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PHYSICOCHEMICAL CHARACTERISTICS OF LIQUID FERTILIZER FROM FISH VISCERA

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ABSTRACT

A study was conducted to develop a liquid fertilizer from fish viscera and analyse its physicochemical character. Effectiveness on the growth of plants was verified by estimating the amount of nutrients in onion and garlic. Liquid fish fertilizer obtained by three methods represents an alternative process for the use of viscera; 1 M NaOH treatment as first method, natural enzyme treatment as second method and alcalase treatment as third method. The results showed alcalase treatment fertilizer contain 13.16% of protein, 79.80% of moisture, 0.20% of lipid and 1.91% of ash. Alcalase treatment fertilizer has high macronutrient such as 2.11% of nitrogen, 0.22% of phosphorus, and 0.25% of potassium. Comparing fish fertilizers with industrial fertilizer (Maxicrop) showed the latter contained less nitrogen (0.72%). Fish viscera can be applied as a fertilizer because it contains enough nutrients for plants. Onion and garlic treated with alcalase treatment fertilizer showed highest total growing ratio (92.60% for onion and 105.55% for garlic) and plant height (38.50 cm for onion and 50.13 cm for garlic). Alcalase treatment fertilizer was very effective to be used as fertilizer for growing onion and garlic as it produced higher total growing ratio and yields, compared with industrial fertilizer (Maxicrop). Solid fish silage has the potential to be developed into a dry fertilizer and could be a suitable substitute for fishmeal in producing high quality and nutrient-rich powder fish silage.

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1 INTRODUCTION

The catch of milkfish in Indonesia 2010 was 421,757 tonnes, which increased to 621,393 tonnes in 2014, a growth of 10.4% per year. The government target was reached when catch of milkfish reached 1.2 million tonnes in 2015 (Directorate General of Aquaculture 2015).

Deboned milkfish products have become popular and common in stores and markets in Indonesia. By-raw material from milkfish such as viscera and cut-offs are approximately 20-30% of the fish (Suseno and Suhono 2007).

These raw materials from fish contain various components, such as, Nitrogen (N), Phosphorus (P) and Potassium (K), and are constituent components to be used in natural fertilizer. Protein in this material can be degraded into peptides or amino acids by enzymatic hydrolysis.

Fish silage is a natural liquid product made entirely from cut-offs and viscera, such as heads, guts, skin and cartilage. The by-raw materials contain minerals, trace elements, nutrients and amino acids, and can be used as a fertilizer for soil or as a supplement to animal feed (McNeill *et al.* 2008).

The main advantage of fish silage process is the utilization of the material in valuable products which is otherwise thrown away. The advantages of making fish silage instead of fish meal can be summarized as follows; c*apital cost* of fishmeal plant is fairly high; the cost of silage equipment is fairly low, *processing* of fishmeal requires engineers and technical staff; silage can be made by unskilled workers, and *smell is* a problem when making meal, unless specially equipped plant is used; there is only minor smell when making fish silage (FAO 2001).

The Indonesian Government Program "Go Organic 2010" must be continued with the program "Go Organic 2020" to ensure the sustainability of the livelihood of the people, through the formulation of the vision, mission and activities undertaken with a broader circle of involved, organic farmers, organic entrepreneurs, non-governmental organizations, as well as colleges that are entirely commanded by the government to fulfil the people's mandate (Susanto 2015).

The use of fertilizers in Indonesian agricultural sector increased five-fold between 1975 and 1990 and continued to increase. However, as a result of the Asian economic crisis in 1998, the government reduced the subsidies for fertilizers, resulting in increasing agricultural input costs. Since then, farmers have been reducing the use of chemical fertilizers and have started to utilize more organic fertilizer and improve the methods for its application (Syuaib 2006).

The benefits of organic farming in support for environmental health gives a positive impact on public health, because it does not cause environmental pollution (water, air, soil) by the residues of synthetic chemical fertilizers and synthetic chemical pesticides. Besides, organic farming creates healthy communities through the provision of agricultural products that are free of pesticides and chemical fertilizer residues (Susanto 2015).

This study started from a plan to utilize the remaining of deboned milkfish processing such as bones and viscera. As research shows, there is similar amount of the nutritional content between milkfish viscera and cod viscera (without liver and roes). Therefore, viscera from cod which is the most common species in Icelandic waters was used to develop the fertilizer in this study. However, the process can be applied in Indonesia, not only for viscera from milkfish, but also for other by-raw materials from this species.

The aim of this study was to develop and produce liquid/dried fertilizers from the viscera of cod with different methods, to optimize the highest nutrient content in the final product for horticultural plants, and to support the Government's program "Go Organic". The objectives of this study were to (a) analyse the physicochemical character of the various liquid fertilizer and (b) to verify the effectiveness on the growth of plants by estimating physical growth.

2 LITERATURE REVIEW

2.1 Milkfish (*Chanos chanos*)

Currently milkfish (Figure 1) is an important brackish water aquaculture species in Southeast Asia and represents an important component of the fisheries sector and national economy in Indonesia, the Philippines, and Taiwan (Martinez *et al.* 2006).

Figure 1. Milkfish (Chanos chanos).

The raw material for silage production can include viscera and cut-offs from milkfish. These by-raw materials are potential source for silage. Supartinah (2012) reported data on the nutrient content of milkfish viscera. The results showed that milkfish viscera contained up to 66.8% moisture, 1.2% ash, 9.7% lipid, and 8.7% protein.

This amount is similar to the nutritional content of cod viscera (without liver and roes) based on research conducted by Bechtel (2003).

2.2 Production of fish silage

Bechtel (2003) reported data on the nutrient content of cod viscera. The results showed that cod viscera (without liver and roes) contained up to 76.5% moisture, 2.0%, 8.1% lipid, and 13.0% protein.

Commonly, high quality cod roes are used in the food industry and high-quality liver is used for cod liver oil production. The rest of the viscera are mainly processed to fish meal or silage, two low value products (Horn *et al.* 2007).

Traditional methods to produce fish hydrolysates, such as fish silage exploit the endogenous enzymes of the fish intestines and are still the most commonly used methods for adding value to fish by-products, such as viscera (Arason 1994).

Sodium hydroxide pre-treatments were shown to be effective at a lower temperature (15-55 °C), compared with acid pre-treatment. But, the time required is in hours or days rather than minutes or seconds needed for acid pre-treatment (Sambusiti *et al.* 2013a).

The method for producing silage is to acidify fish viscera, cut-offs or even a whole fish, after mincing the raw material. Generally, the material is acidified to pH 3.6-3.8 to increase the shelf life of the viscera, at the same time autolysis takes place in the fish viscera by present enzymes. Formic acid (CH_2O_2) 85% is added to lower the pH to 3.6-3.8 of the silage, to increase the shelf life. Formic acid at the recommended pH inhibits bacterial growth in feeding stuff and water for drinking and is recognised as an efficacious silage additive (EFSA FEEDAP Panel, 2014).

Formic acid is the best choice for the preparation of fish silage and the silage becomes liquid and homogenous in several days (Oetterer 2002). Fish viscera have wide biotechnological potential as a source of digestive enzymes, especially proteinases. The biological diversity of fish species provides a wide array of enzymes with unique properties (Klomklao 2008).

The effects of initial inactivation of endogenous enzymes, water and different enzymes on the yield of proteins and oil from cod (*Gadus morhua*) were studied by Slizyte *et al.* (2005). The enzymes used in the hydrolysis were Alcalase and Lecitase ultra. The results revealed that initial heating of raw material changed its composition and inactivated the endogenous enzymes. The yield of fish protein hydrolysate had higher amount of lipids such as phospholipids and other polar lipids. Kristinsson and Rasco (2000) reported that Alcalase was the best enzyme to use for the degradation of proteins from fish and fish waste. They also stated that Alcalase is prominently used in the hydrolysis of proteins from fish due to its high degree of hydrolysis in a relatively short time.

