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EFFECT OF DIFFERENT CONCENTRATION SALT AND TRYPSIN ON THE PHYSICOCHEMICAL PROPERTIES OF FISH SAUCE MADE FROM SEA CATFISH (*Arius* sp.) VISCERA

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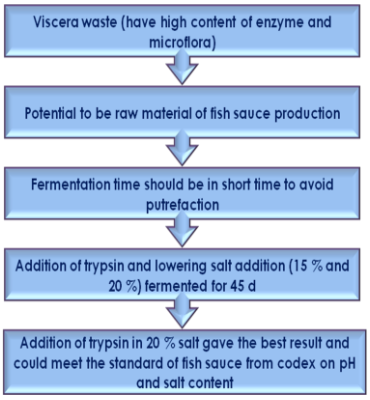
Graphical abstract



Abstract

Fish sauce is a fermented product for seasoning that is popular in Asian country. One of the problems to produce this product is a long fermentation time up to 1 yr to 2 yr. Addition of trypsin as proteolytic enzyme and decreasing salt concentration is done to accelerate the fermentation time. This research examined the effect of trypsin addition and different salt concentrations (15 %) and the physicochemical properties of fish sauce made from sea cat fish (*Arius* sp.) after 45 d of fermentation. The result showed that the higher salt and enzyme concentrations gave higher yield (48.54 %), color intensity (33.6 %) and salt content (24.78 %) but also gave lower TVBN (26.1 mgN per 100 g), TMA (11.14 mgN per 100 g), Ammonia (12.87 mgN per 100 g) and pH level (5.4). This result could meet the standard of fish sauce from codex on pH and salt content.

Keywords: Fish sauce, physicochemical properties, salt, sea catfish viscera, trypsin

1.0 INTRODUCTION

Semarang, a city in Central Java, is famous on smoked fish production with sea catfish (*Arius* sp.) as commonly-used raw material, thus resulted a huge viscera waste each day. This waste is rich of digestive enzyme but also rich in micro flora.

Fish viscera are known to be a rich source of digestive enzymes. Fermentation and curing fish (fish sauce production), selective tissue degradation, production of hydrolyzed products, extraction of pigments are examples of application of enzyme from fish and aquatic invertebrates [1]. Various proteinases can be found in fish viscera and can be a potential source of proteinases enzyme to hydrolyze the protein, for example, in fish sauce production [2-4].

Fish sauce is a clear brown liquid product obtained from heavily salted fish fermented in closed tank on tropical temperature. The raw material that is usually used in the fish sauce process is anchovy. The important thing is that the raw material can give sufficient enzyme to solubilize tissue and protein hydrolysis [5].

Traditionally, fish sauce is made by mixing one part of salt with two or three part of fish (can be different in different regions) and fermented at room temperature for 6 mo to 12 mo or longer [6]. Hydrolysis of protein is conducted by endogenous proteinases in fish and also proteinases produced by bacteria especially halophile bacteria [3]. Fish sauce production normally takes 6 mo to 12 mo. The long fermentation time enables flavor and color development of fish sauce and also for the solubilization of raw material tissue. The fish

sauce volatile compounds which contributes to flavor are produced by non-enzymatic reactions of various components and enzymatic reactions from endogenous enzymes of fish origin and microorganisms surviving during fermentation [7].

The use of viscera as raw material for fish sauce or as a supplementary source of enzymes in the process of making fish sauce also has the potency to cause putrefaction because of the high content of microorganism in the viscera. Chemical, enzymatic or microbial activities can cause the spoilage of food product. 25 % of gross primary agricultural and fishery product every year loss due to the chemical deterioration and microbial spoilage [8]. Microbial activity alone can cause 30 % lost of landed fish [9].

Addition of fish viscera would accelerate the autolysis of fish protein during fermentation of fish sauce [3]. Effort that have been made to speed up or to accelerated the fermentation of fish sauce are reduction of salt concentration (< 20 %), addition of fish viscera 5 % to 10 % enzyme-rich trypsin and chymotrypsin from cod intestines or proteinases (trypsin and chymotrypsin, spleen of skipjack tuna (*Katsuwonus pelamis*) [3, 10–13].

The enzyme that is essentially responsible for tissue solubility in fish sauce production is trypsin enzymes [14–16]. Trypsin enzymes have maximal activity in alkaline conditions. Fermentation process of fish sauce can be accelerated by a moderate initial alkalification and did not affected pH of the final product, improves protein yield and stabilizes the pyloric caeca proteases [12].

