight of glass tube with fat after evaporation
G_1 = Weight of empty glass tube
G_2-$G1$ = Fat in 0.25 ml extract
F_1 = Fat in 50 ml extract
S = Weight of sample

3.15 Free fatty acids content

Free fatty acid content was determined on the total lipid extract according to Lowry and Tinsley (Lowry & Tinsley, 1976) with modifications from Bernardez et al. (Bernardez, Pastoriza, Sampedro, Herrera, & Cabo, 2005).

After weigh of the fat in 0.25 ml extract was calculated, 3 ml of cyclohexane and 1 ml of cupric-acetate-pyridine agent was added to each glass tube to dissolve the fat and was vortex for 40 sec. Thereafter, the fat solution was centrifuged for 10 min at 2000 g at 4°C. The upper layer of the solution was read at 710 nm in spectrophotometer. The FFA concentration in the sample was calculated as µmol oleic acid based on a standard curve spanning a 2-14µmol range. Results were expressed as grams FFA/100 g of total lipids. The formula for calculation is as follows:

$$\frac{Oleic\ acid \times 282.46 \times 1 \times 10^{-6}}{Sample} \times 100$$

3.16 Data analysis and interpretation

The data was analysed and interpreted by MS- Excel.

4 RESULTS

4.1 Visual observation

The gradual changes in appearance and odour of the untreated raw material (RM) and silage samples from day-0 to week-5 are given in Table 2. There was no change in appearance, smell and colour of the untreated raw material until end and no putrefaction was observed. All the silages became liquified by day-5. Along with liquefaction, the initial light pink colour gradually changed into dark-grey in LA 25, bright-orange (surface oil) and grey (bottom solid) in LA 45, grey in FA 25 and orange (surface oil) and grey (bottom solid) in FA 45. All the changes in visual observation are presented in Appendix 4.

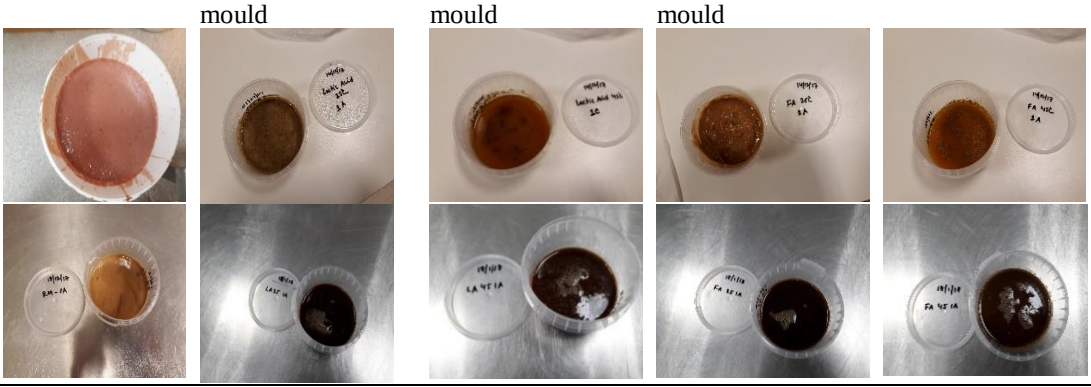
In the beginning, the odour of the untreated raw material (fish viscera) was pungent and characteristic of fish which changed into mild fishy smell in the silages as those become ensiled. It was evident that ensilation was completed for LA 25 and FA 25 by day-3 to day-5, while on the other hand, LA 45 and FA 45 were ensiled faster i.e. on day-1. This rapid liquefaction may be an effect of higher temperate that accelerated the enzymatic activity. All the silage samples remained in acceptable condition until end of storage and no putrid odour or growth of mould was observed.

4.2 pH value of raw material and fish silages

The pH value of untreated raw material (RM) initially was 6.5 which fluctuated a little bit but did not went below 6 (Figure 6). In case of lactic acid silage (LA 25 and LA 45), the initial pH was found 4.18 and 4.14 respectively which was almost same throughout the experiment and became stable at 4.09 and 4.12 respectively on day 36. For formic acid silage (FA 25 and FA 45), the initial pH value was measured at 3.40 and 3.47 respectively which became stable at 3.38 and 3.26 in the end of storage. Thus, lactic acid silage was found to be stabilized below 4.5 and formic acid silage below 3.5 respectively as expected.

Table 2: Changes in appearance, odour, and sign of putrefaction in raw material and fish silages during ensilation and storage.

Day	Raw Material	Lactic Acid 25^{0}C (LA 25)	Lactic Acid 45^{0}C (LA 45)	Formic Acid 25^{0}C (FA 25)	Formic Acid 45^{0}C (FA 45)
0	Thick, light pink colour, raw pungent smell	-	-	-	-
1	same	Semi-liquid, grey colour, mild fishy smell	Liquified, orange (surface oil layer) and grey (bottom solid) colour, mild fishy smell	Semi-liquid, pale grey colour, strong fishy-acid smell	Liquified, orange (surface oil layer) and grey (bottom solid) colour, mild fishy smell
2	same	-	-	-	-
5	Thick, no change in colour and smell *no putrefaction or growth of mould	Liquified, dark-grey colour, mild fishy smell	Liquified, bright orange (oil layer) and grey (bottom solid) colour, mild fishy smell	Liquified, blackish-grey, fishy acidic smell	Liquified, orange (oil layer) and grey (bottom solid) colour, mild fishy smell
36	-	Same *no putrefaction or growth of mould	Same *no putrefaction or growth of mould	Same, *no putrefaction or growth of mould	Same, *no putrefaction or growth of mould



4.3 Dry matter and moisture content

In untreated raw material (RM), the dry matter (DM) and moisture (M) content was found 44% and 56% in the beginning and on day-5, the proportion was 50% for both of them (Figure 7). For lactic acid silage (LA 25 and LA 45), the initial ratio for DM and M content were found as 45%, 55% and 52%, 48% respectively. Then DM content was observed to be decreased (35%, 29%) with a corresponding increase in moisture content (65%, 71%) for both lactic acid silages

at the end of storage (Appendix 5). Similar trend was found for formic acid silages (FA 25 and FA 45).