2.3 Fertilizer

Fertilizers are characterized by nitrogen, phosphorous and potassium content (N-P-K). Generally, by-raw materials from fish, like cut-offs, are used in the production of mince, while materials like viscera are used for production of fishmeal or fertilizer in agriculture or just thrown away. Fish fertilizer are sometimes classified as organic or natural (Bimbo 2009). Fertilizer from fish viscera, like other organic fertilizers is environmentally friendly, and does not leach readily and stays longer in the soil, hence the risk to pollute the aquatic ecosystems is negligible (Lema and Degebassa 2013).

Fertigation is the injection of fertilizers and other water-soluble products into an irrigation system and is widely used in horticultural systems in the world. However, it is necessary to know how much fertilizer is needed for optimum yield for horticultural plants. Chemical fertilizers imported in developing countries are very expensive (Ortas 2013).

3 MATERIALS AND METHODS

3.1 Experimental design

This project includes two tasks; Task 1 was to develop and produce a liquid fertilizer from cod viscera using three different methods, ready to be used for horticultural plants, and Task 2 was to estimate growth in onion and garlic (Figure 2).

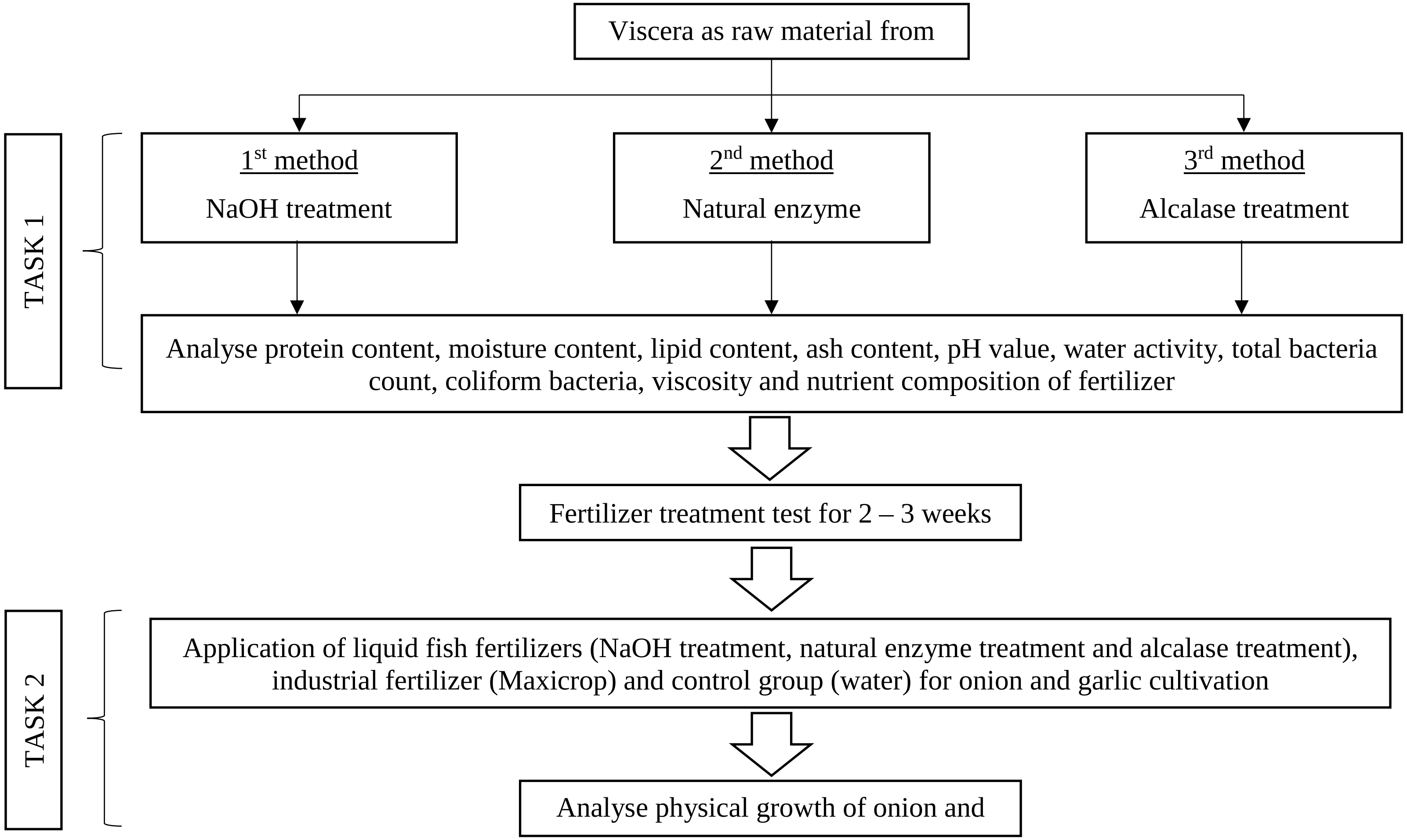

Figure 2. Experiment design diagram of producing liquid fish fertilizers and its application.

Three different methods were developed and tested for ensilaging cod viscera, with the aim to produce a liquid fertilizer with the highest nutrition yield.

3.1.1 Treatment with 1 M sodium hydroxide (NaOH)

This treatment was based on creating a stabilized silage from the viscera by dissolving it in NaOH and decreasing the pH to 3.6 by addition of formic acid, to increase the shelf life and to be used as a fertilizer for horticultural plants (Figure 3).

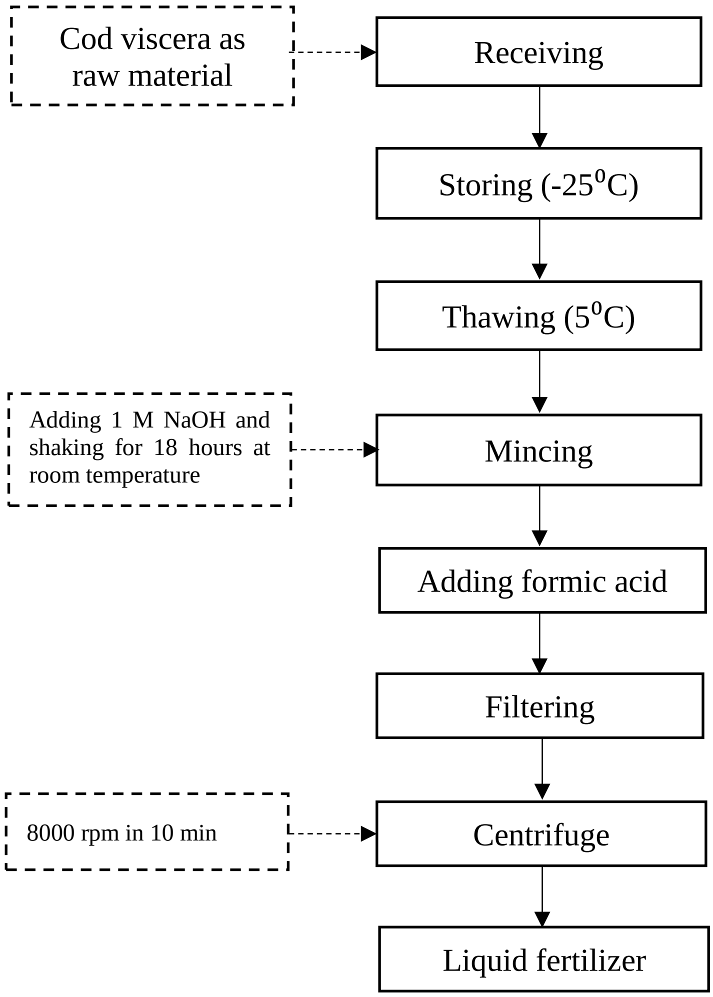

Figure 3. Flow chart of alkali treatment in liquid fish silage production.

