Vitamin E dosage variations as antioxidants for improving the quality of fish oil derived from processing waste of Pangasius catfish

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Abstract: The limited availability of tubifex poses a challenge in obtaining green catfish fry. Fish oil, comprising saturated and unsaturated fatty acids, serves as an alternative fat source in commercial feed. Utilizing by-products from catfish processing, fish oil production has garnered attention; however, its rich unsaturated fatty acid content renders it prone to oxidation. Vitamin E emerges as a potential antioxidant to curb this oxidation. This study aims to evaluate fish oil quality with vitamin E supplementation. Peroxide number (PV) and storage duration (0, 3, 6, 9, 12, and 15 days) were analyzed. Fish oil received varying doses of vitamin E (0, 1, 3, 5, and 7 mg/100 g), with each treatment replicated twice. Findings indicated that without vitamin E (0 mg/100 g), PV reached 10.0 meq/kg after 15 days of storage. Conversely, with vitamin E (1, 3, 5, and 7 mg/100 g), PV values after 15 days were 7.2, 6.0, 5.4, and 4.0 meq/kg respectively. The study establishes that higher vitamin E doses effectively prolong fish oil shelf life while conforming to the Codex PV standard (≤ 5 meq/kg).

1. Introduction

The rapid development of aquaculture in this decade continues to face several challenges, including the limitation of adequate seed availability due to obstacles in providing live natural feed such as silkworms for green catfish larvae [1]. One alternative to address this issue is providing commercial feed enriched with imported fish oil. However, imported fish oil has a very high price tag [2,3,4]. The increase in the price of imported fish oil directly impacts the rise in feed costs. As a solution, efforts to replace imported fish oil can be undertaken by utilizing the processing waste of pangasius catfish, which currently has limited utilization, as a raw material for fish oil [5,6].

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Fish oil and fish meal are crucial sources of fatty acids in global fish feeds [7,8,9]. Nevertheless, the accessibility of fish oil and fish meal is progressively becoming restricted [10,11]. Hence, there is a need to discover novel alternative substances that can serve as fish oil sources within the aquafeed sector [12,13].

Pangasius catfish is a type of fish that has been cultivated on a large scale in Koto Mesjid Village, Kampar Regency, Riau Province. The yield reaches 36 tons monthly, as I know from personal communication with the head of the catfish processing group. The catch of catfish is processed into smoked fish products. Before processing, the entrails of the fish are removed, including the liver, spleen, intestine, belly fat, and other components.

Unfortunately, the stomach contents of this fish have not been utilized optimally as a source of additional income for fish processors. This condition has the potential to result in waste that can pollute the environment. One of the components of the stomach contents is mesenteric tissue, which has the potential as a raw material for fish oil.

The Pangasius catfish oil produced as a by-product of the smoked fish processing process contains various types of essential fatty acids. These fatty acids include palmitic, meristic, oleic, stearic, arachidic, and behenic, which fall into the saturated fatty acids (SFAs) category. In addition, this oil also contains a type of fatty acid that belongs to the group of monounsaturated fatty acids (MUFAs) and polyunsaturated fatty acids (PUFAs) [14, 15].

Fish oil has a tendency to oxidize faster due to its high saturated fatty acid content. This oxidation can be inhibited by using antioxidants, such as Butylated Hydroxyanisole (BHA), Butylated Hydroxytoluene (BHT), as well as vitamins C and E. This action helps extend the shelf life of fish oil. While it is important to add antioxidants to fish oil to maintain its quality, keep in mind that if fish oil is of poor quality and then added to fish feed, it can damage the overall quality of the feed.

The purpose of this study was to determine the best shelf life of fish oil produced from catfish processing waste with different dose variants of vitamin E. In this way, this study aims to find a balance between the addition of antioxidants and an effective dose of vitamin E so as to maintain the quality of the fish oil for a longer period of time without compromising the quality of the fish feed produced.

This kind of research is very relevant for the fisheries and fish feed industries because it can help increase the efficiency of using catfish processing waste and ensure that the final product is of optimal quality and has an adequate shelf life.