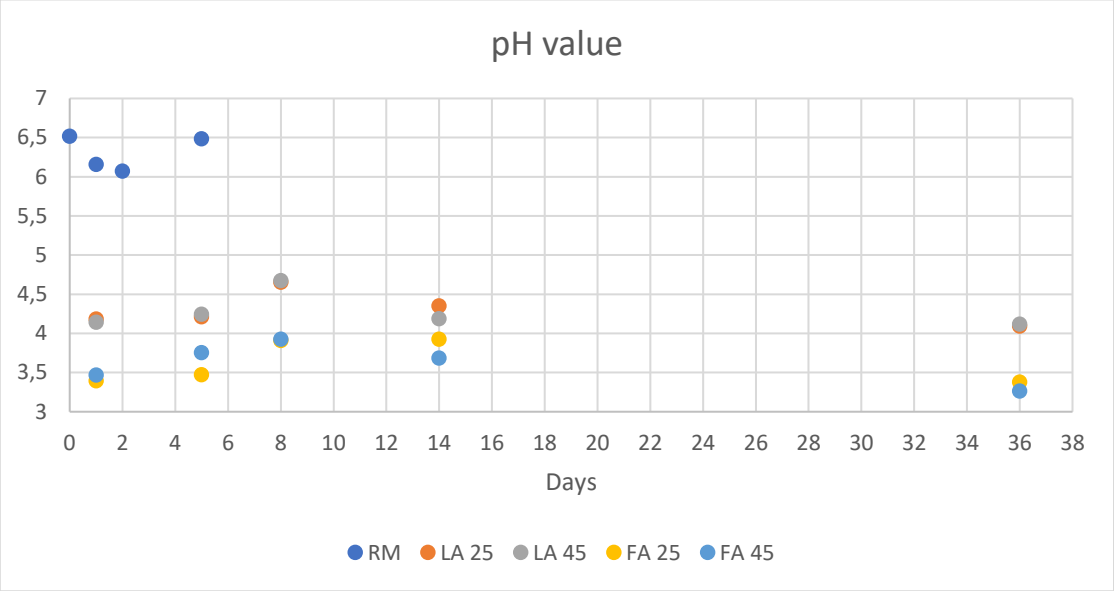


Figure 7: Changes in pH value of raw material and fish silages during ensilation and storage.

4.4 Proportion of different phases of raw material and silages

The untreated raw material and silage samples were centrifuged to determine the ratio of soluble phases due to hydrolysis or liquefaction with time. The weight of the moisture (aqueous) phase is expressed as percentage of the of the total weight of the sample. There were 4-5 layers found from surface to bottom after centrifugation as follows: oil, solid-1, moisture, solid-2 and bottom solid (Figure 8). In untreated raw material, surface oil and bottom solid layer was found to have almost same ratio from the beginning to the end. Moisture phase was not observed to increase though it was expected due to enzymatic activity. The phases in untreated raw material and silages are shown in Appendix- 6, 7, 8, 9 and 10. Both lactic and formic acid silage (LA 25 and FA 25) were found to have 5 layers (with layer solid-2). This layer was not so distinctly separated from moisture phase and easily get mixed up. In contrast, both LA 45 and FA 45 were found to have 4 layers (without the layer solid-2) which may be an influence of higher temperature. The proportion of aqueous phase was expected to increase in both LA 25 and FA 25, however, it was found to be less in LA 25 than FA 25. The differences in degree of liquefaction can be an effect of the strength of the acids as formic acid is known to be stronger than lactic acid.

In case of LA 45 and FA 45, increase in soluble phase as a result of hydrolysis was clearly visible and showed similar trend in both the silages. This may be a direct influence of higher temperature assisting the smaller units of protein molecule to be solubilized in the aqueous phase (Figure 8).

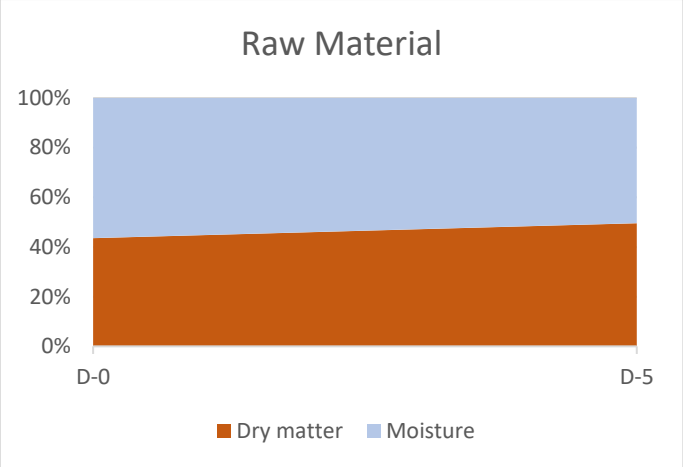


Figure 8: Dry matter and moisture content of untreated raw material

4.5 Chemical composition

To evaluate the chemical composition of raw material and silages; total fat, mineral and moisture was determined first to calculate the relative proportion of protein. In untreated raw material, the protein and mineral content was found to be consistent (around 13%, 1.8%) from day-0 to day-5 (Figure 9). While slight increase in fat (from 29% to 35%) and decrease in moisture content (from 56% to 50%) was observed in the end. Fish viscera is usually higher in fat content (depending on the composition) and lower in protein content than other parts of the body.

Conceptually, the silages in this experiment were supposed to have similar proportion of fat, protein, mineral and moisture content conforming with those of the raw material. However, varied results were found in all the silage samples. For lactic and formic acid silage (LA 25, FA 25), the proportion of mineral contents showed slight increase from initial value i.e. from 1.80% to1.90% and from 2.20 to 2.30% respectively (Appendix 11).

Both LA 25 and FA 25 were found to have lower amount of protein (around 9.57% and 5.63%) than the untreated raw material (13%) in the beginning. Then the protein content decreased further in LA 25 (6.32%) but increased little more in FA 25 (5.88%) in the end. Tissue proteins are gradually broken down by the protease enzyme into smaller peptides and free amino acids in silages which might be the cause of lower proportion of protein. Similar trend was found for lipid or fat content for these two silages. In silage, the enzyme lipase breaks down triglycerides into free fatty acids which increases with time and is dependent on temperature. The reduction in fat may be a result of formation of more free fatty acids. However, the moisture content was found to increase from initial proportion for both the silages (54.53% to 65.38% in LA 25 and 58.97% to 63.42% in FA 25) which may be related to the degree of hydrolysis during ensilation (Appendix 11).

Almost similar trend in LA 45 and FA 45 were found for fat, mineral and moisture content. In contrast, protein content was found to increase in both the silages (LA 45; from 2.61% to 11.01% and from 3.26% to 14.43% respectively) which may be the influence of accelerated temperature and nature of acid. The hydrolysis process in silages at 25^0C was allowed to continue till end of storage which may be the probable cause of breaking down of protein and

fat more and thus reflecting a corresponding decrease in their proportion. On the other hand, the hydrolysis process in silages at 45^0C was more due to higher temperature but then by heat treatment the enzymatic activity was stopped on day-5. This was supposed to inhibit further breakdown of protein which can be a cause of increased amount of protein in the end products (Figure 9).

