

THE EFFECT OF STORAGE ON PHYSICAL, CHEMICAL AND MICROBIOLOGICAL CHARACTERISTICS OF FISH WASTE ACIDIFIED USING FERMENTED VEGETABLES WASTE EXTRACT

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ABSTRACT

Fish waste (“ikan rucah”) is part of discarded fishing product, which is composed by non-food categorized fish (NFC-fish). Quality of NFC-fish meal that was made by dipping in extract of fermented vegetable’s waste has been reported better than commercial fish meal, but the effect after storing remained in question. Experiment was conducted to study the effect of different time of storing on physic-organoleptical, chemical and microbiological characteristics of the acidified NFC-fish meal that was made by dipped in extract of fermented vegetable’s waste (FVW-extract). The NFC-Fish was soaked in the FVW-extract by the ratio 1:1 (w/v) for 4 hours, and then it was dripped out and dried. The dried fish was ground passed through 20 mesh, kept into plastic bags and stored at the room temperature (23-25 °C and 70-80% RH) for 0, 1, 2 and 4 months. Physic-organoleptical, chemical and biological characteristics were parameters observed. Experiment was conducted by completely randomized design (CRD). Data were analysed by the GLM of SAS. Actual number of moisture, crude protein, extract ether, and proteolytic bacteria of fish meal were significantly influenced by time of storing ($P<0.05$). Dipping NFC-fish in the FVW-extract effectively maintain the physical characteristics, pH, moisture, crude protein, extract ether and the number of proteolytic bacteria of fish meal up to 2 months of storage. Dipping NFC-fish in the FVW-extract provide better characteristics on physical, chemical and microbiological than the commercial fish meal at the same condition of storage.

Keyword : acidified, extract vegetable, storage, fish meal, characteristics

INTRODUCTION

Fish waste (“ikan rucah”) is part of local fishing product waste that was composed of non-food categorized of fish. That is almost 5-10% of fishing product and it contains high protein (>36%). Fish waste has been recognized as source of protein for animal feeding. The conventional method of fish meal production in farmer’s scale is the technology constrain and its quality. To obtain high yield and quality of fish meal, it was necessary method skills and experience in cooking, pressing and drying. These technologies were not easy and high cost in operation (Barlow and Windsor, 1984; Solar *et al.*, 2005; Folador, 2006; Amiza *et al.*, 2011). Organic wastes of traditional vegetable markets were potential local resources. Our previous study noted that fermentation of vegetable waste provided product which was rich in organic acid and total number

of lactic acid bacteria were 3.5×10^9 cfu/mL. Acidified fish meal has advantages in terms of application technology and quality in compared to conventional fish meal.

Soaking is an alternative acidifying technique in preservation. Soaking fish meal in the Sauerkraut-extract (FVW-extract) was reported increasing microbiological and physical performances than that of commercial fish meal (Sulistiyanto and Nugroho, 2009), and prevented the growth of bacteria in the smoked products of silver catfish (*Pangasius sp*) (Amin and Leksono, 2001). It was reported that solution extract of fermented vegetables could be applied as a sources of organic acids which was rich in lactic acid bacteria, and those play role in maintaining the physic-organoleptical and microbial performance of the product of acidified fish meal. Lactic acid bacteria were known to have potential for preservation as well as suppressing the

development of microbial pathogens such as NFC-fish (Utama and Mulyanto, 2009; Sumarsih *et al.*, 2010). The process of soaking is expected to provide acid atmosphere so that impedes autocatalytic process in the fish meat and prevent activity of decomposing microorganisms, so it will produce of acidified fish meal with a good performance.