2. Material and Method

2.1 Materials and tools

This study utilized various materials, including the mesenteric fat segment of catfish, acquired as a by-product during the catfish processing. The chemicals employed encompassed vitamin E (Nature–e 300), glacial acetic acid, distilled water, sodium hydroxide (NaOH), starch, potassium iodide (KI), chloroform, and 0.01 N sodium thiosulfate.

2.2 Methods

The raw material for the oil used is fat derived from the belly of catfish, known as mesenteric fat. This fat is obtained from the waste of catfish processing in Koto Mesjid Village, Kampar Regency. To process this fat, the following steps are performed:

The raw material for the oil used is fat from the stomach of catfish, known as mesenteric fat. This fat is obtained from catfish processing waste in Koto Mesjid Village, Kampar Regency. The process of processing this fat involves the following steps: (1) Mesenteric fat is separated from the fish, then cleaned of all dirt and blood that may stick to it using tissue and a soft cloth. (2) Meenteric tissue (the part related to the stomach and intestines) is cut

into small sizes of around 1-1.5 cm. This cut is carried out to prepare the mesenteric tissue so that it is easier to process further.

The process of extracting oil from mesenteric fat is carried out in the following steps: (1) heating using an electric oven: mesenteric fat is heated using an electric oven at 70°C for one hour. This heating aims to melt the fat and allow the oil to escape from the network. (2) Filtration of Extracted Liquid: After the heating process, the extracted liquid containing oil is separated from tissue and dirt. The filtering process is carried out using a filter device to separate the oil from other components that may be present in the liquid. (3) Oil storage: Oil that has been successfully extracted and separated is put into a container or storage container. This step is important to keep the oil clean and ready for further analysis.

Each treatment involved 100 grams of catfish oil acquired through dry rendering. This oil was subsequently augmented with varying concentrations of vitamin E: 0.0 mg (control group), 1.0 mg, 3.0 mg, 5.0 mg, and 7.0 mg per 100 grams of oil. This experimental setup was duplicated twice. The catfish oil enriched with vitamin E was stored at room temperature for distinct durations: 1, 3, 6, 9, 12, and 15 days. The analytical parameter under scrutiny was the Peroxide Number [16].

The peroxide value was analyzed using the AOCS Cd-8b-90 method, which involved determining the peroxide number through the principle of iodine titration. In this method, the potassium iodide compound releases iodine when it reacts with the peroxide in the fish oil sample. The titration was carried out using a standard thiosulfate solution as the titrant, while a starch solution was used as an indicator. This method is capable of detecting various substances capable of oxidizing potassium iodide in an acidic environment.

Samples weighing 5 g were weighed and put into a 250 ml Erlenmeyer flask. Next, 30 mL of acetic acid and chloroform were added with a ratio of 3:2. Then, 0.5 mL of potassium iodide (KI) solution was added to the mixture. The solution is then shaken carefully to ensure good mixing. After that, 30 mL of distilled water (distilled water) was added.

The next step involved titrating the solution using 0.01 N sodium thiosulfate (Na2S2O3) solution until the color of the solution changes to yellow. After that, 0.5 mL of 1% starch indicator solution was added to the solution, which would change the color of the solution to blue. The titration was continued while continuing to shake the solution until the color of the solution changed to light blue, indicating the release of iodine from the chloroform layer. The titration is continued carefully until the blue color in the solution disappears, indicating the end point of the titration. Peroxide value calculation is done by using the following equation [16]:

$$Peroxide\ Number = \frac{S \times M \times 1000}{Sample\ Weight\ (g)}$$

where:
S : total sodium thiosulfate (mL)
M: sodium thiosulfate concentration (0.01 N)

3. Result and discussion

The crude oil extracted from the mesenteric fat of catfish has a distinctive brownish-yellow color and a specific fishy aroma. In addition, there is also a clear precipitate in the oil. This characteristic can be seen from the striking brownish-yellow color and distinctive aroma [15, 17, 18].