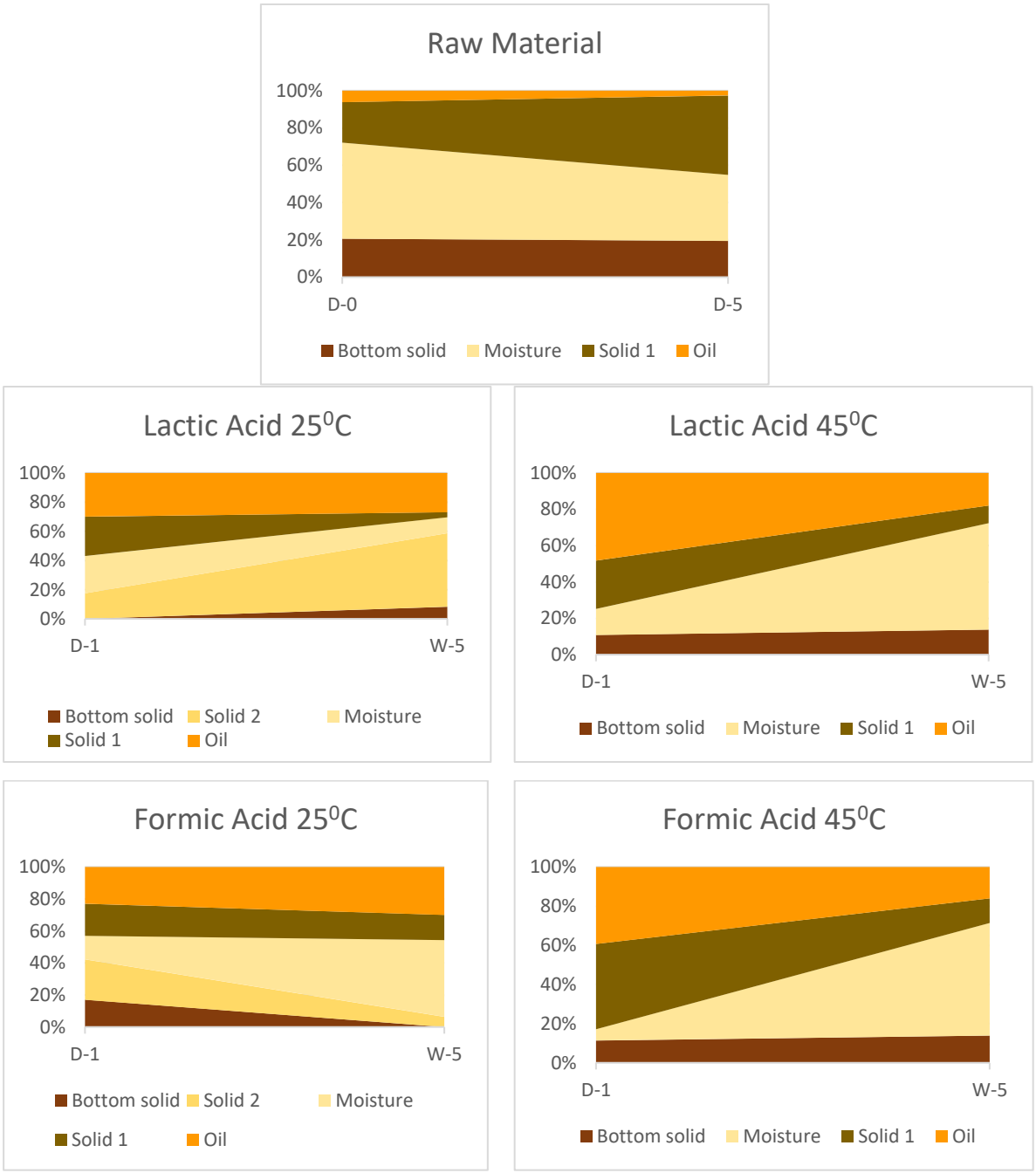


Figure 9: Changes in the rate of hydrolysis in raw material and silages during ensilation and storage.

4.6 Protein in the soluble phase

The proportion of protein solubilized in the aqueous phase of raw material and in the silages (LA 25, LA 45, FA 25 and FA 45) was determined since this protein is important from nutritional point of view (Figure 10). The untreated raw material was found to consist of 10.40% protein in soluble form (expressed as % of total N) which showed an increase in concentration for all of the end products. However, the proportion of protein content in the aqueous phase of the lactic acid silages (LA 25-11.40% and LA 45-11.30%) were more than those of the formic acid silages (FA 25- 11.30% and FA 45- 11.20%).

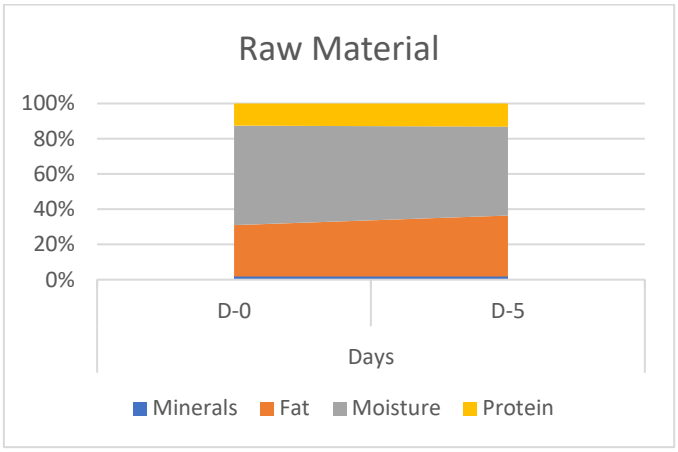


Figure 10: Chemical compositions of untreated raw material.

4.7 Amino acid composition

Amino acid composition of the raw material in the initial state (day-0) and the lactic and formic acid silages (LA 25 and FA 25) in the end (week-5) was determined to assess the concentration of functional units of protein from hydrolysis and possible breakdown of tryptophan. Tryptophan is one of indispensable amino acids that easily breaks down with high temperature and becomes a limiting factor in the end product of silage (Arason, 1994). In the present experiment, tryptophan was found to sustain until the end of storage and the amount of it increased in the silages (LA 25- 0.063 and FA 25- 0.0655 g/100g) than in the untreated raw material (0.0445 g/100g) (Figure 11).

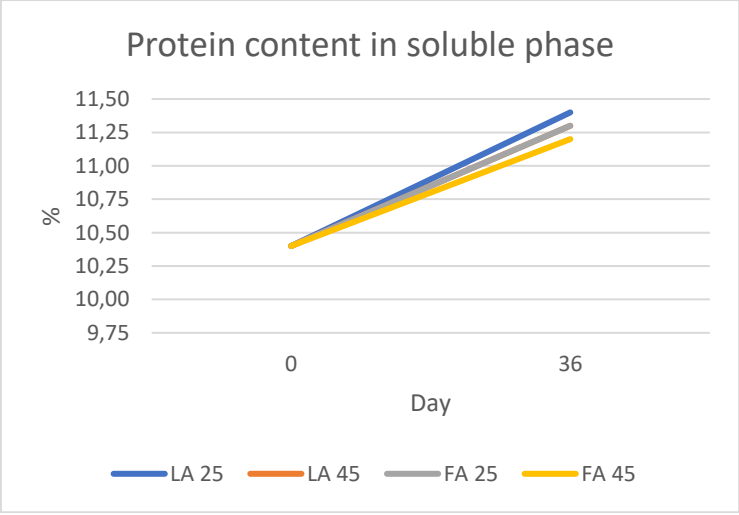


Figure 11: Chemical compositions of untreated raw material.

