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Effect of Steaming Process on New Formulation and Physical Properties of Earthworm-based Fish Pellets for African Catfish (*Clarias gariepinus*)

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Abstract: Fish feed has been recognized as one of main part/unit in aquaculture industry. However, current fish feed faces few challenges in terms of health aspects and cost issues. Alternatively, new nutritional and economical/low cost formulation of fish pellets was designed by combination of earthworm powder and other economical ingredients such as fishmeal, soybean waste, rice bran and tapioca flour. The formulation was calculated using Pearson's square and optimized by One-Factor-At-Time (OFAT) method. The effect of steaming processing on the water stability, soaking experiment, protein leaching test and breaking force of the earthworm-based fish pellets was investigated. Results indicate steam pellet at 80°C for 40 min has higher water stability, less protein leaching and more durable than unsteam pellets. Introduction of this new formulation of fish meal is expected to provide essential nutrient, energy and improved the quality of pellets to fuel the growth of aquaculture industry.

Key words: Fish pellets, steaming, water stability, aquaculture

INTRODUCTION

In Malaysia, fisheries sector is one of the important industries which offers job opportunities for 89,453 fishermen and 21,507 fish farmers (MDoF, 2004). Owing to the factors, Aquaculture as one of the sub sectors in fisheries is now becoming a new engine for Malaysia economic growth (Saalah *et al*., 2010). In year 2010 report stated that Malaysian aquaculture industry increased 7.93% as compared to year 2009 (MDoF, 2012).

In Malaysia, the majority fish feed for aquaculture industries is formulated from agriculture by-products, poultry waste, trash feed and formulated commercial feed (Saalah *et al*., 2010). Few of the ingredients affect the health aspect of the fish and economical aspect of the fish industries. Utilization of raw fish as main ingredient in fish feeds has found to be risky to the health and growth of fish due to the presence of compound which could destroy the essential nutrients in the fish feed (Royes and Chapman, 2003).

Approximately 50% of the production cost of aquaculture industry depends upon the fish feed (Vielma *et al*., 2000). Commercial nutritious feed is expensive and hence incur more processing cost in aquaculture industry. Owing to these factors, there are growing interest among the researchers to formulate an economical and nutritional fish feed.

In previous study conducted by Zakaria *et al*. (2012), earthworm powder based-fish feed showed significant protein content as compared to other ingredients. In addition, earthworm contain various types of amino acid that necessary for fish growth (Dedeke *et al*., 2010). An Phu Earthworm Farm (2010) found earthworm to be effective in developing muscles and weight gain for the fish.

Apart from that, quality of fish pellet is another important factor need to be considered for fish feed manufacturer. Extrusion is one of the conventional methods for producing fish pellet. The pellet being produced by compressing the fish feed ingredient using different dies and physical conditions where by this features allow the user to control the pellet density. However, the versatility of the extrusion process also makes it more expensive than other method (Hardy, 2000). In contrast, steaming method has been a preferred choice by small scale farmers for preparing the fish pellet due to less processing cost. The addition of steam after compressing and mixing the necessary ingredients as a fish feed improves pellet quality by providing sufficient condition for the gelatinizing agent to bind the ingredients in the pellet (Gonzalez and Allan, 2007).

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Owing to the factors that fish feed contribute to major cost in the aquaculture industries (Tacon *et al*., 2006), thus, this study will focus on the use of steaming method for pelleting the fish feed as it is cheaper than other fish feed pelleting method. Besides, this method also being an alternative solution for small scale farmer who could not afford to engage in high processing cost aquaculture business.

Thus, in this study, the effect of steaming process on new formulation of earthworm-based fish feed and its properties were investigated.

MATERIALS AND METHODS

Raw material preparations: The earthworm powder was supplied by local company, Parkman Agro Resources. Fish meal, soybean waste and rice bran is taken from individual person and protein analysis was done to determine average protein content. Vitamin mix was purchased from Laman Flora Enterprise. Tapioca flour was purchased from local market. Calcium bis-(dihydrogenphosphate) was purchased from HmbG Chemicals Inc., Germany.