3.1.2 Treatment with natural enzymes, present in the viscera

The treatment was based on using natural enzymes, present in the viscera, to degrade the nutritional components in the silage. This procedure is followed by addition of formic acid to decrease the pH for the preservation of the silage to be used as a liquid fertilizer for horticultural plants (Figure 4).

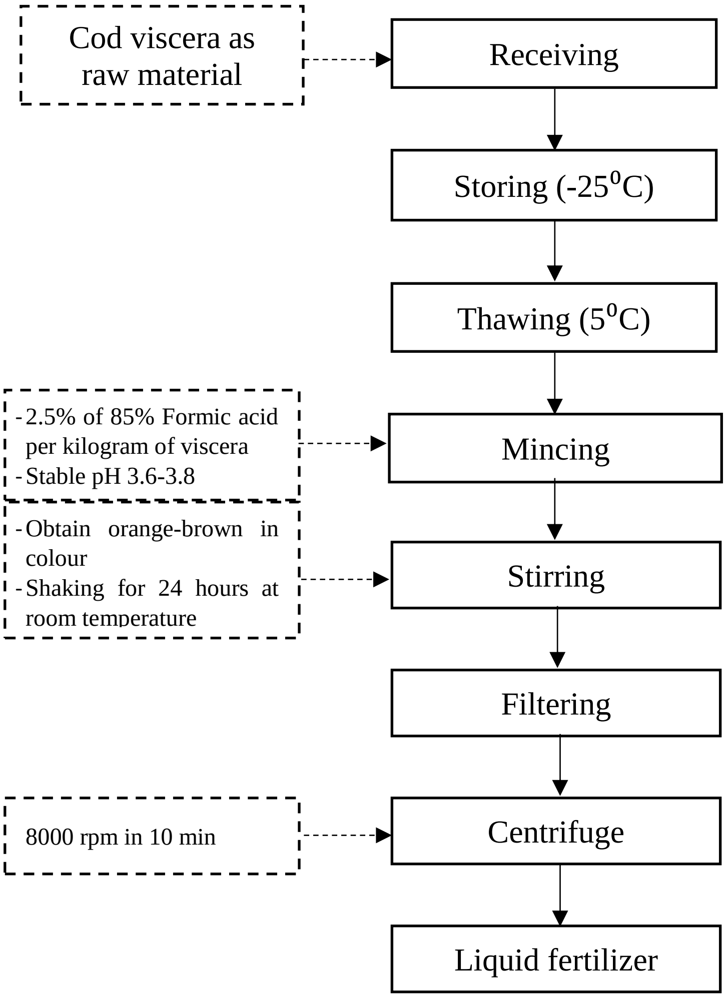

Figure 4. Flow chart of natural enzymes treatment in liquid fish silage production.

3.1.3 Treatment with industrial enzyme (Alcalase)

This treatment was based on using industrial enzyme Alcalase (Novozymes, Bagsvaerd, Denmark) to degrade the viscera for silage production (Figure 5).

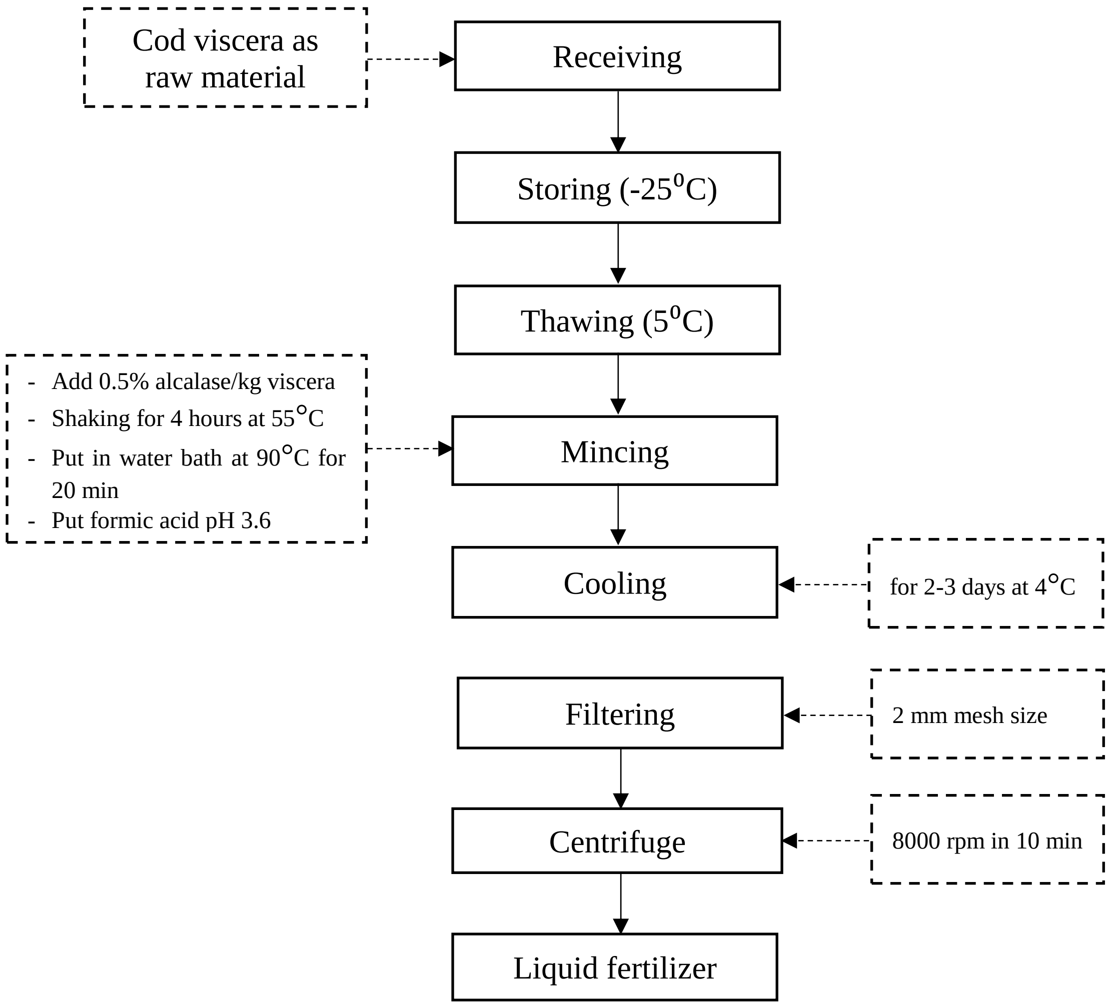

Figure 5. Flow chart of industrial enzyme treatment in liquid fish silage production.

3.2 Raw material

Viscera from cod was the raw material used in developing liquid fertilizer. The raw material was received from processing plants on the south-west coast of Iceland. The viscera contained no liver or roes and it was stored at -25°C until needed.

Two types of plants, onion and garlic were watered with different types of fertilizers produced in this project along with industrial fertilizer (Maxicrop) and a control group (water), to estimate the growth of the plants.

Onions were in the genus Allium, in the Alliaceae family (Maynard and Hochmuth 1997). Onion (*Allium cepa* L.) is one of the horticultural plants consumed by humans as seasoning

(Irfan 2013). Maximum yield and bulb weight uniformity were the two important characters in determining the marketable proportion of onion (*Allium cepa* L.) performance (Krishnamuthy and Sharanappa 2005). Nutrients play a significant role in improving productivity and quality of crops. Tiwori *et al.* (2002) noted nitrogen is an essential element for both growth and productivity of crop.