Decreased level of salt in the manufacture of fish sauce potentially leads to lower product quality as salt has ability to protect product against microorganism. Lowering salt concentration also have other benefit because high salt content in fish sauces had limited nutrient value because fish sauce could not be consumed in large quantities. Therefore it is will be benefit for the society if low salt fish sauce is available in the market [17].

To match the speed of the activity of microorganisms and raise the speed of hydrolysis on raw material of fish sauce, addition of another source enzyme became necessary. Trypsin was choose because it has been understood well that enzyme that have major contribution on the fish sauce process were trypsin and chymotrypsin [13].

The advantages of viscera waste for production of fish sauce are both economical and environmental (18). However, limited information is available regarding the use of sea cat fish viscera as a raw material to produce fish sauce. The purpose of this study was to monitor the effect of enzyme addition and salt concentration on the hydrolysis of fish sauce as well as on the chemical and physical changes of fish sauce after fermentation for 45 d.

2.0 MATERIALS AND METHOD

2.1 Chemicals

Tricloroacetic acid, formaldehyde, K_2CO_3, boric acid, photasium chromat, $AgNO_3$ reagent were obtained from Merck (Darmstadt, Germany).

2.2 Raw Material

The viscera from sea cat fish (*Arius* sp.), obtained from the waste of smoked fish industry in Semarang, Indonesia were packed in plastic bag, then kept in cool box equipped with ice and transported to Fisheries Department, Diponegoro University, Semarang within one hour. The viscera were washed and chopped approximately (1 to 2) cm length. The salt that used in this experiment is solar salt.

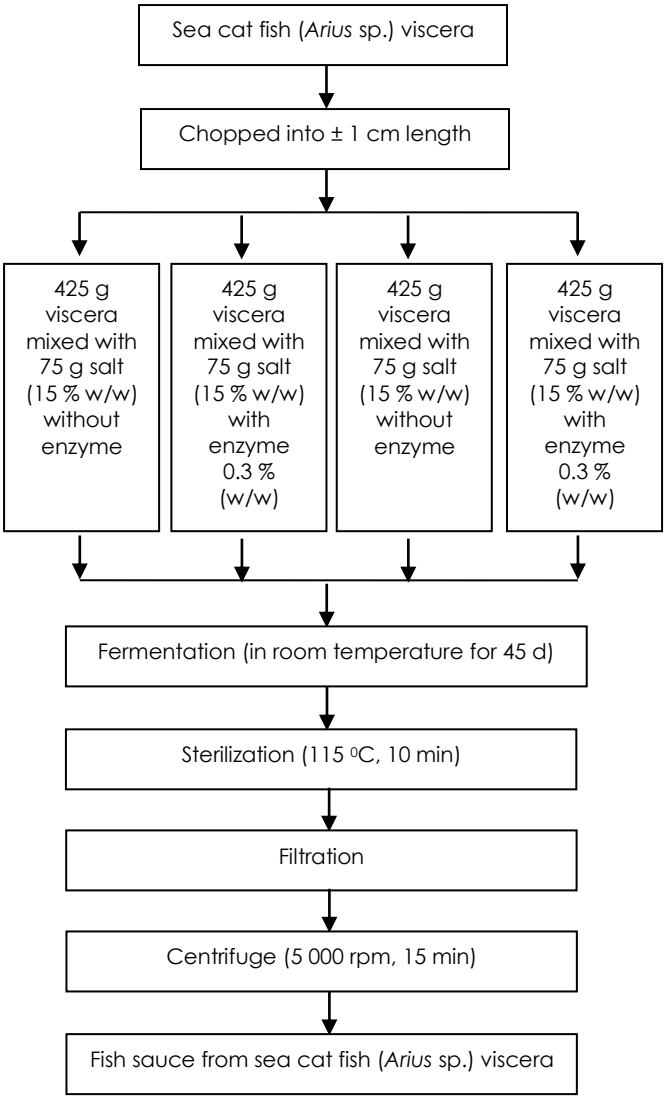


Figure 1 Flow chart of fermented fish sauce from sea cat fish (*Arius* sp.) viscera

2.3 Fish Sauce Fermentation

The viscera was cut into 1 cm length and mixed with different levels of salt (15 % and 20 % w/w). 425 g of chopped viscera was mixed with 75 g of solar salt (15 % w/w) and 400 g of chopped viscera was mixed with 100 g of solar salt (20 % w/w), all final weight in one batch was 500 g. For each salt level, three batches were used as the control and the three others batches were mixed with trypsin enzyme (0.3 %). The mixtures were transferred to glass bottles and covered with polyethylene plastic. The bottles were placed in room temperature. Liquid formed after 1.5 mo (45 d) fermentation was collected. Figure 1 was a flow chart of fermented sea cat fish (*Arius* sp.) viscera.

