THE EFFECT OF FISH VISCERAL ENZYMES ON RIPENING QUALITY CHARACTERISTIC OF SALT-FERMENTED MACKEREL (*Rastrelliger* sp)

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Abstract

This research was intended to improve ripening quality characteristic of salt-fermented mackerel by addition of fish visceral enzyme. Two week ice-stored mackerel weighing 250-350 g each were taken from a fish market in Pekanbaru; and the fish were processed for salt-fermented fish in the Laboratory of Fish Processing Technology, Faculty of Fisheries and Marine Science, University of Riau. Three batches of salt-fermented mackerel were prepared respectively from whole gutted fish + enzyme (A), whole gutted fish (B) and whole ungutted fish (C). The fish were ripened at room temperature for four weeks; and changes in their sensory quality characteristics, NPN, FFA, Halophilic and LAB counts were evaluated every week. The results showed that sensory quality characteristics of salt-fermented fish differed among the three lots during ripening process ($P<0.05$). Lot A had better quality characteristics and shorter ripening time than lot B but less quality characteristics and longer ripening time than lot C. NPN and FFA values in lot A were also higher than lot B but lower than lot C ($P<0.05$). Total halophilic counts in lot A were not significantly different from lot C ($P>0.05$), but higher than that lot B ($P<0.05$). There was an increasing trend in halophilic counts of the three lots during ripening process. Total lactic acid bacterial counts of the three lots decreased gradually up to the end of ripening ($P<0.05$).

Key Words: *Salt-fermented fish, ripening, visceral enzyme, fermentation, sensory analysis, non protein nitrogen, free fatty acid*

1. INTRODUCTION

Fisheries and aquaculture have played an important role in Indonesia, not only for generating income but also for animal protein source of more than 60% Indonesian population. National fish production has increased 12% per year; and the production in 2011 reached 12,4 million tons. Most of the production (60%) is marketed in form of processed products; and the salted fish is account for more than 60% of the total processed products.

Salt-fermented fish is one of the populer traditional processed fish products that has been recognized since long time ago; and the product is easily found in both tradisional food store and super market. The product is usually prepared from fatty fish such as mackerel (*Rastrellinger sp*), herring (*Sardinella sp),* layang (*Decapterus sp*) dan selar (*Caranx sp*); and salt-fermented fish made from mackerel (*Rastrelliger brachysoma*) is more preferable due to its flesh color (red) and flavor (Van Veen, 1965, Hanafiah, 1987 dan Hasan dan Sumarto, 2008). The product is usually consumed with rice, as a food additive

and animal protein source. Since the consumption of the product continues to increase, improvement in product quality is essential.

Production process of the product is divided into two stages. The first stag is diffusion of salt into the fish flesh and reduction of the water; and the second stage is ripening process which involves a series of biochemical process that can be grouped into proteolysis, lipolysis and lipid oxidation. The physical and chemical changes that occur during ripening determine the overall sensory qualities of the product (Voskresensky, 1965 and Hasan dan Sumarto, 2008). Ripening process of the product takes usually 2-9 weeks; and tissue enzymes, especially visceral enzymes are recognized responsible for the process (Van Veen, 1965; Kamil et. 1976; Irawadi, 1979; Syachri, 1979; Murdinah et al., 1983; Irawadi dan Syachri, 1984, Hernandez-Herrero et al., 2002 and Hasan dan Sumarto, 2008).

Traditionally, most of the salt-fermented fish is prepared from whole fish without eviscerated as the visceral enzymes are important for ripening process of the product (Nunes et al, 1997; Olsen and Sakara, 1997; Ritskes, 1971; Nielsen Borresen, 1997). However, the rapid autolytic degradation of the abdominal tissue (belly bursting) caused by visceral enzymes render to low acceptability of the finished product (Love, 1980; Sheu, 1995; Adam et al., 1987; Karmas and Lauber 1987). The product also has low sanitary quality and short shelf live (Hasan and Sumarto, 2008). As the government now emphasis on the important of food industry and the establishment of Food Regulation Act making more entrepreneurs aware of the economic implication of modernizing the traditional food processing industries, the preparation method of salt-fermented fish need to be improved.