The concentration of all other amino acids (cystein+ cystine, methionine, alanine, aspartic acid, arginine) conforming to the similar trend. It was observed that the concentration of all other amino acids in LA 25 were very close to those of the FA 25 which may be attributable to the desirable performance of lactic acid. However, status of tryptophan in the silages with higher temperature (LA 45 and FA 45) was not possible to compare in this experiment.

4.8 Free fatty acid content

In this study, free fatty acid (FFA) content of untreated raw material and the respective silages was determined as a ratio of the total fat. The level of unsaturated fatty acid is important to assess the degree of hydrolysis of triglycerides in lipolysis. In untreated raw material, increase in the concentration of free fatty acid was found i.e. from 5.76% (day-0) to 17.29% (day-5) conforming to the rapid degradation during first few days of storage (Figure 12). The trend was similar for LA 25, as the ratio of FFA was more within 5 days (from 6.85% to 10.26%) and then it slowed down (10.88%) in the end (Figure 13). While FA 25 showed a decrease in FFA concentration on day-5 and then a slow increase to the end was observed. This trend is not comparable, and it may be occurred as sampling or analytical error.

Both LA 45 and FA 45 showed an increase in FFA concentration in this experiment from starting to end i.e. from 6.01% to 13.37% and from 4.46% to 10.33% for LA 45 and FA 45 respectively.

5 DISCUSSION

5.1 Ensilation and storage stability

In this work, two organic acids formic and lactic acid were used to preserve the raw material. Formic acid is more common in silage production while direct use of lactic acid was not found in any literature. However, use of Lactobacilli is quite familiar in fermented silage which produce lactic acid to lower the pH and create the environment for autolysis (Fagbenro, 1994). Formic acid silage usually becomes stable at pH 3.5 to 4 (Batista, 1987) which matches with the pH value 3.38 and 3.26 found for FA 25 and FA 45 respectively. On the other hand, recommended pH range for lactic acid fermented silage is 4 to 4.5 (von Hofsten & Wirahadikusumah, 1972) which is comparable with the pH value found for LA 25 (4.09) and LA 45 (4.12) in the end of storage.

Fish silage is considered as relatively stable under long term storage (Tatterson & Windsor, 2001), however storage for more than 6 months is not recommended for commercial purpose. In this experiment, putrefaction or growth of mould was not observed in the silages in 5 weeks of storage since pH was reasonably low to prevent spoilage. However, chemical changes occurring in protein and fat during the process should be taken into account which can affect the quality.

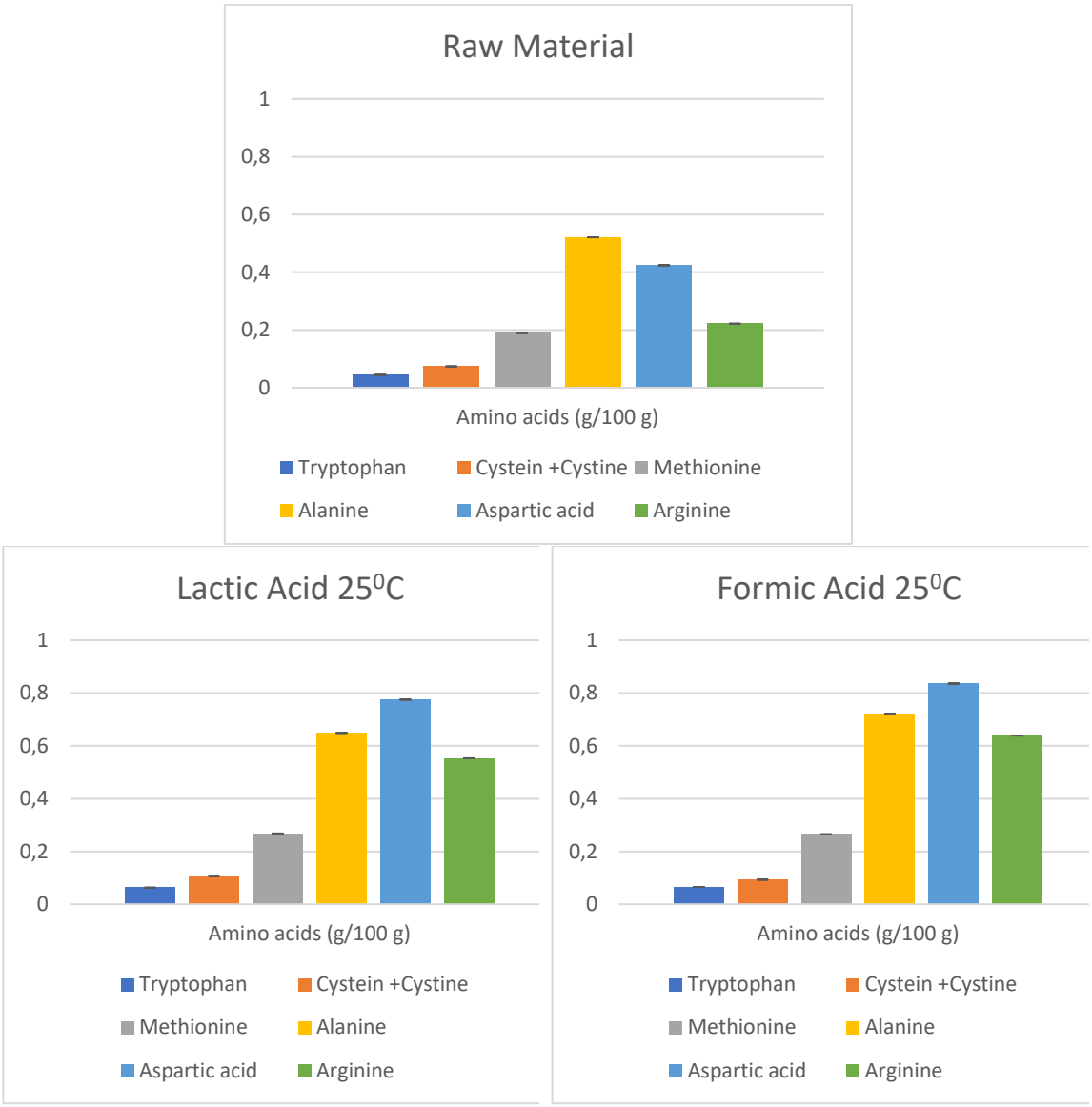


Figure 12: Amino acid compositions in untreated raw material and in silages.