Sulistiyanto *et al.* (2011) noted that the number and viability of LABs of FVW-extract remain stable after pelleting process, and feeding that pellet significantly increased the total number of LABs and suppressed the number of pathogens in the gut of chickens. Storing generally caused characteristics' exchange and decreased the total number of bacteria that may lead to decreasing the quality of product (Malaka and Laga, 2005; Prachyakij *et al.*, 2006; Katikou *et al.*, 2007). The previous study noted that dipping into FVW-extract effectively reduced the content of total bacteria, fungi and proteolytic's bacteria in fish meal. The effect of the FVW-extract was presumably stable to maintain the quality of the fishmeal product during storing. Therefore, the experiment aimed to study the influence of different storage time on physic-organoleptical, chemical and microbiological characteristics of the acidified fish meal that was made through dipped the fish in the fermented vegetable's waste extract.

MATERIALS AND METHODS

Extract of fermented vegetable's waste (FVW-extract) that was made by Chinese green mustard and cabbage wastes, was mixed with 2% of salt (w/w) and 6.7% of molasses (w/w), and was incubated for 6 days. Extraction was done mechanically, it was pressed by 10 kg/cm² for 5 minutes, and the FVW-extract was then used to acidify "Rucah" fish. The Fish (\pm 60 kg) was chopped to 3-4 cm, then was soaked into FVW-extract by ratio 1:1 (w/v) for 4 hours, subsequently it was drained and dried. The dried acidified fish was ground passed through 20-40 mesh, kept into plastic bags (\pm 2.5 kg) and stored at the room temperature (23-25 °C and 70-80% RH) for 0, 1, 2 and 4 months. The commercial fish meal was stored within 4 months storing since it was taken from poultry shop. To that physic-organoleptical characteristics, pH, moisture content, crude protein, crude fat, total fungi and total proteolytic bacteria were observed. Physic-organoleptical characteristics were conducted by

panels (9 panels) to observe odor, color and texture. pH was measured after diluting fish meal with water 1:1 (w/v), mixed well, stand up for 15 minutes, and then measured the pH. The moisture content, crude protein and crude fat were analysed by the method of AOAC (1997). Total fungi and total proteolytic bacteria were determined by the method of Fardiaz(1993). Statistical analysis was conducted by the GLM-SAS (SAS-Institute, 1982).

RESULTS AND DISCUSSION

Result of experiment noted that weight loss from a fresh form to be a fish meal product (moisture content \pm 10%) of acidified with FVW-extract method was lower than that of conventional method, that was 48 vs 62% vs 62% ($P < 0.05$). Table 1 showed the resume a result of the experiment. The result showed that during 2 months of storage, physical characteristics i.e.: odor, color and texture did not change significantly ($P > 0.05$). It was significant after 4 months of storing, in particular of texture which was a bit dense ($P < 0.05$). In general, the physical appearance was better than commercial fish meal that was stored for 4 months under the same conditions. The pH values of fish meal was in acidic conditions that was 3.8 ± 0.2 - 5.5 ± 0.1 . While the commercial fish meal pH tended from neutral to alkaline. The moisture content increased with the time of storage. There were 7.7 ± 2.8 ; 8.7 ± 2.3 ; 8.9 ± 3.2 and $13.3 \pm 2.3\%$ (0, 1, 2 and 4 months of storage). The significant increment was shown in time of 4 months storage ($P < 0.05$). Crude protein content did not significantly change during 2 months of storage (59.54, 61.39 and 60.64%), and it significantly decreased at 4 months of storage (58.14%), although it was higher than those in the commercial fish meal (45.86%) that stored for same period ($P < 0.05$). The content of ether extract tended to decrease with the increasing of storage time (8.82, 8.12 and 8.74%). It was significantly decreased in the time of storage of 4 months (7.84%). The ether extract of acidified fish meal was lower than those of commercial fish meal (11.45%) that was stored for the same condition ($P < 0.05$). Fungi was not found in fish meal made with the technique of dipping in the solution of fermented vegetable extract, while the fungi was found in the commercial fish meal, there was amounted to 2.2×10^2 cfu/g. The total

Table 1. Physical, Chemical and Microbiological Characteristics of Fish Meal Made by Dipping into Extract Pickled Vegetables