This investigation was carried out to evaluate the effect of visceral enzymes on ripening quality characteristics of the salt-fermented fish.

2. MATERIAL AND METHOD

Preparation of sample and visceral enzyme

Salt-fermented fish was prepared from mackerel (*Rastrellinger sp*). The fish weighing 250-350 gram each which had been stored in ice for two weeks were taken from a fish market in Pekanbaru. The fish were transported to the laboratory of Fish Processing Technology University of Riau Pekanbaru; and at the laboratory, the fish were grouped into three batches: Whole gutted fish + enzyme (A), Whole ungutted fish (B) and Whole gutted fish (C). Crude visceral enzymes were extracted from the fish visceral organ (stomach, liver, pancreas and spleen). The salt-fermented fish were made using the method described by Hasan and Sumarto, 2008. Each batch of the fish was placed in 500 ml plastic container and layered with salt crystals at the ratio of fish and salt was 5:1. After salted for 24 hours, the fish was removed from remained salt crystal and swept with liquid crude visceral enzymes. The fish then was ripened at room temperature for 4 weeks. Changes in sensory quality characteristics, NPN, FFA, LAB and Halophilic count of the fish were evaluated every week for 4 weeks.

Sensory analyses

Sensory quality of ripening process was evaluated using the method following salt-fermented ripening developed by Hernandez-Herrero et al., (2002). Five trained panelists assessed the sensory quality. Five parameters were assessed: flavor, flesh color, odor, flesh consistency and adherence to backbone; and the fish were assigned a score for each factor according to the descriptions in Table 1. The maturation scale has its minimum at zero which represent sensory characteristic of raw fish, just before beginning of the ripening process; and point 3 expressed the optimum level of ripening; and point 5 corresponded to spoiled or overripe.

Biochemical analyses

Biochemical quality of ripening process was evaluated for formation of non protein nitrogen (NPN) and free fatty acid (FFA). NPN was determined by trichloroacetic acid (TCA) precipitation using makro-Kjeldahl procedure recommended by (Backhoff,

1976). FFA was determined in 50 ml neutral alkohol and 2 ml phenol phethalein indicator which was titrated with 0.1 N NaOH until the color changes to red for 30 seconds (Meihen Bachere, 1960). FFA was calculated as oleat.

Microbial analyses

Microbial analyses were conducted for total halophilic bacterial count and lactic acid bacterial count. Halophilic bacterial count was calculated on Trypticase Soy Agar (TSA) containing 20% NaCl and incubated at 30^{o}C for 96 hours; and lactic acid bacterial count was enumerated on de Man Rogosa and Sharpe (MRS) agar and incubated at 30^{o}C for 48 hours.

Statistical Analyses

Statistical analysis was carried out using one-way analysis of variance (ANOVA); and Least Significant Difference (LSD) test (Steel and Torrie, 1980) was used to determine differences among treatment means.

Table 1. Sensory quality characteristic scoring scale of salt-fermented fish during ripening process

1. Flash color	**NILAI**
Natural fresh fish	0
Natural around border, deep red in the middle, pink in between	1
Light pinkis meat, deep red or pink in the middle	2
Uniformity in the pink tone distribution	3
Darken red	4
Blacken red	5
II. Odor	
Fresh fish	0
Neutral, smell like brine	1
Smooth of agreeable odor to volatile esters	2
Smells of agreeable volatile esters	3
Rancid, acid off-odor	4
Ammoniacal and Sulfurous off odor	5
III. Flavor	
Raw fish	0
Natural	1
Slightly ham-like	2
Ham-like cured meat	3
Rancid off flavor	4
Intense rancid off flavor	5
IV. Flesh consistency and adherence to backbone	
High elasticity and adherence	0
Less elasticity and adherence	1
Slight elasticity and adherence	2
Very little elasticity and adherence	3
Soft, easily torn	4
Belly bursting	5

3. RESULTS

Sensory quality

Sensory quality characteristic values of salt-fermented mackerel during ripening process (Table 2) differed among the three lots; and the difference were observed from the first week of ripening ($P<0.05$). Lot A ripened faster than that lot B but slower than lot C. Bassed on 3 as an index of optimal ripening, both lot A and C reached an optimal ripening after three weeks and lot B ripened after four weeks. At four weeks of ripening, lot C began to overripe, which the fish was characterized by belly bursting; however lot A and B were still acceptable.