2.4 Collection of Liquid

After incubation for the designated time, the fish sauce mush was sterilized at 115 °C in autoclaves for 10 min. The fish sauce mush was cooled and filtered and then centrifuged for 15 min at 5 000 rpm (1 rpm = 1/60 Hz). The lipid was removed with a spoon. The liquid obtained was used for analysis.

2.5 Chemical Analysis

2.5.1 Determination of Color

Color characteristics of the samples were determined by measuring the L* a* and b* values using a Hunter Lab instrument, according to the CIE Lab scale. The system provides the values of three color components: L* (black to white component, luminosity), a* (red to green component) and b* (yellow to blue component). Samples (15 mL) were pipetted into a glass Petri dish (5 cm diameter). The sample was illuminated with D65 artificial daylight (10 standard angle) according to the procedure provided by the manufacturer of instrument.

2.5.2 Determination of TVB, TMA and Ammonia

TVB and TMA contents were determined using the Conway microdiffusion assay [18]. Sample (4 mL) was mixed with 16 mL of 4 % tricloroacetic acid (TCA). The mixtures were filtered using Whatman No. 41 filter paper and the filtrate was used for analysis. To determine the TMA content, formaldehyde was added to the filtrate to fix ammonia present in the sample. TVB and TMA were released after addition of saturated K_2CO_3 and diffused into the boric acid solution. The titration of solution was performed and the amount of TVB or TMA was calculated. The ammonia value obtained from the difference between TVB and TMA.

2.5.3 Determination of pH

pH was determined directly using a pH meter.

2.5.4 Determination of Salt Content

Sample (5 g) were diluted with 45 mL distilled water and filtered. Ten mL filtrate added with five drops photasium chromat and titrated with $AgNO_3$ 0.1 N [19].

2.6 Statistical Analysis

A completely randomised design (CRD) was used throughout this study and the experiments were done in triplicate. Data were subjected to analysis of variance (ANOVA) and mean comparison was carried out using HSD Test [20].

3.0 RESULTS AND DISCUSSION

3.1 Yield

Volume or yield of fish sauce obtained from different treatments with various levels of salt and enzyme had different value (Table 1). Yield of fish sauce with enzyme addition is higher on all the salt concentration. It is probably due to the hydrolysis degree of the samples. Addition of enzyme will increased the degree of hydrolysis, thus, increased the volume of the final product. Higher salt addition will result higher yield although there was no marked differences in yield result. It is probably due to the diffusion osmosis phenomena. Higher salt addition will result higher salt that enter the tissue of the viscera thus water from viscera will leave the tissue and the fish sauce yield will increased. These phenomena happen in the early stage of fish sauce fermentation, and in this experiment only use 45 d fermentation so the yield is higher with the higher salt addition.

Table 1 Physicochemical characteristic of fish sauce made from sea cat fish (*Arius* sp.) viscera with trypsin addition on different salt concentration after fermented for 45 d

No	Characteristic	15 % Salt concentration		20 % Salt concentration	
		Without enzyme	With 0.3 % enzyme	Without enzyme	With 0.3 % enzyme
1.	Yield (%)	40.55[b]	45.58[a]	43.74[b]	48.54[a]
2.	Color (Hue)	29.83[b]	30.75[b]	33.02[a]	33.56[a]
3.	TVBN (mgN per 100 g)	96.04[a]	50,02[b]	88.04[a]	26.01[b]
4.	TMA (mgN per 100 g)	14.47[a]	9.01[c]	11.14[b]	7.21[d]
5.	Amonia (mgN · mL^{-1})	81.58[a]	43.02[c]	74.83[b]	12.87[d]
6.	pH	6.82[a]	5.43[b]	7.17[a]	5.40[b]
7.	Salt content (%)	19.19[c]	17.94[d]	23.85[b]	24.78[a]

Values represent mean from triplicate determinations.
Different letters in the same row showed significant differences ($p < 0.05$)

3.2 Color

Fish sauce samples obtained from different treatments with various levels of salt and enzyme had different color characteristics (Table 1). As fermentation time increased the color of fish sauce samples developed gradually [13]. Samples with higher salt gave higher color value which was more yellow and lower color value was more red. The formation of low molecular weight compounds and the presence of melanoidins of high molecular weight likely affected the color formation of fish sauce [21]. Same result of higher color intensity was found in fish sauce with higher salt content [13].