Experimental design: Fish pellet was formulated based on ingredients as shown in Table 1. The calculation of ingredients amount is based on Pearson's Square formulation calculation (Hardy, 1980). Protein concentration for each ingredient was determined using Lowry's method and turned into percentage (Table 1). The experiment was done by using One-factor-at-a-time (OFAT) design (Ahmad Anas *et al*., 2012). Optimization of steaming time and temperature were performed separately for maximum water stability determination. The studied time variables were 10, 20, 30, 40 and 50 min at fixed steaming temperature, 90°C (Royes and Chapman, 2003). Temperature variables were 60, 70, 80, 70 and 100°C whereby the optimized time was used.

Preparation of feed pellet: The soybean wastes and rice bran were dried at 60°C for 72 h in the oven and were grinded to get 500 μm of size. All ingredients were weight according to Table 1 and were mixed thoroughly. Distilled water was slowly added to the mixture at room temperature in proportion 2:3 (v/w) mixing to accomplish agglutination (Solomon *et al*., 2011). The dough was pelletized using pelletizer to form pellets. The steamed pellets were prepared according to the studied temperature and time.

Optimization of steaming time and temperature for water stability

Water stability test: Water stability test was conducted in triplicate for each sample. An accurate weight of 1 g each for steam and unsteam pellets was tied in a nylon sieve and mesh with a twine to avoid breakage and immersed in the aquarium agglutination (Solomon *et al*., 2011). Samples were immersed for 10 min and air dry transferred into an oven at 103°C for 2 h. The weight of before and after water treatment were recorded. Water stability were calculated by the following equation:

Table 1: Percentage of protein in each ingredient for the formulation of steam and unsteam pellets

Ingredients	Protein (%)
Earthworm powder	77.12
Fish meal	51.16
Soybean waste	24.26
Rice bran	16.28
Tapioca flour	0.10

$$\text{Water stability} = \frac{\text{Weight after water treatment}}{\text{Weight before water treatment}}$$

and converted to percentage (Fagbenro and Jauncey, 1995).

Physical properties test

Soaking experiments: The dry weights of ten pellets of each type of pellet were determined. The pellets were left sunk by the surface of water and left to submerged for 2, 5 and 10 min. The water was drained out after submerged time and the pellets were dried using adsorbent paper. The particles were re-weighted to obtain weight increment after immersion (Vassallo *et al*., 2006).

Protein leaching test: Leaching of protein from the pellets were measured using Lowry's method. Drained out water from soaking experiment was used for protein leaching test (Fagbenro and Jauncey, 1995).

Breaking force: Breaking force or hardness test was done by using Brookfield CT3 Texture Analyzer, USA. Each types of sample (30 pellets) was measured and valued of length (mm), width (mm) and force (N) was recorded.

Statistical analysis: The result of soaking experiment, protein leaching test and breaking force was studied by mean and standard deviation for both steam and unsteam pellets.

RESULTS

One-factor-at-one-Time optimization: One-Factor-at-One-Time optimization design results are shown as Fig. 1.

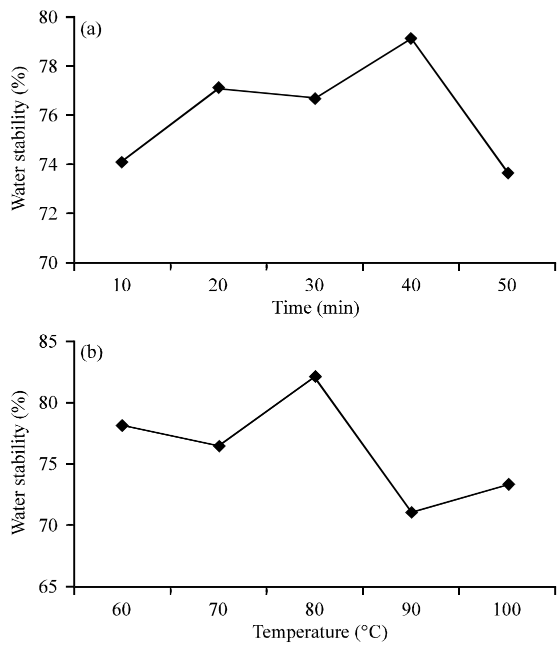

Fig. 1(a-b): Optimization of steaming (a) Time and (b) Temperature for maximum water stability using OFAT method