Table 2. Overall sensory quality characteristic values and changes during ripening process

Salt-fermented fish	Overall sensory quality				
	Ripening time (Weeks)				
	0	I	II	III	IV
Whole gutted fish+Enzyme (A)	0.91[a]	1.45[b]	2.30[b]	3.02[b]	3.25[b]
Whole gutted fish (B)	0.90[a]	1.07[a]	2.05[a]	2.65[a]	3.00[a]
Whole ungutted fish (C)	0.90[a]	1.85[c]	2.75[c]	3.75[c]	4.80[c]

Means in the same column followed by the same letters were not significantly different ($p<0,05$).

Biochemical changes

NPN concentration of the salt-fermented fish (Table 3) increased gradually during ripening; and the values varied among the three lots from initial to the end of ripening process ($P<0.05$). NPN concentration in lot C was the highest, then followed by lot A and B. NPN concentrations reached 30.40%, 43.35% and 57.70% for lot B, A and C respectively at the end of ripening process.

FFA (Table 4) also increased during ripening; and the concentration differed among the three lots ($p<0.05$). FFA concentration of the salt-fermented fish in the lot C was the highest, then followed by lot A and B ($p<0.05$). FFA values of lot A, B and C during ripening process were 8.92-29.22 g oleic/100 g fat, 8.87-22.77 oleic/100 g fat and 8.93-36.25 g oleic/100 g fat.

Table 3. NPN concentration of salt-fermented fish (% Total N) during ripening process

Salt-fermented fish	NPN				
	Ripening time (Weeks)				
	0	I	II	III	IV
Whole gutted fish+Enzyme (A)	9.71[a]	19.95[b]	25.50[b]	39.80[b]	43.35[b]
Whole gutted fish (B)	9.70[a]	13.60[a]	19.60[a]	28.85[a]	30.40[a]
Whole ungutted fish (C)	9.70[a]	23.70[c]	36.50[c]	49.30[c]	57.70[c]

Means in the same column followed by the same letters were not significantly different ($p<0,05$).

Microbial changes

Total halophilic counts of salt-fermented fish prepared from lot A and C (Table 5) were not significantly different but higher than that lot B ($P<0.05$). There was an increasing trend of halophilic counts of the three lots during ripening process.

Table 4. FFA concentration (g oleic/100 g fat) of salt-fermented fish during ripening process

Salt-fermented fish	FFA				
	Ripening time (Weeks)				
	0	I	II	III	IV
Whole gutted fish+Enzyme (A)	8.92[a]	9.33[b]	11.12[b]	23.44[b]	29.22[b]
Whole gutted fish (B)	8.87[a]	9.08[a]	10.55[a]	15.65[a]	22.77[a]

Whole ungutted fish (C)	8.93[a]	10.13[c]	12.75[c]	28.22[c]	36.25[c]

Means in the same column followed by the same letters were not significantly different ($p<0,05$).

Total lactic acid bacterial counts of the three lots (Table 6) decreased gradually during ripening. There were no significant difference in counts among the three lots at the first week of ripening process; and the difference was only observed at second week up to the end of ripening process, which the counts of lot A and C were higher than that lot B ($P<0.05$).

Table 5. Total halophilic counts of salt-fermented fish during ripening process

Salt-fermented fish	Total halophylic counts (log CFU/g)				
	Ripening time (Weeks)				
	0	I	II	III	IV
Whole gutted fish+Enzyme (A)	3.59[a]	3.73[b]	3.92[b]	3.98[b]	4.09[b]
Whole gutted fish (B)	3.51[a]	3,54[a]	3.58[a]	3.75[a]	3.87[a]
Whole ungutted fish (C)	3.53[a]	3,73[b]	3.86[b]	3.94[b]	4.05[b]

Means in the same column followed by the same letters were not significantly different ($p<0,05$).