Garlic (*Allium sativum* L.) belongs to the family Alliaceae and is the second most widely used after onion (Rubatzky and Yamaguchi 1997). Garlic is highly accepted for its flavour enhancing capacity (Lošák and Winiowska-Kielian 2006).

Onion and garlic were planted in soil lacking nutrients. A chamber with fluorescent light was used in this experiment (Figure 6).

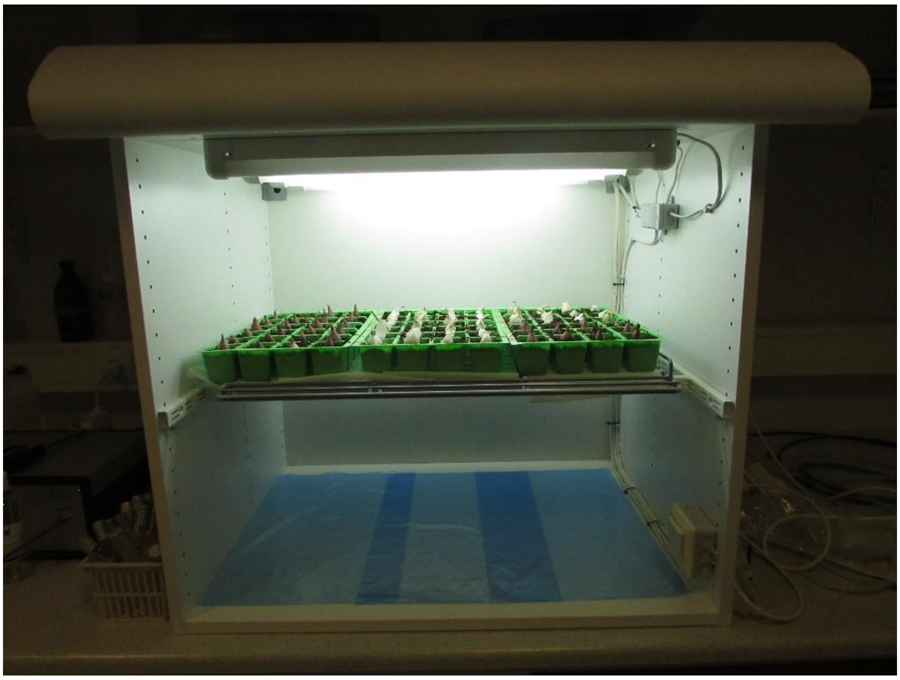

Figure 6. A chamber with fluorescent light used for planting onion and garlic plants for the experiment.

Onion and garlic seeds were planted 3 weeks before being transplanted in soil. The plants were weighed before transplanting. During the experiment, the plants were watered every other day with water and fertilizers.

The fertilizers were prepared by diluting concentrated liquid fish fertilizer and industrial fertilizer to concentrations 0.5% in 100 ml water. In this experiment, feeding the plants took 28 days. The plants that were only watered, was the control group, the others were fed with the developed and industrial fertilizers (Figures 7-9). Furthermore, total growing ratio, number of leaves and plant height of onion and garlic were analysed.

NaOH treatment fertilizer (A)		Natural enzyme fertilizer (B)		Alcalase treatment fertilizer (C)	
A	A	B	B	C	C
5.6 g	2.9 g	2.6 g	7.4 g	6.6 g	3.4 g
3.5 g	4.5 g	3.2 g	4.7 g	2.7 g	2.8 g
7.2 g	2.4 g	3.5 g	5.9 g	4.0 g	5.7 g
2.4 g	8.2 g	4.8 g	5.2 g	4.4 g	3.2 g

Figure 7. Onion planting scheme using liquid fish fertilizer (The original weight of each plant when they were transplanted is shown below the plant)

NaOH treatment fertilizer (A)		Natural enzyme fertilizer (B)		Alcalase treatment fertilizer (C)	
A	A	B	B	C	C
8.9 g	5.2 g	4.2 g	4.9 g	6.0 g	4.9 g
6.9 g	4.3 g	5.3 g	5.2 g	4.8 g	6.3 g
5.4 g	6.5 g	5.4 g	8.4 g	7.9 g	5.5 g
5.9 g	4.4 g	6.5 g	3.8 g	4.3 g	4.0 g

Figure 8. Garlic planting scheme using liquid fish fertilizer (The original weight of each plant when they were transplanted is shown below the plant).

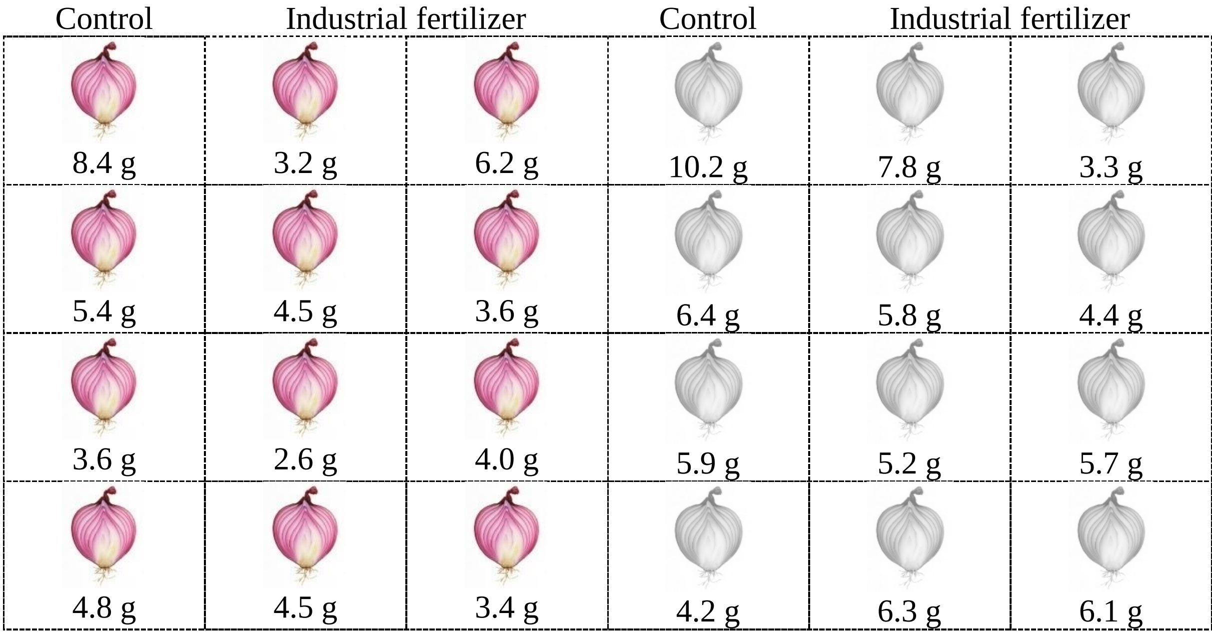

Figure 9. Onion and garlic planting scheme for control and industrial fertilizer (Maxicrop) (The original weight of each plant when they were transplanted is shown below the plant).

The growing rate of the plants were estimated together with the weight and height of the leaves.

3.3 Physicochemical analysis

Physicochemical analysis such as protein content, moisture content, lipid content, ash content, pH value, water activity, total bacteria count, *E. coli*, viscosity, and nutrient composition of fertilizer were measured on the different liquid fertilizers.

Chemical analysis

3.3.1 Protein

Protein contents of the fertilizers were determined according to Kjeldahl method (ISO 5983-2:2005).

3.3.2 Moisture

Moisture content was determined according to ISO 6496:1999.

3.3.3 Lipid

Crude lipid was determined gravimetrically following ethyl-ether extraction from a dried sample according to Ba 3-38 (AOCS, 1997) in a Soxhlet extractor.