The oil's color and degree of turbidity are affected by several factors, including the free fatty acid content, the type and amount of adsorbent used, temperature, and the duration of

the extraction process. The crude oil yield extracted from this process reached 58.75% [18]. The peroxide value (meq/kg) of unrefined catfish oil has been detailed in Table 1.

Table 1. Peroxide value of crude catfish oil with different concentrations of vitamin E (n = 2)

Concentration of vitamin E (mg/100g)	**Peroxide Value (meq/kg)**					
	Observation (day)					
	0	3	6	9	12	15
0	0	0	6.40 ± 0.00	8.80 ± 0.00	9.20 ± 0.00	10.0 ± 0.00
1	0	0	4.80 ± 0.03	5.40 ± 0.00	7.20 ± 0.00	7.20 ± 0.00
3	0	0	3.80 ± 0.03	4.00 ± 0.03	5.40 ± 0.02	6.00 ± 0.00
5	0	0	3.40 ± 0.03	3.60 ± 0.00	5.40 ± 0.02	5.40 ± 0.02
7	0	0	3.20 ± 0.00	3.40 ± 0.02	4.40 ± 0.00	4.50 ± 0.02

Table 1 presents the initial findings after storage for 0, 3, and 6 days of crude catfish oil. In both samples without vitamin E supplementation and those enriched with vitamin E as an antioxidant, the initial peroxide value (PV) registered as 0.0 meq/kg.

However, after a 9-day storage period, the catfish oil treated with a 0.0 mg dose demonstrated an escalation in peroxide value, reaching 6.4 meq/kg. In contrast, the samples treated with vitamin E exhibited peroxide values of 3.2, 3.6, 3.0, 2.0, and 3.6 meq/kg, respectively, corresponding to the given vitamin E doses.

On the 15th day, the control group (0.0 mg vitamin E) witnessed an inclination towards an increase in peroxide value. Conversely, as the vitamin E dosage increased, the peroxide value decreased, consistently adhering to the Codex standard, which stipulates a peroxide value of ≤ 5 meq/kg.

In this study, catfish crude oil was treated with the addition of bentonite at different doses, namely 1.0%, 4.0%, and 7.0%. The results showed that the oil had a peroxide value of 0.0 meq/kg (undetectable) and was not stored [17].

Furthermore, in this study, catfish crude oil, which was the control group and was not given vitamin E on day 0, also had a peroxide value of 0.0 meq/kg. However, after nine days of storage, there was an increase in the value of the peroxide value. However, the higher the dose of vitamin E given, the lower the peroxide value tends to decrease. The decrease in peroxide value at treatment with doses of 3 mg/100 g, 5 mg/100 g, and 7 mg/kg can be explained by the ability of vitamin E to reduce fat oxidation products, such as peroxides, aldehydes, and ketones, in these oils [18]. The Pangasius catfish oil, whose Peroxide number will be measured, is shown in Figure 1.

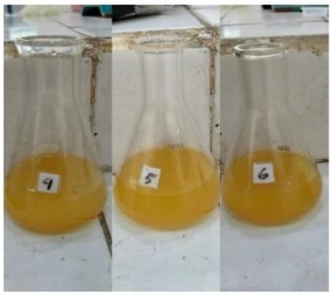

Fig.1. The Pangasius catfish oil.

Noted: 4 = Added of vitamin E 3 mg/100 g fish oil; 5 = Added of vitamin E 5 mg/100 g fish oil; 6 = Added of vitamin E 7 mg/100 g fish oil

4. Conclusions

The characteristics of crude catfish oil extracted from mesenteric fat include a distinctive brownish-yellow hue, a specific fishy aroma, and the presence of visible sediment.

In the control group (without vitamin E supplementation), the peroxide value displayed an increasing trend after 15 days of storage, reaching 10.0 meq/kg. Conversely, when administering a dose of 7 mg vitamin E per 100 g of oil, the peroxide value only escalated to 4.5 meq/kg. The outcomes of this study affirm that a higher vitamin E dosage correlates with an extended shelf life of catfish oil, maintaining oil quality in accordance with Codex standards, specifically a peroxide value ≤ 5 meq/kg.

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