Table 6. Total lactic acid bacterial counts of salt-fermented fish during ripening process

Salt-fermented fish	Total lactic acid bacterial counts (CFU/g)				
	Ripening time (Weeks)				
	0	I	II	III	IV
Whole gutted fish+Enzyme (A)	1.92[a]	1.86[a]	1.81[b]	1.68[b]	1.57[b]
Whole gutted fish (B)	1.83[a]	1.79[a]	1.67[a]	1.63[a]	1.41[a]
Whole ungutted fish (C)	1.94[a]	1.88[a]	1.82[b]	1.71[b]	1.61[b]

Means in the same column followed by the same letters were not significantly different ($p<0,05$).

4. DISCUSSION

Ripening process of salt-fermented fish is a series biochemical process which involves proteolysis, lipolysis and lipid oxidation; and their products contribute to the overall sensory quality of the products (Huss, 1995 and Voskrezensky, 1995 and Hernandez-Herrero et al., 2002). The ripening process was accelerated by fish enzymes (visceral and tissue enzymes) and enzymes originated from microorganisms (Van Veen, 1965 and Heu et al., 1991). In this study, 3 lots of salt-fermented fish were prepared from whole ungutted fish, gutted fish and gutted fish + visceral enzyme; and the ripening quality was evaluated for sensory characteristics, proteolysis (NPN), lipolysis (FFA), halophilic counts and lactic acid bacterial counts.

The result indicated that the sensory quality characteristics of the three products gradually developed during ripening; and the development was better and faster in the salt-fermented fish prepared from whole gutted fish+ enzyme than that of whole gutted fish but slower than that prepared from whole ungutted fish. This fact indicated the role of additional enzymes to ensure the sensory quality development of the product. Hernandez-Herrero et al., (2002) reported that development of sensory characteristics of salt-fermented fish was an accumulation of physical and chemical changes during ripening process; and the changes depended on the concentration and activity of the enzymes mainly visceral enzymes. Enzymatic hydrolysis during ripening was reported to create a soft textured product and

taste-active peptides and free amino acids (Del-Rosario and Maldo, 1984; Nielsen and Borresen, 1997; Olsen and Sakara , 1997; Saisithi, 1994; Shenderyuk and Bykowski, 1990).

Visceral enzymes contain proteolytic enzymes which accelerate breakdown of the fish tissue during ripening proses, shorten fermentation time and ensure good quality products. However excessive degradation of abdominal tissue due to visceral enzymes lead to belly bursting, soft tissue and low acceptable product. Enzymes originated from pyloric caecae are recognized as the most active enzymes which breakdown protein and lipid of the abdominal tissue to cause belly bursting (Love, 1980; Sheu, 1995; Adam et al., 1987; Karmas and Lauber 1987). Extracted visceral enzyme which is treated to the whole body of gutted fish will lead to the distribution of proteolytic and lipolytic enzymes to the whole fish muscle, therefore ripening process undergoes rapidly and thoroughly, promoting good quality of end products.

For salt-fermented fish prepared from whole gutted fish, proteolysis and lipolysis activities only caused by muscle enzymes; and their activities are not as high as visceral enzymes. This may be a reason why ripening process of salt- fermented fish prepared from whole eviscerated fish slower than that eviscerated fish. Some researchers isolated various proteases from muscle tissue of fish (Reddi et al, 1972; Siebert and Schmitt, 1965; Haard, 1994; Kolodziejska and Sikorski, 1996; Asgeirsson et al., 1995 and 1988). Cathepsin D was a dominant protease in fish muscle tissue and it concentration was ten times more than mammalian muscle (Wojtowicz and Odense 1972; Gilberg, 1988). Cathepsin D was reported to be an important protease which initiated the degradation of cell protein to peptide; and further breakdown was accelerated by others (A, B and C). Cathepsin was also reported to play important role in ripening process of fish pickle (Lerke et al., 1967). Some peptidases were also found in fish muscle tissue (Siebert dan Schmitt, 1965; dan Konagaya, 1978).