3.3.4 Ash

The amount of ash was determined according to AOAC 938.08-1990.

3.3.5 Minerals and heavy metals

Minerals and heavy metals were determined using ICP-MS (Agilent 7500ce, Waldbronn, Germany).

<u>Physical analysis</u>

3.3.6 pH

The pH of the sample was measured with combined electrode SE 104- Mettler Toledo, Knick Berlin Germany.

3.3.7 Water activity (a_w)

An Aqua Lab Water Activity Meter (Decagon Devices, Inc.) was used to measure water activity (a_w) of the fertilizers.

3.3.8 Viscosity

NDJ-8S viscometer was used to measure viscosity of the fertilizers.

3.3.9 Microbiological analysis

The microbiological analysis was done on the fertilizers at day 0, day 15 and day 30 after preparation. The total bacteria count was measured in the fertilizers according to NMKL 86, 5th Ed. 2013. *E. coli* was measured according to NMKL 96, 4th Ed. 2009, mod. Freeze drying for preservation was also options for the final product of the fertilizer and was tested in this project.

3.4 Statistical analysis

All measurements were performed in triplicate per sample. The collected data was analysed by using one-way ANOVA with Least Significant Differences (LSD) as post-hoc test.

4 RESULTS

4.1 Chemical analysis of liquid fish fertilizers

The chemical analysis data of liquid fish fertilizers were presented as proximate content of protein, moisture, lipid and ash contents.

4.1.1 Protein content

Protein content of liquid fish fertilizers were observed in the three methods. For the NaOH treatment the protein content was 8.18% in average. For the natural enzyme treatment, the protein content was 12.69%, and for the Alcalase treatment the protein content was significantly higher compared to other methods ($p<0.05$) or 13.16% (Table 1).

4.1.2 Moisture content

Results showed that all moisture contents were significantly different for the three methods. The highest moisture content was in the fertilizer with the natural enzyme treatment with an average of 81.2%, followed by the alcalase treatment with an average of 79.8%, then NaOH treatment with an average of 76.3%.

4.1.3 Lipid content

The lipid content of the different treatment of the fertilizers was between 0.20% - 0.30%. The lipid content was significantly different for all treatments ($p<0.05$) with the highest content for the natural enzyme treatment (0.3%) (Table 1).

4.1.4 Ash content

The ash content also showed significantly differences ($p<0.05$) between the different treatments with the highest amount for the NaOH treatment with an average of 3.34%.

Table 1. Proximate analysis data of different liquid fertilizers.

Liquid fertilizers	Proximate analysis (%)			
	Protein content	Moisture content	Lipid content	Ash content
NaOH treatment	$8.18{\pm}0.13^{b}$	76.30 ± 0.23^{a}	$0.20{\pm}0.01^{a}$	$3.34{\pm}0.08^{c}$
Natural enzyme treatment	$12.69{\pm}0.04^{c}$	81.20 ± 0.21^{c}	$0.30{\pm}0.02^{b}$	$2.29{\pm}0.01^{b}$
Alcalase treatment	$13.16{\pm}0.42^{d}$	79.80 ± 0.17^{b}	$0.20{\pm}0.01^{a}$	$1.91{\pm}0.01^{a}$
Industrial fertilizer	$4.50{\pm}0.01^{a}$	85.70 ± 0.10^{d}	$0.30{\pm}0.01^{b}$	$2.22{\pm}0.06^{b}$

Values were mean±stdev. Superscripts a,b,c each column showed the significant difference, ($p<0.05$)

4.1.5 Minerals

The results of the amount of minerals showed the highest content of nitrogen in the fertilizer treated with Alcalase (2.11%), compared with the NaOH treated fertilizer (1.31%), the natural enzyme fertilizer (2.03%), and industrial fertilizer (0.72%). Other minerals as phosphorus and potassium were highest in the industrial fertilizer (Table 2).

Table 2. Minerals content of different liquid fertilizers.

Minerals (%)	Liquid fertilizers			
	NaOH treatment	Natural enzyme treatment	Alcalase treatment	Industrial fertilizer
Nitrogen (N)	$1.31{\pm}0.01^{b}$	$2.03{\pm}0.00^{c}$	$2.11{\pm}0.05^{d}$	$0.72{\pm}0.00^{a}$
Phosphorus (P)	$0.09{\pm}0.00^{a}$	$0.21{\pm}0.00^{b}$	$0.22{\pm}0.00^{c}$	$2.00{\pm}0.00^{d}$
Potassium (K)	$0.11{\pm}0.01^{a}$	$0.23{\pm}0.00^{b}$	$0.25{\pm}0.00^{c}$	$3.00{\pm}0.00^{d}$
Natrium (Na)	$0.99{\pm}0.03^{b}$	$0.17{\pm}0.00^{a}$	$0.18{\pm}0.00^{a}$	$18.9{\pm}0.00^{c}$
Calcium (Ca)	$0.13{\pm}0.00^{a}$	$0.52{\pm}0.01^{d}$	$0.30{\pm}0.01^{b}$	$0.44{\pm}0.00^{c}$
Magnesium (Mg)	$0.02{\pm}0.00^{a}$	$0.04{\pm}0.00^{c}$	$0.04{\pm}0.00^{b}$	$0.58{\pm}0.00^{d}$

Values were mean±stdev. Superscripts a,b,c,d each row showed the significant difference, ($p<0.05$)

4.2 Physical analysis of liquid fish fertilizers

4.2.1 pH value

The pH of the fertilizers ranged between 3.60-3.62 during storing time (Figure 10).

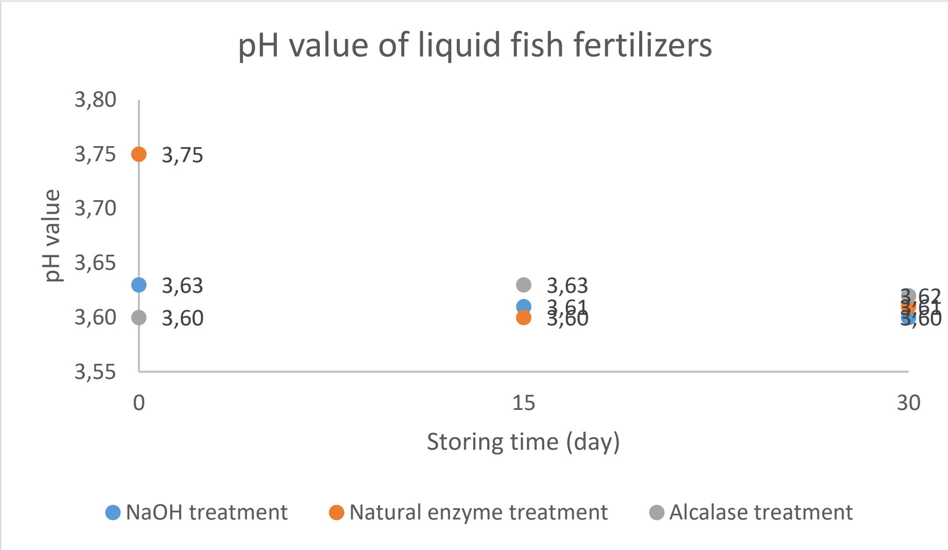

Figure 10. pH value of liquid fish fertilizers.

4.2.2 Water activity (a_w)

The water activity of fish silage for each treatment was 0.96 (Table 3).

Table 3. Water activity (aw) of liquid fish fertilizers.

Liquid fish fertilizers	Water activity (a_w)
NaOH treatment	0.96
Natural enzyme treatment	0.96
Alcalase treatment	0.96

4.2.3 Total bacteria count and coliform bacteria

Total bacteria count and *E. coli* was negligible in the products (Table 4).