5.2 Degree of hydrolysis

The rate of hydrolysis depends on a number of factors such as composition and freshness of raw materials, activity of digestive enzymes, pH, temperature and characteristics of the preservative acid (Batista, 1987). In this study, the degree of liquefaction was found to be less in LA 25 while more in FA 25 with time while it was expected to increase in both. Lactic acid is a weak organic acid in comparison to formic acid which can be a cause of this difference in liquefaction. Besides, the relative proportion of the layer solid-2 (consisting suspended particles) in the silages can also affect the ratio of soluble phase. Liquefaction in the 45^0C silages (LA 45 and FA 45) was more visible in this work that can be attributable to maximum activity of digestive proteases at temperature 45-50^0C (Arason, 1994). In general, fish viscera liquifies more rapidly than the other body parts which could be better understood from the non-protein nitrogen (NPN) content of the silage samples as the NPN value increases with the rate

of liquefaction (Jayawardena & Poulter, 1980). However, NPN content was not measured within the scope of this experiment.

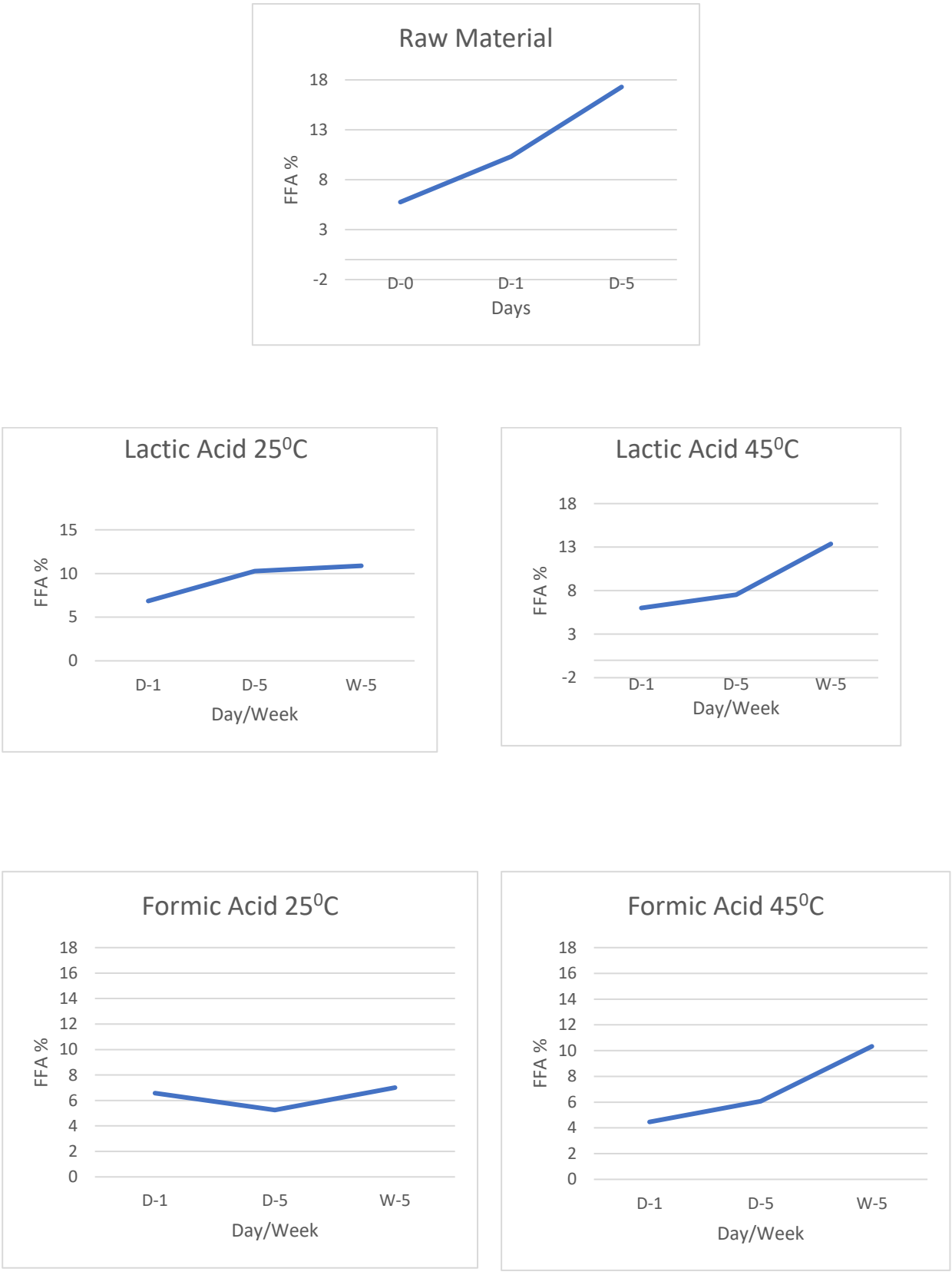


Figure 13: Free fatty acid content of untreated raw material and silages during ensilation and storage.

5.3 Composition of silage

The composition of fish silage is virtually similar to that of the raw material (Tatterson & Windsor, 2001). The moisture, protein, fat and mineral content of the raw material (red fish viscera) found in this study are comparable with the composition of white fish viscera (Arason, 1994). The protein and fat content was found to decrease and moisture content increased in LA 25 and FA 25 in storage. This may be related to break down of protein and fat into more functional units and increase in the volume of moisture phase with liquefaction. In contrast, protein content was found to increase in LA 45 and FA 45 which may be attributable to inactivating the protease enzymes and thus further break down was controlled after day-5. The biochemistry of the hydrolysis process needs detail study to understand the chemical changes thoroughly. Moreover, in this work, the samples consisted higher level of fat than expected which may be because the silages were not constantly stirred to get a homogenous sample.

5.4 Protein in soluble form and stability of amino acid

It has been estimated that up to 70% of the nitrogen bound in polypeptides in any type of silage becomes soluble within 1 week depending on the right temperature (Arason, 1994). This increase was found particularly rapid in the first few days of storage of silage from sprats (Tatterson, 1982). In this study, it was only possible to know the ratio of protein in aqueous phase increased in the end products. But most importantly, lactic acid silage independent of temperature was found to have higher proportion in comparison to formic acid silage which may be a performance indicator of protein hydrolysis for lactic acid.

Amino acids in acid fish silage are reported to be very stable (Batista, 1987). However, continued autolysis will degrade the essential amino acids like tryptophan and produce ammonia (Arason, 1994). Tryptophan was found to be available in both lactic and formic acid silages stored at room temperature (25^0C) for 5 weeks but the effect of higher temperature could not be assessed within the scope of this experiment. In this study, total volatile nitrogen (TVN) was not measured which increases with NH_3-N in fish silage resulting from degradation of amino acids mainly glutamine and asparagine and gives important insights on nutritional value (Arason, 1994). However, the concentration of all amino acids in lactic acid silage are comparable with that of the formic acid silage meaning that lactic acid is at least as good as formic acid in silage production.