Table 2: Weight increment percentage in 2, 5 and 10 min for steam and unsteam pellets

	Soaking weight increment (%)	
Time (min)	Steam pellets	Unsteam pellets
2	59.86±0.03	26.00±0.01
5	67.77±0.05	26.65±0.03
10	71.16±0.10	28.79±0.04

Table 3: Leaching protein concentration (mg mL^{-1}) 2, 5 and 10 min for steam and unsteam pellets

	Leaching protein concentration (mg mL^{-1})	
Time (min)	Steam pellets	Unsteam pellets
2	0.02±0.002	0.04±0.003
5	0.03±0.003	0.06±0.005
10	0.04±0.001	0.10±0.001

Soaking experiment: Mean and standard deviation of sample were recorded in Table 2. There were increments in weight for steam pellets compare to unsteam pellets. Weight increments were observed as 32.86, 7.91 and 2.39% for 2, 5 and 10 min soaking time, respectively.

Protein leaching test: Standard curve for Lowry's Method was plotted and protein leaching concentration in 2, 5 and 10 min was determined according to standard curve. Mean and standard deviation of sample were recorded in Table 3. The data showed significant differences in leaching protein concentration (mg mL^{-1}) in unsteam soaking pellets compared to steam pellets. There is higher protein content in immersion water of unsteamed pellets ranging from 0.1053 to 0.0411 mg mL^{-1} compared to steam pellets that was 0.0411 to 0.0255 mg mL^{-1} only. The concentrations of protein leached out into the water are proportional to immersion times.

Table 4: Force requirement for breaking down steam and unsteam pellets

Pellets type	Breaking force (N)	Width (mm)	Length (mm)
Steam	12.38±1.14	0.50±0.01	10.00±0.09
Unsteam	1.90±0.40	0.50±0.01	10.00±0.04

Hardness or breaking force test: Table 4 showed mean and standard deviation of force (N) need to break each pellets with recorded width (mm) and length (mm). High breaking force of 12.38 N was needed to break down the steam pellets compared to just 1.90 N for unsteam pellets. The similar values of width and length of pellet showed the end sizes after broken.

DISCUSSION

The earthworm-powder based fish pellets were formulated using Pearson's square for 32% protein content using earthworm powder, fishmeal and soybean waste as protein source, rice bran as energy and fat source and tapioca flour as gelatinization agent. Other ingredients like vitamin mix and dicalcium phosphate are use as micronutrient supply. The ratio of earthworm powder used in formulation is in smallest ratio among three protein sources and maximizes the use of soybean waste to control the cost of production and to increase agriculture waste usage.

The fish pellet was pelletized from uncooked dough and therefore caused unstable pellet condition in water. The pellets were cook with optimum steaming temperature and time to achieve maximum water stability. The optimum steaming time and steaming temperature were obtained at 40 min and 80°C, respectively as shown in Fig. 1. Pellets exposed to temperature below 80°C failed to gelatinize completely and therefore less interaction within the starch structure. On the other hand, at temperature above 80°C, starch became over gelatinized and this condition also reduces the interaction within the starch structure. The increasing of steaming time caused greater forces of shear that paste the starch. The greater the shear and the longer time exposed to these forces, the more swollen the granule and the more fragile and susceptible it is to rupture (National Starch and Chemical Company, 1996). Shear forces are recognized to stimulate endodegradation of the high molecular weight compounds in starch (Baud *et al*., 1999; Davidson *et al*., 1984).