Table 4. Total bacteria count and Coliform bacteria of liquid fish fertilizers during storing time.

Liquid fish fertilizers	Total bacteria count (Log number/g)			*E. coli* (MPN)		
	Day 0	Day 15	Day 30	Day 0	Day 15	Day 30
NaOH treatment	< 10	< 10	< 10	< 3	< 3	< 10
Natural enzyme treatment	< 10	< 10	< 10	< 3	< 3	< 10
Alcalase treatment	< 10	< 10	< 10	< 3	< 3	< 10

4.2.4 Viscosity of liquid fish fertilizer

Viscosity of liquid fish fertilizers were observed in the three methods. The results showed that the viscosity of the three treatments were significantly different ($p<0.05$), with the highest viscosity for the natural enzyme treatment (Table 5).

Table 5. Viscosity of different liquid fish fertilizers and industrial fertilizer.

Liquid fish fertilizers	Viscosity (mPa.s)
NaOH treatment	1.79 ± 0.02^{a}
Natural enzyme treatment	6.30 ± 0.33^{c}
Alcalase treatment	2.19 ± 0.03^{b}
Industrial fertilizer	1.58 ± 0.04^{a}

Values are mean±stdev. Superscripts a,b,c in each column shows the significant difference ($p<0.05$)

4.3 Solid fish silages as potential nutrient

Protein content of freeze dried fish silages showed the highest content in the natural enzyme treated silage (67.5%) (Table 6).

Table 6. Protein of solid/dried fish silage.

Solid/dried fish silages	Protein content (%)
NaOH treatment	34.4
Natural enzyme treatment	67.5
Alcalase treatment	65.1

4.4 Application of different fertilizers for onion and garlic cultivation

The growth of the plants of onion and garlic cultivation using different fertilizers were presented as total growing ratio, number of leaves and plant height (Table 7 and 8).

Onion treated with alcalase treatment fertilizer produced highest total growing ratio; 92.60±18.57% while the lowest growing ratio was the control which was 38.40±18.76% after 28 days of cultivation (Table 7). Number of leaves of onion treated with different fertilizers were not significantly different ($p>0.05$) in comparison with the control set. On the other hand, natural enzyme treatment, alcalase treatment, and industrial fertilizers showed significantly ($p<0.05$) higher plant height compared to plants receiving NaOH treatment fertilizer and control treatment.

Table 7. Total growing ratio, number of leaves and plant height of onion for 28 days cultivation in different fertilizers.

Liquid fish fertilizers	Total growing ratio (%)	Number of leaves	Plant height (cm)
Control	38.40 ± 18.76^{a}	16.25±2.63	31.75 ± 1.71^{a}
NaOH treatment	73.69 ± 24.66^{b}	18.25±6.55	35.38 ± 1.11^{b}
Natural enzyme treatment	86.66 ± 18.82^{b}	15.00±3.37	40.00 ± 1.83^{c}
Alcalase treatment	92.60 ± 18.57^{b}	18.25±0.96	38.50 ± 1.91^{c}
Industrial fertilizer	75.90 ± 6.32^{b}	11.75±1.71	40.00 ± 2.68^{c}

Values were mean±stdev. Superscripts a,b,c in each column shows the significant difference ($p<0.05$)

Garlic treated with alcalase treatment fertilizer also produced highest total growing ratio; 105.55±14.83% while the lowest growing ratio was the control which was 56.07±10.94% after 28 days of cultivation (Table 8). Growing ratio, number of leaves and plant height of garlic treated with NaOH treatment, alcalase treatment and industrial fertilizers were significantly different ($p<0.05$) to natural enzyme treatment fertilizer and control. On the other hand, garlic receiving alcalase treatment fertilizer produced highest plant height, which is 50.13±6.51 cm while the lowest plant height was obtained in natural enzyme treatment fertilizer (40.63±2.75 cm.)

Table 8. Total growing ratio, number of leaves and plant height of garlic for 28 days cultivation in different fertilizers.

Liquid fish fertilizers	Total growing ratio (%)	Number of leaves	Plant height (cm)
Control	56.07 ± 10.94^{a}	5.75 ± 0.50^{a}	45.50 ± 2.86^{a}
NaOH treatment	85.29 ± 8.49^{b}	6.75 ± 0.50^{b}	49.50 ± 2.94^{b}
Natural enzyme treatment	56.43 ± 23.25^{a}	5.75 ± 0.96^{a}	40.63 ± 2.75^{a}
Alcalase treatment	105.55 ± 14.83^{b}	7.00 ± 0.82^{b}	50.13 ± 6.51^{b}
Industrial fertilizer	99.84 ± 14.74^{b}	7.00 ± 0.00^{b}	49.25 ± 1.19^{b}

Values were mean±stdev. Superscripts a,b,c in each column shows the significant difference ($p<0.05$)

5 DISCUSSION

5.1 Preparation of liquid fish fertilizers

The most important factor in liquid fish fertilizer production was to start with fresh raw material. Viscera where some spoilage or bacterial breakdown has occurred was not suitable for silage-making, because the resulting product would be poor in quality, with a high bacterial content and an unpleasant odor. Haaland and Njaa (1990) found that silage quality depends on the quality of the raw material, on proper ensiling and on reactions in the silage during storage. The type of raw material may also be important. The necessity for high quality viscera products means that the fish industry must look upon its waste by-raw materials not as trash but as valuable resources and treat them accordingly.

Before mincing, cod stomachs should be cleared of the content in the stomach. This was done to avoid bias when chemical analysis was measured. Whereas, if applied in Indonesia with milkfish as raw fish, the stomach does not need to be cleaned, because milkfish stomach does not have the same content as cod. Fish visceral waste was an excellent source of proteolytic enzymes and marine peptones for supporting bacteria production (Vázquez *et al.* 2004). Artificially produced enzymes such as alcalase, which work much faster than the natural enzymes, can be purchased and added to speed-up the autolysis process. This could be useful in certain continuous industrial plants. As the temperature increases, up to 55ºC, the process speeds up. A heat source can be applied to warm the silage and accelerate this process if it was developing too slowly naturally.

The three methods in this study used to make fish silage was based on grinding or mincing the fresh raw material and adding formic acid for preservation.

5.2 Physicochemical of liquid fertilizers

The nutrient contents of liquid fish fertilizers were similar to raw viscera. There was a slight decrease in protein, lipid, ash contents in liquid fish fertilizers than viscera. This might be due to the breakdown of nutrient components during hydrolysis. This can also be attributed to the low pH, which enhanced the action of fish digestive and muscle enzymes on fish nutrients. Whereas, the moisture content of liquid fish fertilizers was slightly higher than viscera. This might be due to liquefaction of viscera during silage preparation. Tanuja *et al.* (2014) stated that low pH helps in the activation of these enzymes favoring the liquefaction of the waste.

<u>Protein content</u>

The higher amount of protein content in liquid fish fertilizer using alcalase treatment (13.16±0.42%) compared to natural enzyme treatment (12.69±0.04%) and NaOH treatment (8.18±0.13%) indicated higher amount of nitrogen content. Shahidi *et al.* (1995) reported that fish protein hydrolysate produced by alcalase had better functional properties, a high protein content with an excellent nitrogen yield, an amino acid composition comparable to that of muscle, and a higher nutritional value than those produced by other enzymes such as Neutrase.

Protein content in liquid fish fertilizer tended to decrease with 1M NaOH treatment. Flipot *et al.* (1976) stated that decrease of protein content might be due to loss of free ammonia. Similar results were obtained by Nishino *et al.* (1993).