5.5 Formation of free fatty acid and influence of temperature

In this study, the rapid increase in free fatty acid for first 5 days of storage for the untreated raw material and silages conforms to the previous works reported by Batista and Arason (Batista, 1987; Arason, 1994). The silages of 45^0C (LA 45 and FA 45) were found to contain more FFA than the silages of 25^0C (LA25 and FA 25) which was expected as hydrolysis of fat is temperature dependent (Arason, 1994). Moreover, this higher amount of FFA could be attributable to the time of heating process during pasteurization at 80^0C which was retained in the end products. The temperature logger showed that it took 40 mins to cool down to room temperature which can have affect the formation of FFA. It was observed in this experiment that the rate of FFA formation was more in lactic acid silage (LA 45) than in formic acid silage (FA 45) of 45^0C. This may indicate that lactic acid is more active in breakdown of fat to produce FFA.

However, unsaturated free fatty acids have the risk of oxidation producing hydroperoxides and secondary reaction products and ultimately affect the nutritional value.

6 CONCLUSION

The production of fish silage using formic acid has been practiced for decades and although the basic method remained same, improvements can be done in the use of other organic acids and nutritional quality assessment during ensilation and storage.

Results obtained in this study indicated that lactic acid can be used as effectively as formic acid in producing silage from fish viscera. The stability of pH below 4.5 without putrefaction gives indication of good storage stability. Moreover, increase in amino acid composition including tryptophan reflects that lactic acid silage by no means is inferior to formic acid silage. The concentration of protein in soluble phase and the ratio of unsaturated free fatty acid in lactic acid silage is comparable to that of the formic acid silage.

Although pH gives indication if the silage is deteriorating or not; other quality parameters such as hydroperoxides (PV), thiobarbutaric acid reactive substances (TBARS), total volatile nitrogen (TVN) and trimethylamine (TMA) values of the silage during storage need to be considered.

Handling of fish waste is a big challenge for the fish industry of Bangladesh. Indiscriminate disposal of this waste materials is not only causing severe environmental pollution but also loss of valuable nutrients. First of all, we need to develop proper technology to preserve the raw materials immediately after processing. The technology of silage production is simple, low-cost and applicable in our country context. This preservation process serves two purposes: prevent environmental degradation and ensure the quality in raw waste products in first place.

Findings from this study can provide some useful guidelines on using lactic acid to produce silage efficiently. Lactic acid is likely to be a better alternative to formic acid since it is cheap in price, not corrosive and will be easy to use in small-scale production. Once, the quality in raw fish materials is maintained it can be taken forward to further value addition to be used in aquaculture, nutritional and other prospective uses.

7 RECOMMENDATIONS

Further research is required to determine the nutritional quality of fish silage by lactic acid and formic acid by incorporating the analyses of fat oxidation, trimethylamine, total volatile nitrogen and non-protein nitrogen content. Moreover, in next research design, sampling should be carried out on every single day particularly during first few days of ensilation since the chemical changes are more rapid during this time.

In order to ensure homogenous sample, the silage should be maintained in constant stirred condition. Besides, sampling technique should be improved so that components from each layer is collected in representative proportion. This is very important precisely for silage prepared from fatty raw materials like fish viscera in which dry matter content is lower than the oil phase.

Silage from fish viscera is quite susceptible to rancidity in storage, therefore, addition of anti-oxidant should be taken into account. Further analysis is needed to assess the effectiveness of heat treatment of the digestive enzymes in silage.

Since the knowledge on the biochemistry of the silage process is not fully studied, detail research is required to standardize the nutritional composition in the final product to be used as feed resource.

Finally, it can be said that to meet the management challenge of fish waste in Bangladesh and to convert it into revenue generating resource, proper preservation technology is the first step. Therefore, government has to take initiative to support the research in this field and facilitate collaboration with the industry as well.

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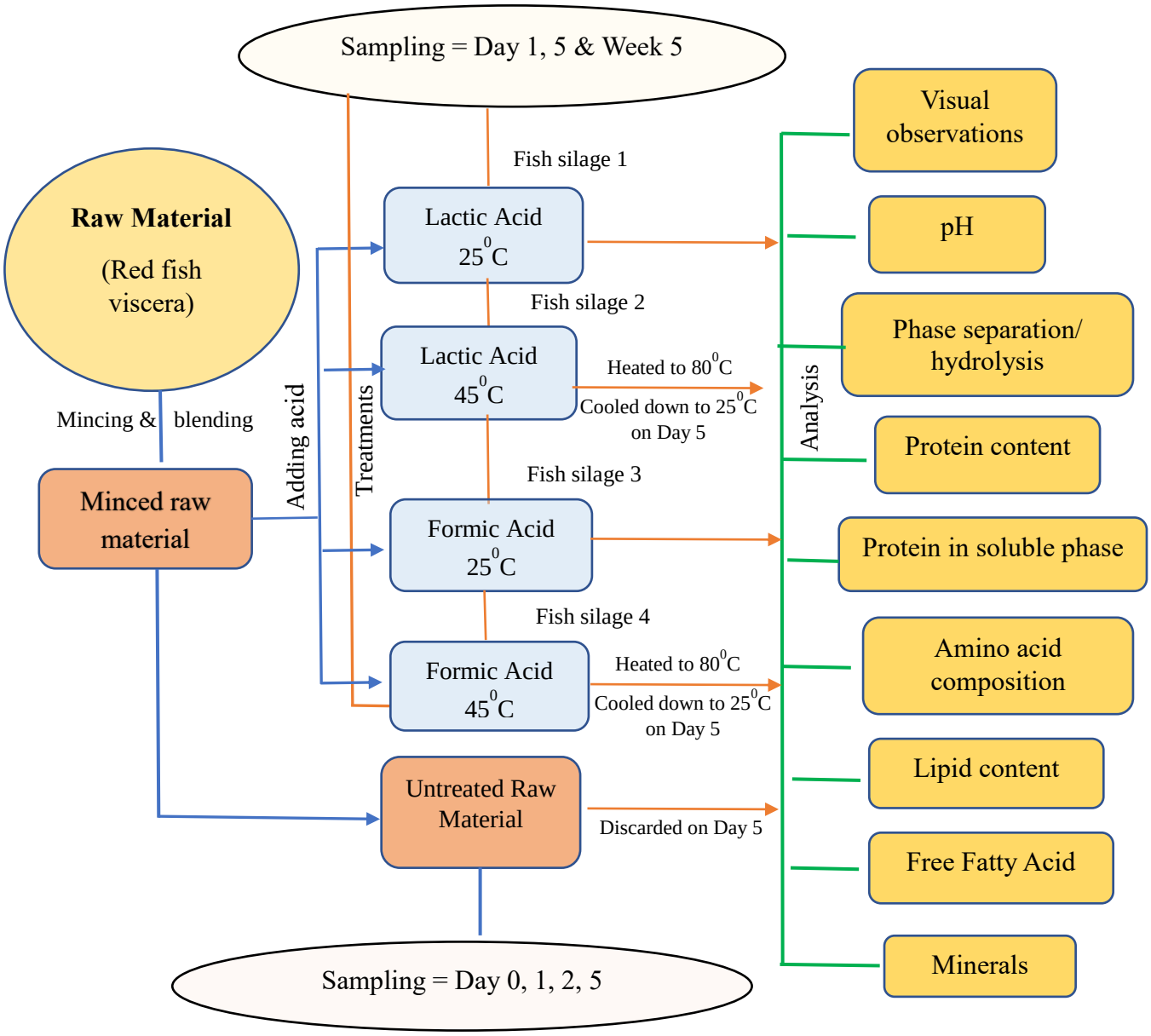
APPENDICES

Appendix 1. Experimental Design



Appendix 2. Collection of raw material from processing factory, HB Grandi.