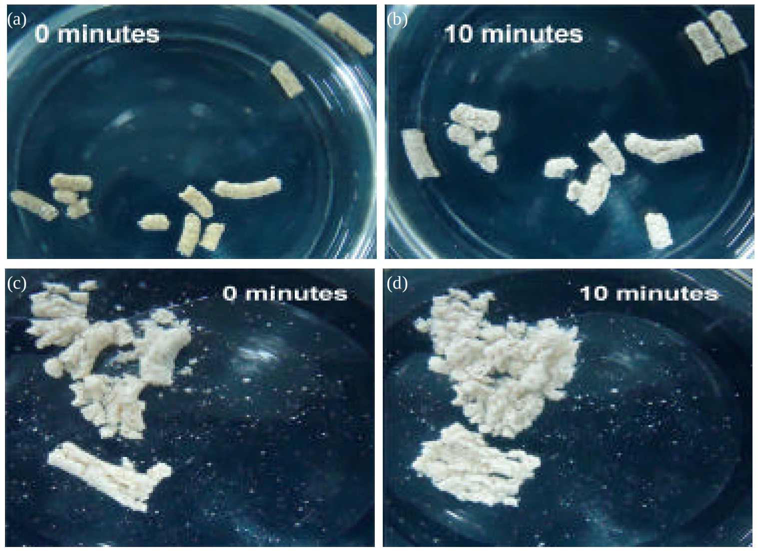

Fig. 2(a-d): Appearance of pellets at 10 min soaking experiments Steams pellets (a) Before soaking, (b) After soaking, Unsteam pellets (c) Before soaking and (d) After soaking

Soaking experiment: Less weight increment caused by breaking of pellets during soaking experiment was due to unstable structure of pellets during water treatment. Stable pellets will absorb more water and could retain the water content without breaking up the pellets up to 10 min of soaking as illustrate in Fig. 2a and b. Unsteam pellets was broken immediately (Fig. 2c and d) after immersed in water and therefore unable to absorb water. However, steam pellets made better binding of starch during gelatinization to firm the structure pellets compared to unsteam pellets which the starch do not undergo gelatinization. Firm structure of steam pellets will not easily break during soaking (water fully absorbed into pellets) compared to unsteam pellets that break immediately after immersed into water because of the fragile structure.

Protein leaching test: The leaching protein concentration was increased when more protein from pellets leached into soaking water. Stable pellets had showed less leaching protein concentration and less different of leaching protein concentration in 10 min time. Steam pellets have more stable structure to avoid leaching of protein from pellet during soaking. The starch gelatinization that occurred made the structure of firm and provided good binding properties to the pellets. The firmness of steam pellets structure give less protein leaching compared to unsteamed pellets with more pores for water to fully absorb into pellets and drained out more protein into the water. According to Brown (2007), gelatinization had caused the starch granule to swell and leave a narrow space among pellets particles and hence prevent the protein from leaching out of the pellets.

Hardness or breaking force test: Stable pellets required more forces to break down compare to less stable pellets. Cooked starch have maximum gelatinization to enhance the firmness of the structure in steam pellets. Steam pellet required obviously more breaking force than unsteam pellets. Steam pellets have better stucture of binding during steaming process (heat transfer to the mash of moist pellets). After drying process, the firmness of pellets stucture is maintain to withstand force applied compared to unstable stucture of unsteam pellets than increase potential of breakness after drying process since all moisture was dried out and leaved the porous stucture of dry pellets.

CONCLUSION

A new earthworm-based fish pellets was formulated using earthworm powder, soybean waste, fishmeal, rice bran and tapioca flour using Pearson's square feed formulation to achieved 32% protein content. Steaming process was successfully stabilized the pellets through starch gelatinization with optimum steaming time and temperature responded on water stability. Tested physical properties such as soaking experiment, leaching protein test and hardness showed that steam pellets obviously more stable compared to unsteam pellets.

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REFERENCES

Ahmad Anas, N.G., D. Arbain and M.S. Ahmad, 2012. Effects of selected medium components for production of glucose oxidase by a local isolate *Aspergillus terreus* UniMAP AA-1. APCBEE Procedia, 2: 125-128.