Biochemical changes during ripening process of salt-fermented fish in this study were indicated by development of non protein nitrogen and free fatty acid. Non protein nitrogen is low molecule weigh nitrogenous compounds resulted from breakdown of fish protein and other nitrogenous compounds (Durrand, 1982). Non protein nitrogen concentration for the three lots of salt-fermented fish in this study increased during ripening process. This was also in accordance with the results found by Filsinger et al., 1984, Hernandez-Herrero, et al., 1999; Duran, 1982; Perez-Villarreal and Pozo 1992 and Hasan and Sumarto 2008); Salt fermented fish prepared from uneviscerated fish and eviscerated + enzymes resulted in higher non protein nitrogen than that prepared from eviscerated fish. Non protein concentration during ripening process was 6.70-30.40%, 6.71-57.70% respectively for total nitrogen. Endogenous enzymes were reported to be responsible in ripening process of salt-fermented fish, and protein autolysis is probably caused mainly by trypsin-like enzymes (Heu at al., 1991); thus salt-fermented fish prepared by additional visceral enzymes or whole uneviscerated fish resulted in higher non protein nitrogen that of eviscerated fish.

There was an identical trend of increase in non protein nitrogen and sensory quality development during ripening process, illustrating the contribution of non protein nitrogen to the development of sensory characteristics in salt-fermented mackerel. The same findings were also reported by other researchers (Filsinger et al., 1984, Hernandez-Herrero, et al., 1999; Duran, 1982; Perez-Villarreal and Pozo 1992 and Hasan and Sumarto 2008); and they concluded that the non protein nitrogen a good indicator for ripening rate in salt-fermented fish. These compounds especially free amino acid such as lysine, leucine, histidine, glutamic acids, arginine and alanine play the important role of mayor taste compounds in most salt-fermented fish products (Cha et al., 1983).

Free fatty acid concentration also increased during ripening process; and their concentration at the end of four week ripening reached 22.77 - 36.25 gram Oleat/100 gr fat. The highest free fatty acid values were found in salt-fermented mackerel prepared from whole ungutted fish then followed by those prepared from whole gutted fish+ enzymes and whole gutted fish. Formation of free fatty acid during ripening process was a result of lipid

hydrolysis and oxidation. Lipolysis may be initiated by lipoxygenase dan microprotease, mainly those originated from fish viscera (Hernandez-Herrero et al., 2002). This may be a reason why salt-fermented made from ungutted fish undergo lipolysis more intensive than that prepared from gutted fish. Free fatty acid production in a certain food is not desirable due to its rancid flavor and odor; however, these compounds were identified as a contributor of a specific flavor and odor of salt-fermented fish (Wheaton dan Lawson, 1985).

Halophilic counts showed an increasing trend; but lactic acid bacterial counts decreased gradually during ripening process. The same results were also found in salt-fermented fish by Hasan and Marto, 2008. The present of NaCl stimulated the growth of halophilic and halotolerant bacteria (Campello 1985; Verez-Villareal and Pozo 1992; Hernandez-Herrero et al., 1999). NaCl concentrations of 10 to 20% have stimulatory effect on halophilic bacterial activities; while levels in excess of 20% inhibit the growth and activities of the bacteria (Hernandez-Herrero et al., 1999 and Hernandez-Herrero et al., 2002).

Lactic acid organisms, however, are sensitive toward high salt concentration and their presence in salt-fermented fish may be through contamination during processing and ripening process. Formation of glycogen during post-mortem glycolysis may stimulate the occurance of lactic acid bacteria at the beginning of ripening process; and their number decreased as the glycogen concentration shortage.

5. CONCLUSON

An Addition of visceral enzymes improved ripening process and quality of salt-fermented mackerel. Salt-fermented mackerel prepared from whole gutted + enzyme has a better quality and shorter ripening time than that prepared from gutted fish. Salt-fermented made from whole unggutted fish although has a similar ripening time with that prepared from gutted fish + enzyme, but its quality decreased faster which was indicated by belly bursting. Sensory quality characteristic development during ripening process was identical with non protein and free fatty acid values. There was an increasing trend of total halophilic counts and decreasing trend of lactic acid bacterial counts during ripening process.

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