Storage Time (month)	Odor	Color	Texture	pH ²⁾	Moisture ³⁾	Crude Protein ³⁾	Extract Ether ³⁾	Total Fungi ⁴⁾	Total Proteolytic Bacteria ⁴⁾
					----- % -----			----- cfu/g -----	
0	Fresh sourish smell, typical fish meal	Light brown	crumbly	3.8±0.2 ^c	7.7±2.8 ^c	59.54±2.3 ^a	8.82±2.8 ^b	ND	4.8 x 10 ^{5b}
1	Fresh sourish smell, typical fish meal	Light brown	crumbly	4.8± 0.2 ^{bc}	8.7±2.3 ^c	61.39±5.2 ^a	8.12±2.8 ^c	ND	5.2 x 10 ^{5b}
2	Fresh sourish smell, typical fish meal	Light brown	crumbly	5.5± 0.2 ^b	8.9±3.2 ^c	60.64±4.3 ^a	8.74±1.8 ^b	ND	5.5x 10 ^{5ab}
4	Typical fish meal	Light brown	Crumbly, little bit compacted	5.5± 0.1 ^b	13.3±2.3 ^b	58.14±4.8 ^{ab}	7.84±3.2 ^c	ND	4.8 x 10 ^{5b}
Commercial fishmeal ¹⁾	Typical fish meal, slightly fishy and smelties	Redish slightly dark brown	Crumbly, little bit compacted	6.7± 0.1 ^a	16.2±2.8 ^a	45.86±2.8 ^b	11.45±2.8 ^a	2.2 x 10 ^{2 b}	7.8 x 10 ^{6a}

Different superscript in the same collum shows the significantly different (p<0.05)

ND : not determined (not identified from the sample)

¹⁾Commercial fishmeal within 4 month storing since it was taken from poultry shop

²⁾pH was measure by diluted fish meal with water 1:1 (w/v), mixed well and stand up for 15 minutes then measured the pH

³⁾Moisture content, crude protein and crude fat analysed by the method of AOAC (1997)

⁴⁾Total fungi and total bacteria determinated by the method of Fardiaz(1993)

number of proteolytic's bacteria could be maintained up to 2 months of storage. Even though shown an increasing trend in the last 2 months of storage (4.8 x 10⁵, 5.2 x 10⁵, 5.5x 10⁵ cfu/g), the total number of proteolytic's bacteria was still lower than that in the commercial fish meal(7.8 x 10⁶ cfu/g) which was stored at the same condition(P<0.05). It can be noted in this study, the increase of the moisture content during storage promoted the growth of the mold and proteolytic's bacteria, and the activity of proteolytic's bacteria causing changes of nutrient composition.

Loss of weight of fish meal that was calculated from fresh, after acidification and drying was lower than that was made by conventional method. Barlow and Windsor

(1984), noted that to obtain high yield and quality of fish meal, it is necessary the method skill and experience in cooking, pressing and drying. Cooking temperature, screening materials, pressing and drying are factors related to the decreasing of yielding and quality of fish meal (Marki, 1990; Babbitt *et al.*, 1995; Opstvedt *et al.*,2003). In the manufacture of fish meal with acidification technique, intra-/extra-cellular fluid flows out as a result of penetration of FVW-extract through osmotic pressure differences. In the conventional techniques, the liquid is discharged as a result of mechanical pressure, therefore the fluid was much more loosed. Marki (1990) and Jensen (1990) reported that increasing the pressure may lead to the weight loss because the mixture of water, oil and some solids was

squeezed out through the perforations.