Atlantic Red Fish (*Sebastes marinus*)

Fish processing line

Fish processing line

Baader gutting machine (153) in operation

Baader gutting machine (open)

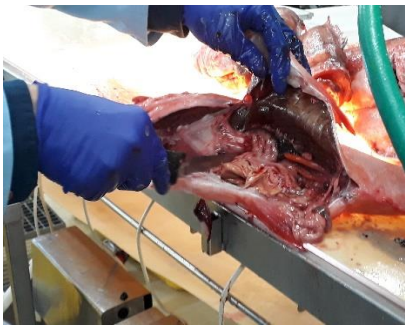
Collection of raw materials from Baader machine

Collection of raw materials in plastic basket

Raw materials put into plastic container (1)

Raw materials collected in plastic container (2)

Red fish degutted manually (1)



Visceral compsitions

Red fish degutted manually (2)

Fish liver putting into collection container

Red fish ready for degutting

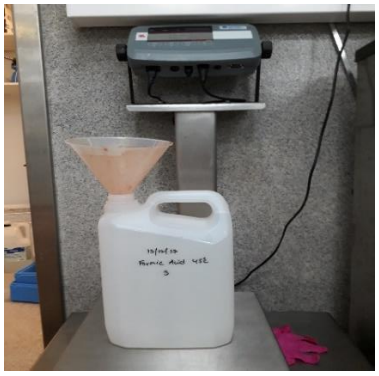
Red fish viscera being collected

Appendix 3. Mincing and preparing raw material for ensilage.

Electric mincer (VCB-62, Hellde) used for mincing raw material

Inside view of electric mincer (VCB-62, Hellde)

Minced raw material



Silage container on weigh machine

Minced raw material taken for weighing

Minced raw material weighed into silage container

Silage cotainers after weigh

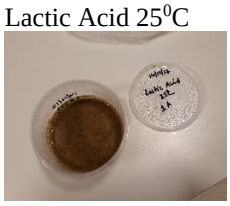
Raw material samples day-0)

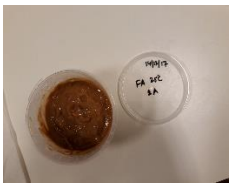
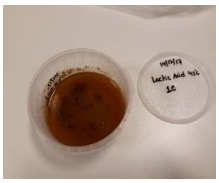
Silage containers after adding acid

Silage containers in water bath at 45^0C (Formic acid and Lactic acid))

Temperature reading from temperature logger

Appendix 4. Changes in appearance of Raw Material and Fish silages during ensilation and storage.

Raw Material	Lactic Acid 25^{0}C	Lactic Acid 45^{0}C	Formic Acid 25^{0}C	Formic Acid 45^{0}C
				
Day-0	Day-1	Day-1	Day-1	Day-1
				
Day-1	Day-5	Day-5	Day-5	Day-5
				
Day-2	Week-5	Week-5	Week-5	Week-5
				
Day-5				

Lactic and Formic Acid 25^{0}C on day-1		Lactic and Formic Acid 45^{0}C on day-1	
			
			
Lactic and Formic Acid 25^{0}C on day-5	Lactic and Formic Acid 45^{0}C on day-5	Lactic and Formic Acid 25^{0}C on week-5	Lactic and Formic Acid 45^{0}C on week-5

Appendix 5. Phase separation of Raw material on day-0.

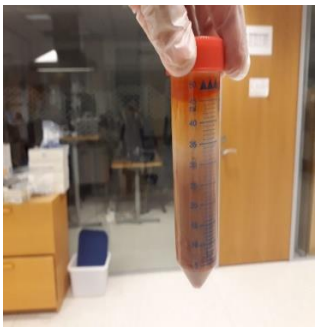
Weighing of sample (1)

Weighing of sample (2)

Samples inside centrifuge machine

Centrifuge machine in operation

Raw material after centrifuge



Phases in raw material

Appendix 6. Phase separation of Raw material and Silages on day-1.

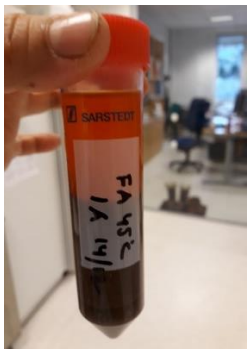
Raw material after centrifuge

Phases in raw material

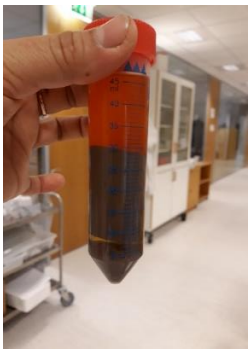
Lactic acid silage 25^{0}C after centrifuge

Phases in Latic acid silage 25^{0}C

Lactic acid silage 45^{0}C after centrifuge



Phases in Lactic acid silage 45^{0}C



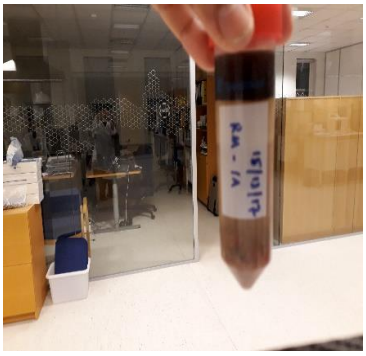
Formic acid silage 25^{0}C after centrifuge

Phases in Formic acid silage 25^{0}C

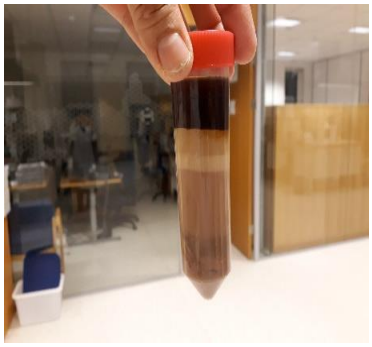
Formic acid silage 45^{0}C after centrifuge

Phases in Formic acid silage 45^{0}C

Appendix 7. Phase separation of Raw material on day-2.