<u>Moisture content</u>

The higher percentage of moisture content was obtained in liquid fish fertilizer using natural enzyme treatment (81.20±0.21 %) compared to alcalase treatment (79.80 ± 0.17%) and NaOH treatment (76.30 ± 0.23%). This could be because the moisture content in liquid fish fertilizer was influenced by the enzymes in the viscera or by the additional enzyme given. In the experiment with the NaOH treatment, an enzyme present in the viscera was damaged in part because of the mixing of the viscera with 1M NaOH. Thus, the liquefaction process was not running smoothly. While on trial with the natural enzyme treatment and alcalase treatment, the enzymes in the viscera were still working optimally, especially in the natural enzyme treatment. This was slightly different from the alcalase treatment using additional enzyme that produces a more uniform product. The bacteriological activity was thereby stopped, but the fish enzymes will break down the protein chains so that the fish takes on a liquid consistency (Arason *et al.* 1990).

In addition, the high moisture content in liquid fish fertilizer using the natural enzyme treatment might be due to liquefaction process being longer to achieve a stable pH at 3.6, compared to the NaOH treatment and alcalase treatment. Also, higher moisture content of natural enzyme treatment and alcalase treatment might be due to autolysis. The silage gradually becomes more liquid or reaches a higher moisture content. Jangaard (1987) stated the fish silage becomes liquid because the tissue structures were degraded by a process called autolysis by enzymes that are naturally present in the flesh.

In the NaOH treatment, moisture content was lowest compared to other methods. It might be because some components of enzymes were inactive due to alkali treatment. Normally, there were sufficient enzymes in all types of fish silage, either from viscera, guts or skin. Cod viscera also consist of many different types of enzymes. Even in fish meat there were normally sufficient enzymes to start the liquefaction process. Klomklao (2008) reviewed that fish digestive proteolytic enzymes most commonly found include pepsin and trypsin. These enzymes from fish viscera may have the advantages for the applications in the food industry since their temperature and other characteristics differ from homologous proteinases from warm-blooded animals.

Lipid content

Related to data, liquid fish fertilizers have low lipid content (0.2-0.3%). This indicates that the product was not very susceptible to the presence of lipid oxidation. Due to the lipid oxidation that can compromise the nutritional value of the ration, the removal of the lipid fraction during the elaboration of the silage was recommended to obtain a uniform and more stable product (Ferraz de Arruda *et al.* 2007).

In this study, liquid fish fertilizers were centrifuged at 8000 rpm for 10 minutes to get final product. Ferraz de Arruda *et al.* (2007) reviewed that centrifugation was the best extraction method for preserving the physical-chemical characteristics of the oil resulting in a larger yield.

The reduction of the lipids content in the preparation of silage will allow for the production of a more uniform and adequate fish silage for the formulation of rations.

Ash content

This study used same raw material. The ash content from natural enzyme treatment and alcalase treatment were approximately 2%. But, NaOH treatment has a higher ash content, which might be due to the addition of 1M NaOH on silage preparation, therefore, affecting sodium content in the final product. Ash content was higher for the NaOH-treatment compared with the untreated straw in evaluation of sodium hydroxide treatment on chemical composition and digestibility of straw (Arisoy 1998).

The ash content would be very suitable to fulfill the mineral requirements of plants. The results showed especially liquid fish fertilizer using NaOH treatment had higher ash content (Table 1) and sodium content (Table 2). Sambusiti *et al.* (2013b) stated when sodium hydroxide pretreatment was performed, sodium concentration in the ensiled sorghum forage reactor increases.

Macronutrient of liquid fish fertilizers

Results showed that the alcalase treatment has highest macronutrient such as nitrogen (2.106±0.05), phosphorus (0.218±0.00), and potassium (0.246±0.00). The quantity of nitrogen obtained would be suitable for the growth of plants. According to the experiment done by Karim *et al.* (2015), liquid fish silage from threadfin seabream by-products has 1.84±0.38% of nitrogen, 0.50±0.09% of phosphorus and 0.41±0.05% of potassium and can be very effective for growing plants as it would produce same yields and quality when treated with commercial fertilizer.

However, the NaOH treatment contained highest ash content. It was significantly different from other methods especially sodium content. It might be due to the effect of adding 1M sodium hydroxide. Santos da Silva *et al.* (2014) explained that the ash quantity increased significantly ($p < 0.05$) with the addition of urea and sodium hydroxide. The result can be explained by the addition of mineral Na, through sodium hydroxide.

The liquid fish fertilizers had a higher protein content compared to the industrial fertilizers. It can be applied as a fertilizer which contains enough nutrients for plants. The results showed macronutrient element of alcalase treatment was significantly different from other methods. Liquid fish fertilizers from viscera can be a good source of minerals. Tatterson and Windsor (2006) stated the composition of fish silage were similar to the material from which it was prepared. However, silage made from viscera was lower in mineral content than silage made from whole fish or heads and shrimp head silage (Srour 2009). Since the silage was acidic, and most of the material liquefied, a large portion of the minerals should be readily available to plants.

pH value

The pH value of final product of fish silage ranged between 3.6-3.8 that keep the bacteria from growing. For the fish silage to be stable, it was important that viscera came into contact with the acid to ensure the correct pH level throughout the mixture. Formic acid was used as acid preservative. Nuria *et al.* (2004) described that formic acid as organic acids have been used for decades in feed preservation, for protecting feed from microbial and fungal destruction or to increase the preservation effect of fermented feed such as silage. This was obtained by adding the correct amount of formic acid to the viscera and by grinding and mixing this all together until a homogeneous mixture was achieved. The duration of increased pH depends on how finely the particles were grinded, the proportion of viscera and the temperature of the mixture.

The acidity needs to be maintained between pH 3.6 and 3.8. At a pH lower than 3.0 the enzymes were unable to work properly. The silage at pH 4.5 and above was always susceptible to spoilage caused by high bacterial load (Tanuja *et al.* 2014). This leads to a rapid increase in pH, which inactivates the enzymes and leads to putrefaction of the entire load. In this event, other microorganisms take over all functions.

Water activity (a_w)

Water activity was the amount of water in a food/material that was available for the growth of microorganisms, including pathogens. It determines the storage life of fish. The water activity of fish silage for each treatment was stable during storing time at between 0.96 and 0.97. Generally, there was microbial growth or toxic formation in food/material when the water activity was highest than 0.85. Results of this study indicate that even though water activity of fish silage was higher than 0.85, the tolerance of individual micro-organisms to water activity was in general lower if other factors in the foodstuff such as temperature, pH value, redox potential, and oxygen and carbon dioxide concentration deviated from the optimum, or if the product was treated with preservatives (Rodel 2001).

For the liquid fish fertilizers with a high level of water activity, the shelf life was limited mainly by microbiological activity. Rodel (2001) stated the product with a_w levels below 0.70 may well be stable microbiologically and consequently have a longer shelf life, but now the slower, enzyme-related breakdown processes come to the fore. It was mainly chemical reactions that determine the quality and stability of product. Lorenzo and Kiely (2008) also founded formic acid applied at moderate rates of application such as low water-soluble carbohydrate (WSC) concentration, high water activity and high buffering capacity was particularly effective at inhibiting the activity of undesirable bacteria.

Total bacteria count and coliform bacteria

As shown in Table 4, either total bacteria count or *E. coli* was not found in liquid fish fertilizers for all methods. It might be due to the usage of formic acid to maintain pH within the range of 3.6-3.8, at the same time preventing bacterial spoilage. Randby (2000) stated formic acid was used as the preservative. When applied to wet herbage it dissociates and at the same molar rate of acid has been shown to be as effective as formic acid at assisting preservation (Randby 2000).

Viscera was especially vulnerable to spoilage because of the large amounts of enzymes and bacteria in the intestinal system. McDonald *et al.* (1991) reviewed that one of ensiling objective was to discourage the activities of undesirable microorganisms such as Clostridia and Enterobacter. Clostridia was present on crops and in the soil in the form of spores. Enterobacter also have the ability to degrade amino acids. The critical pH at which growth of Clostridia and Enterobacter were inhibited depends on the moisture content and the temperature.