The increase in redness might be resulted from Maillard reaction. Fish sauce is a liquid product made from hydrolysis of fish and most of the nitrogenous compounds are small peptides and free amino acids that will contribute to the development of brown color [6]. Even though reducing sugar content in fish is low, carbohydrate derivatives, such as glucose-6-phosphate and other substances present in the metabolic pathways, can also act as reactants to initiate the Maillard reaction [22]. From the results, the fish sauce sample with 15 % of salt showed the greater value. The addition of enzyme did not affect the color which probably due to inhibition activity of the salt on the enzyme activity.

3.3 pH

The pH of fish sauce with and without trypsin in the different salt concentration are presented in Table 1. The pH value of fish sauce without trypsin was higher compared to pH fish sauce with trypsin ($p < 0.05$). Different salt concentration gave the same pH value ($p < 0.05$) in samples with or without addition enzyme. Thermostable proteinases in salted anchovy processed with 16 % to 17 % of salt were still active and able to hydrolyze myofibrillar protein [23]. Addition of enzyme will lead to higher degree of hydrolysis and it will decrease the pH value. With the high degree of hydrolysis, it might inhibit the putrefaction microorganism that will produce volatile base substances. Lower volatile base production will gave lower impact to pH changes to the product. The accumulation of TVB, TMA and ammonia produced during fish muscle spoilage led to increase in pH [24]. TVB and TMA were produced during fermentation of fish sauce, thus it also influenced the changes of pH during fermentation process [25].

3.4 TVB, TMA and Ammonia

The value of TVB, TMA and ammonia of fish sauce with and without trypsin in the different salt concentration are presented in Table 1. TVB, TMA and ammonia content of fish sauce from sea cat fish (*Arius* sp.) viscera generally lower with higher of salt and enzyme addition ($p < 0.05$). Deterioration of fish is reflected by the increasing of the TVB, TMA and ammonia contents. Higher salt and enzyme addition will gave lower value of TVBN. The value of TVB of all samples were lower than 200 mg per 100 gram which is a maximum limit for levels of TVBN in fish processed with salt, according to the legislation of certain countries [26]. The growth of specific spoilage bacteria such as *Shewanella putrefaciens*, *Photobacterium phosphoreum*, and *Vibrioaceae* is generally associated with the formation of TVB and TMA [27]. The increase in pH was in accordance with the increase in TVB, TMA and ammonia (Table 1). TMAO is decomposed to TMA due to bacterial spoilage and enzymatic activity [24]. Protein breakdown products, peptides and amino acids during storage represent precursors for amine formation used by spoilage microorganisms. Very high concentration of salt also inhibited the growth of microorganisms that could decarboxylate histidine to form histamine [28]. High salt concentration might also inhibit the growth of putrefaction bacteria include bacteria that produce TMA and ammonia.

3.5 Salt Content

Fish sauce samples obtained from different treatments with various levels of salt and enzyme had different value of salt content (Table 1). Addition of 20 % of salt with trypsin showed the highest salt content while the addition of 15 % of salt with trypsin enzyme showed the lowest salt content ($p < 0.05$). Fish sauce in all treatment showed increased the value of salt content compare to the addition of salt before fermentation

process. The 15 % addition of salt in the raw material gave value 19.19 % and 17.94 % for salt content in the final product while 20 % addition of salt in the raw material gave value 23.85 % and 24.78 % for salt content in the final product. This phenomenon probably happened because of the relatively short fermentation time (45 d). This short fermentation time caused the fish sauce was not much so the salt that dissolved in a liquid fish sauces becomes thick and produces a high salt content, thus that it looked like there was an increase in the salt content of the product.

4.0 CONCLUSION

Fish viscera are known to be a rich source of digestive enzymes that is important in the process of making fish sauce but also rich in micro flora that potential to decay the material. Fish sauce made from sea cat fish (*Arius* sp.) viscera added with 20 % of salt and trypsin enzyme showed higher in yield and more yellow but and lower in TVB, TMA, Amonia and pH. Therefore, sea cat fish (*Arius* sp.) viscera can be processed in to fish sauce product.

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