An Phu Earthworm Farm, 2010. The benefit of earthworm. http://earthwormvietnam.com/?frame=news_detail&id=107.

Baud, B., P. Colonna, G. Della Valle and P. Roger, 1999. Macromolecular degradation of extruded starches measured by HPSEC-MALLS. Les Colloques de l'INRA, 91: 217-221.

Brown, A.C., 2007. Understanding Food: Principles and Preparation. 3rd Edn., Thomson Learning Incorporation, USA., ISBN-13: 9780495107453, Pages: 696.

Davidson, V.J., D. Paton, L.L. Diosady and G. Larocque, 1984. Degradation of wheat starch in a single screw extruder: characteristics of extruded starch polymers. J. Food Sci., 49: 453-458.

Dedeke, G.A., S.O. Owa and K.B. Olurin, 2010. Amino acid profile of four earthworms species from Nigeria. Agric. Biol. J. N. Am., 1: 97-102.

Fagbenro, O.A. and K. Jauncey, 1995. Water stability, nutrient leaching and nutritional properties of moist fermented fish silage diets. Aquacult. Eng., 14: 143-153.

Gonzalez, C. and G.L. Allan, 2007. Preparing Farm-Made Fish Feed. NSW Department of Primary Industries, Australia, ISBN: 9780734718020, Pages: 21.

Hardy, R., 1980. Fish Feed Formulation. In: Fish Feed Technology, FAO (Ed.). Chapter 15, Food and Agriculture Organization of the United Nations, Rome, Italy.

Hardy, R.W., 2000. New developments in aquatic feed ingredients and potential of enzyme supplements. Proceedings of the 5th International Symposium on Nutrition and Food Technology, November 19-22, 2000, Merida, Mexico, pp: 216-226.

MDoF., 2004. The condition of fishery industry in Malaysia. Annual Fisheries Statistic 2004. Malaysian Department of Fisheries. http://www.dof.gov.my.

MDoF., 2012. Scenario of Malaysian fishery industry 2012. Malaysian Department of Fisheries. http://www.dof.gov.my.

National Starch and Chemical Company, 1996. Food starch technology. http://eu.foodinnovation.com/pdfs/foodstarch.pdf

Royes, J.A.B. and F. Chapman, 2003. Preparing your own fish feeds. Series of Fisheries and Aquatic Sciences Circular 97, Florida Cooperative Extension Service, Institute of Food and Agriculture Sciences, University of Florida, USA.

Saalah, S., R. Shapawi, N.A. Othman and A. Bono, 2010. Effect of formula variation in the properties of fish feed pellet. J. Applied Sci., 10: 2537-2543.

Solomon, S.G., G.A. Ataguba and A. Abeje, 2011. Water stability and floatation test of fish pellets using local starch sources and yeast (*Saccahromyces cerevisae*). Int. J. Latest Trends Agric. Food Sci., 1: 1-5.

Tacon, A.G.J., M.R. Hasan and R.P. Subasinghe, 2006. Use of Fisheries Resources as Feed Inputs to Aquaculture Development: Trends and Policy Implications. FAO Fisheries Circular No. 1018. Food and Agriculture Organization of The United Nations, Romes.

Vassallo, P., A.M. Doglioli, F. Rinaldi and I. Beiso, 2006. Determination of physical behaviour of feed pellets in Mediterranean water. Aquacult. Res., 37: 119-126.

Vielma, J., T. Makinen, P. Ekholm and J. Koskela, 2000. Influence of dietary soy and phytase levels on performance and body composition of large rainbow trout (*Oncorhynchus mykiss*) and algal availability of phosphorus load. Aquaculture, 183: 349-362.

Zakaria, Z., N.H.M. Salleh, A.R. Mohamed, N.G. Ahmad Anas and S.N.A. Idris, 2012. Optimization of protein content in earthworm-based fish feed formulation in catfish (*Clarius gariepinus*). Sains Malaysiana, 41: 1071-1077.