The FVW-extract has been indicated having a potential lactic acid bacteria that possibly be used as a natural control to the nutrition damage of "Ikan rucah" (Sulistiyanto and Nugroho, 2009). Lactic acid bacteria's (LABs) has been applied in feed preservations as a bio-control agents of *E.coli* and *S. aureus* (Rahayu *et al.*, 2004). Bacteriocins that are produced LABs such as : acidophilin, acidolin, lactobacillin, and lactocidin have had demonstrated an inhibitory activity against *Klebsiella sp* , *Pseudomonas sp*, *Bacillus sp*, *Proteus sp*, *Salmonella sp*, *Shigella s*, *Staphylococcus sp*, *Vibrio sp* and enteropathogenic *E. coli* (RisØen *et al.*, 2004 ; Rehman *et al.*, 2009). Agustina *et al.* (2011) and Sulistiyanto *et al.* (2011) noted a number and viability of LAB's of FVW-extract could be maintained in the pellet form. The number LAB's of FVW-extract remained high in the pellet, even the moisture content was 8-14%. That was significantly increased the total number of the LABs and suppressed the number of *Salmonella sp.* and *Coliform* in both digesta of the small and large intestines of the chickens. Lactic acid bacteria helps to develop and stabilize a competent microflora in the gastrointestinal tract that should successfully avoid the proliferation of pathogens (Fuller, 1977; Khaksefidi and Ghoorchi, 2006). It could be considered in this experiment that the conditions in the storage period of 2 months allegedly as a result of group activity of LABs. LABs are groups of proteolytic facultative bacteria, the activity of LABs produces lactic acid that may play a role in inhibition of the growth decomposing microbes, so the pH and total number of proteolytic bacteria remain no significant changes. In term of commercial fish meal, activity of non-LABs proteolytic's bacteria presumably increased after 2 months of storage, as shown the increase in the bacteria population and the pH that leads to alkaline. Decomposition of proteins that lead to damage was marked from the release of ammonia thus encourage an increasing of pH value.

The lactic acid bacteria numbers play the important role on the preservation effect of fish product. Increasing number of LABs reduced the number of non-LABs of fermented fish. Crude protein and extract ether of fermented fish increased in line with the increasing of FVW-extract (Sumarsih *et al.*, 2010; Utama and Sumarsih (2010). *Lactobacillus sp* may inhibit the growth of decomposing bacteria

(*Enterobacteriaceae* and *Pseudomonas spp.*) of the fish fillet (Katikou *et al.*, 2007). The accumulation of metabolic products of cell may toxic to the cells, and the cells became death, therefore it eliminated the contamination of fermented fish from *Coliform* and *Salmonella sp.* up to 2 weeks (Ndaw *et al.*, 2008). Inhibitory mechanism of LABs to the growth and development of pathogenic and decomposing bacteria considered be the causative effect of the LAB's number and its organic acids. The organic acid of the LABs inhibited the growth and metabolism of non-LABs in particular non acidic resistant bacteria (Lindgern, 1992; Amin and Leksono, 2001; Carey *et al.*, 2008; Christine *et al.*, 2002). *Salmonella sp.*, *E.Coli* and *Clostridium sp.*, are the pathogenic and decomposing bacteria that are sensitive to acidic condition (Jay *et al.*, 2005). Organic acids and its concentration affected the effectiveness of organic acid to the preservation (Woolford, 1992). Introducing organic acid and decreasing pH are constitutes effective method for feeds preservation. Application of organic acid to control bacteria and fungi, provide good impact on animal that consume acidified feedstuffs (Seerly, 1993 and Gerritsen *et al.*, 2010). This research has been proven that organic acid in the extracts of FVW-extract provide acid condition during the process of soaking and the moisture content of the acidified fish meal was bellow 12%, so the fungi did not grow. Thus could be argued that the LABs and FVW-extract maintaining the nutrient content such as CP and EE during storing is conducted by the role of lactic acid bacteria and the role of pH during the process of dipping and storing simultaneously. Therefore, dipping in the FVW-extract effectively maintained physical performances, pH, moisture, crude protein, extract ether and number of proteolytic bacteria of fish meal up to 2 months of storing. Dipping in the FVW-extract provide better characteristics of physical, chemical and biological of the "rucah" fish than that of commercial fish meal.

CONCLUSION

In conclusion, the acidified "rucah" fish with FVW-extract technically effective reduced the weight loss in fish meal processing and effectively maintain characteristics of physic-organoleptical, chemical and microbiological up to 2 months of storing. Dipping of "rucah" fish

into FVW-extract provided better physico-organoleptical, chemical and microbiological characteristics than that of commercial fish meal.

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