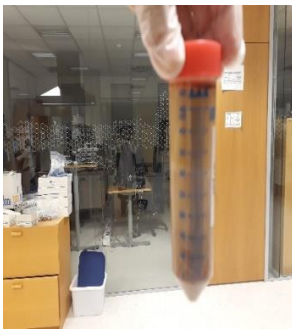
Raw material after centrifuge



Phases in raw material

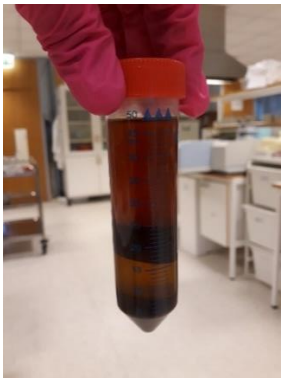
Appendix 8. Phase separation of Raw material and Silages on day-5

Raw material after centrifuge

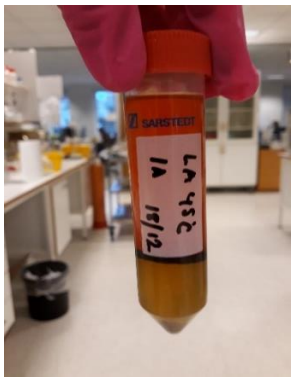


Phases in raw material

Lactic acid silage 25^{0}C after centrifuge

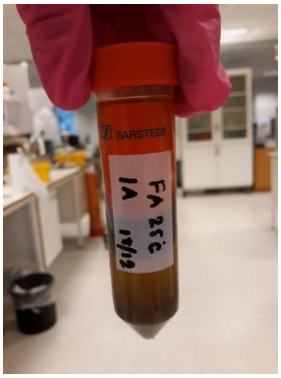


Phases in Latic acid silage 25^{0}C



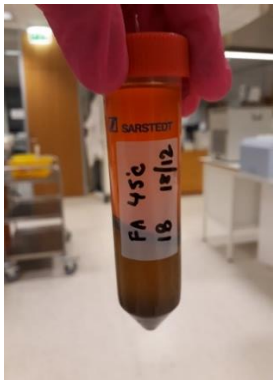
Lactic acid silage 45^{0}C after centrifuge

Phases in Lactic acid silage 45^{0}C



Formic acid silage 25^{0}C after centrifuge

Phases in Formic acid silage 25^{0}C



Formic acid silage 45^{0}C after centrifuge

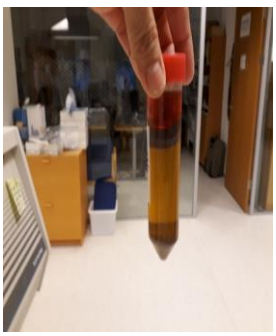
Phases in Formic acid silage 45^{0}C

Appendix 9. Phase separation of Raw material and Silages on week-5.

Lactic acid silage 25^{0}C after centrifuge

Phases in Latic acid silage 25^{0}C

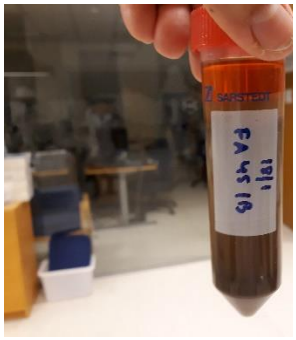
Lactic acid silage 45^{0}C after centrifuge



Phases in Lactic acid silage 45^{0}C

Formic acid silage 25^{0}C after centrifuge

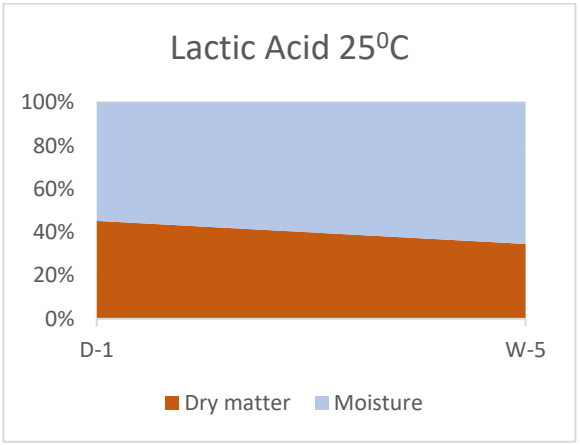
Phases in Formic acid silage 25^{0}C

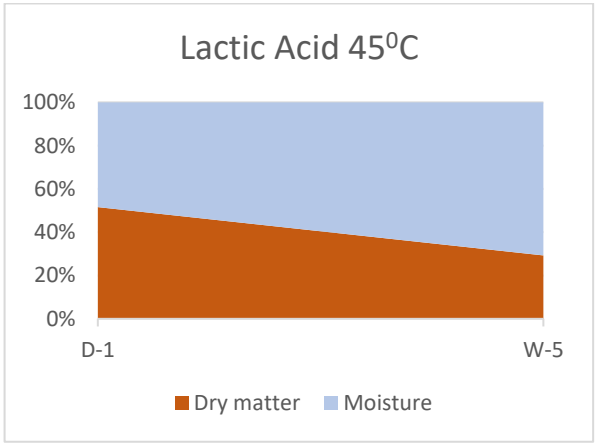


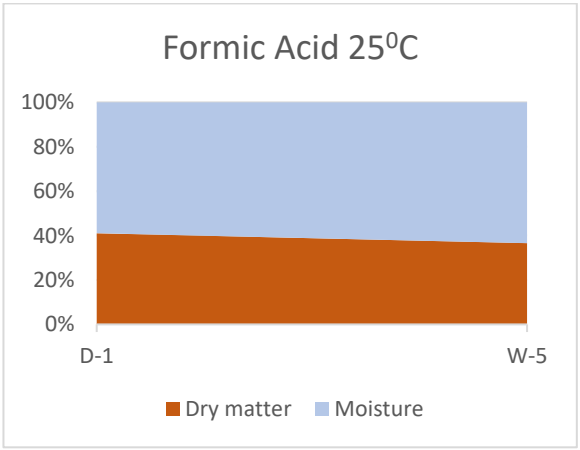
Formic acid silage 45^{0}C after centrifuge

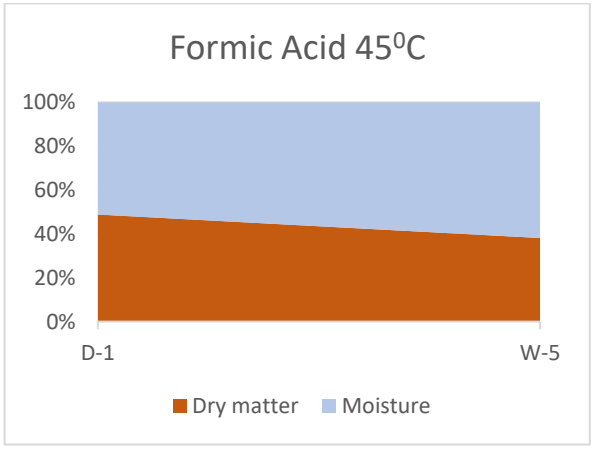
Phases in Formic acid silage 45^{0}C

Appendix 10. Dry matter and moisture content of fish silages during ensilation and storage.









Appendix 11. Chemical compositions fish silages during ensilation and storage.