During the storage period of more than one month, there were no total bacteria count and *E. coli* in liquid fish fertilizers for all methods. It might be due to the pH level of all liquid fish fertilizers which were in the range of 3.6 to 3.8. A sufficient dose of formic acid will increase the lactic acid fermentation, reduce the fermentation to acetic, propionic and butyric acids, reduce proteolysis and reduce silage pH compared with untreated silage. Treatment with high doses of formic acid, will restrict all microbial activity, including lactic acid fermentation, and produce silage with a high concentration of water-soluble carbohydrates (WSC) and low concentrations of fermentation acids (Randby 2000).

Viscosity

Liquid fish fertilizer using alcalase treatment shows a lower viscosity than natural enzyme treatment. This might be because the ensiling temperature for alcalase treatment was 55°C and the boiling temperature was 90°C during enzyme deactivation. However, for the NaOH treatment, the sample was added to sodium hydroxide solution with a ratio equal to the weight of the sample, thus enabling more dilute solution compared with the alcalase treatment. Liquid fish fertilizer using NaOH treatment was not significantly different compared with the industrial fertilizer used in this study. Silage was processed by addition of formic acid to low pH and stored in room temperature. The final products were thinner or thicker liquid fish fertilizers containing more nutrients. Jangaard (1987) stated the viscosity of fish silage was more dependent on the temperature that the silage was produced and stored. The higher the temperature, the more liquid the silage. A thin silage can therefore easily contain more nutrients than a more viscous product. It was often more important for the user that the silage was well stirred and not separated into layers.

Digestive enzymes were always present in cod viscera and other fishes. All methods in this study used digestive enzymes to hydrolyze protein to produce silage. When temperature rises, ensiling process starts, autolysis starts, and the silage gradually becomes more liquid. Tomczak-Wandzel and Medrzycka (2013) stated that protein solubilisation proceeded during the first five days in all hydrolyzates (independent of the temperature and composition of silage). As expected, higher temperature resulted in the higher degree of hydrolysis. The degree of hydrolysis in the processes carried out at 32 °C was higher than at 15 °C.

Further study is needed to find a good way to make liquid fish fertilizer to minimize particles or components to achieve a pure appearance. The end product of liquid fish fertilizers is shown in Appendix 1.

Solid fish silage

Solid fish silage also has the potential to be developed into a dry fertilizer according to its protein content. However, the method of freeze drying requires a relatively large cost to the household scale. Thus, it can be used for large-scale industry with high production. Enke *et al.* (2009) stated powder fish silage can be vitally used as a feed supplement in aquaculture to convert nutrients into flesh. Inclusion of fish silage in fish diets increased body weight gain, total body length and specific growth rate without any adverse effects on survival and water quality.

Solid fish silage could be a suitable substitute for fishmeal in producing high quality and nutrient-rich powder fish silage. It was found possible to partially replace expensive fish meal in fish and animal feed preparation. Solid fish silage should be tested for the growth performance of fish and other animals in farm culture condition. Research needs to be carried out on high potential use in aquaculture. The end product of solid/dried fish fertilizers is shown in Appendix 2.

5.3 Impact of the different fertilizers for onion and garlic cultivation

In this study, onion and garlic were cultivated for 28 days. Onion and garlic treated with alcalase treatment fertilizer produced highest total growing ratio than other fertilizers because the fertilizer provide suitable nutrient requirement (Table 2). Irshad and Javed (2006) found high yield was obtained for mung bean and okra cultivation when using fish as fertilizer compared to Nitrogen-Phosphorous-Potassium (NPK) and urea fertilizer.

Onion and garlic were treated with alcalase treatment showed significantly ($p<0.05$) higher plant height compared to plant receiving other fertilizers because the fertilizer provide suitable nitrogen. Karim *et al.* (2015) reported that application of nitrogen increases leaf dimensions (plant height and number of leaves) that may lead to yield rise. Meanwhile, Li-zhilin *et al.* (1997) stated that plant height was increased significantly due to nitrogen application.

Alcalase treatment fertilizer was very effective when used as fertilizer for growing onion and garlic, as it would produce higher total growing ratio and yields then with industrial fertilizer (Maxicrop).

6 CONCLUSIONS

- Liquid fish fertilizer obtained by three methods represents an alternative process for the use of viscera; 1 M NaOH treatment as first method, natural enzyme treatment as second method and alcalase treatment as third method.
- Alcalase treatment fertilizer contain 13.16% of protein, 79.80% of moisture, 0.20% of lipid and 1.91% of ash.
- Alcalase treatment fertilizer using alcalase treatment has high macronutrients with 2.11% of nitrogen, 0.22% of phosphorus, and 0.25% of potassium. Comparing fish fertilizer with industrial fertilizer (Maxicrop), the latter contained less nitrogen (0.72%). Fish viscera can be applied as a fertilizer which contains enough nutrients for plants.
- Onion and garlic treated with alcalase treatment fertilizer showed highest total growing ratio (92.60% for onion and 105.55% for garlic) and plant height (38.50 cm for onion and 50.13 cm for garlic).
- Alcalase treatment fertilizer was very effective to be used as fertilizer for growing onion and garlic as it produced higher total growing ratio and yields, compared with industrial fertilizer (Maxicrop).
- Solid fish silage can be developed into a dry fertilizer and could also be a suitable substitute for fishmeal in producing high quality and nutrient-rich powder fish silage.

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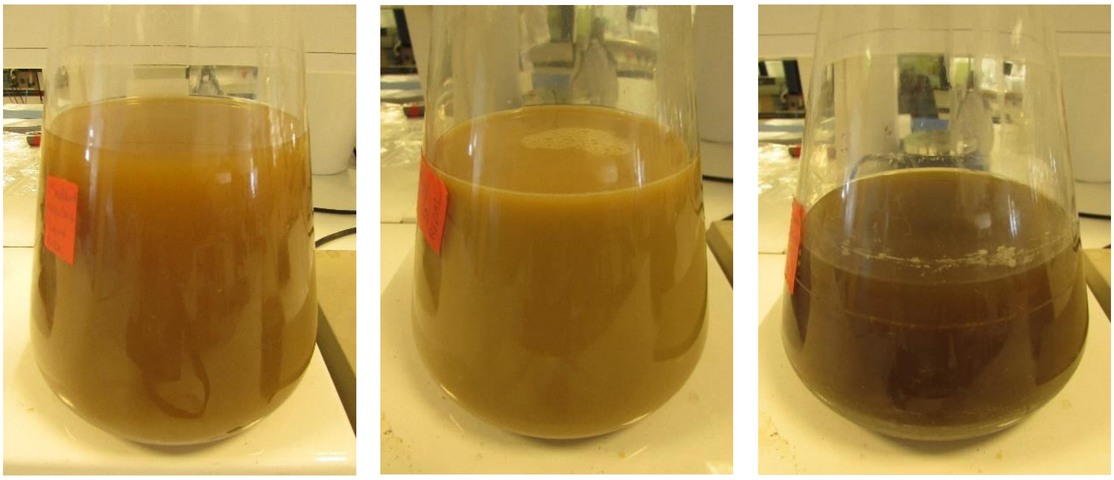
APPENDIX



NaOH treatment | Natural enzyme treatment | Alcalase treatment

Figure 11. Liquid fish fertilizers using NaOH treatment, natural enzyme treatment and alcalase treatment.

NaOH treatment | Natural enzyme treatment | Alcalase treatment

Figure 12. Final product of solid/dried fish silage using NaOH treatment, natural enzyme treatment and alcalase treatment.