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The changes in the physical and chemical quality of catfish oil (*Pangasius* sp.) with rice bran oil addition during room temperature storage

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Abstract

Catfish oil (CFO) is an oil product that can be obtained through catfish extraction by the dry rendering method and followed by a purification procedure. Fish oil has a high content of unsaturated fatty acid and is susceptible to oxidation and hydrolysis processes leading to a decrease in physical and chemical quality. Rice bran oil (RBO) which is rich in phenolic acid has a good antioxidant potential to inhibit the deterioration of fish oil during storage. This study aimed to determine the effect of the CFO and the RBO ratio on the physical and chemical quality of the CFO with the RBO addition during room temperature storage. This research used a completely randomized design with one factor. Data were analyzed by one-way analysis of variance method and continued by Duncan's Multiple Range Tests ($p<0.05$). The ratio of CFO:RBO was 100:0, 90:10, 80:20, 70:30 and 60:40. Data were taken at storage times of weeks 0, 2, 4 and 6. Based on the results obtained, the ratio of 60(CFO):40(RBO) at week 0 has the best physical and chemical quality. It can be concluded that the addition of RBO on CFO effectively inhibits the hydrolysis and oxidation damage up to 6 weeks of room temperature storage.

1. Introduction

Generally, most of the processing of catfish (*Pangasius* sp.) focuses on producing food products such as meatballs, nuggets, shredded fish, sausages, smoked fish and many more. Catfish has thick meat and savory taste, so it is starting to be widely used in food production. Several studies and counseling have also begun to explain different types of processing food products made from catfish (Oktavianawati and Palupi, 2017; Rifa'i *et al.,* 2021). Naturally, processing food products of catfish will produce waste that can lead to environmental pollution. Therefore, a lot of studies were conducted to solve that problem by processing the waste into high-value products such as catfish oil. The high-fat content of the catfish by-product is very profitable in oil production so the produced yield is in large quantities (Hastarini, 2012).

The fat content in a whole catfish is high so that oil production can be carried out not only in the waste part. The fat content in catfish is higher (40%) than in other freshwater fish (Lestari, 2010). Several species of catfish were analyzed by (Hashim *et al*., 2015) and showed a crude fat content in a range of 24.5% to 37.7%. The high -fat content greatly facilitates oil production resulting in high oil yields. Oil production can be carried out through several extraction methods. The easiest and most effective method is dry rendering. Dry rendering is an extraction method that uses heating treatment without water addition and then continues with the pressing step to separate the oil and other compounds (Fitriani *et al*., 2021). The heating treatment directly affects the protein compound and leads to protein and cell membrane damage, so deposited fat more easily comes out and is extracted (Nugroho *et al*., 2014).

The fish oil production through dry rendering will produce crude fish oil that consists of a lot of impurities and undesirable compounds, therefore purification stage can be done to improve the oil quality. The major steps in fish oil purification (refining) are degumming, neutralizing, bleaching and deodorizing. These steps can be carried out in combination or individually, and adjusted to the needs of the final oil product (Chakraborty and Joseph, 2015). The refinement process aimed to separate the phospholipids by degumming and then continued with a neutralizing step to decrease the acidity. The bleaching step is carried out to remove the

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unwanted color or clear the oil color. Fish oil has a unique flavor and tends to have a fishy smell. Therefore, a deodorizing step could be carried out to remove an unpleasant smell but the refining process can be done without deodorizing step (Simat *et al*., 2019).

Like other fish oil, catfish oil is known to be rich in unsaturated fatty acids. Lestari *et al*. (2020) explained that catfish oil has 35.48% of monounsaturated fatty acids and 11.91% of polyunsaturated fatty acids at 80°C of extraction temperature. These characteristics are widely known to be susceptive to lipid oxidation. Lipid oxidation is an oxidative deterioration process that causes rancidity, loss of nutritional value, and decreased food quality as well as food safety (Ladikos and Lougovois, 1990). The antioxidant addition is used to stabilize and inhibit oxidative damage in oil products (fish oil is no exception). However, antioxidant addition is not always effective in inhibiting oxidative damage. Moreover, if the oil has a high concentration of metals. In general, the addition of antioxidants showed good results in oil oxidative stability, even in small amounts. Further studies are needed to conduct because a lot of antioxidants can be pro-oxidant at a higher concentration (Simat *et al*., 2017).

Rice bran oil is known as a natural source of tocols and oryzanol which are known as antioxidant compounds. The highest γ-oryzanol was found in rice bran oil as well as tocopherol and tocotrienol. Overall, most in vitro antioxidant assay results of rice bran oil have exhibited good results and indicated that rice bran oil could be used as an antioxidant agent (Pengkumsri *et al*., 2015). The ratio of rice bran oil addition to catfish oil will affect the quality of physical and chemical characteristics of catfish oil during room temperature storage. The main aim of this work was to investigate the effect of the percentage ratio of catfish oil: rice bran oil on the physical (slip melting point, specific gravity, and color) and chemical (thiobarbituric acid value, peroxide value, free fatty acid, and total phenolic content) quality of the catfish oil with the rice bran oil addition during room temperature storage.

2. Materials and methods

2.1 Materials

This study used raw materials such as fresh catfish (patin fish) of 45 cm in length or more. Catfish were obtained from the fish distributor in Pajang, Central Java, Indonesia. The rice bran oil was obtained from Oryza Grace Brand produced by Kasisuri Co. Ltd, Thailand, that was purchased from PT. Indoglobal Distribusi Nusantara, Karanganyar, Central Java, Indonesia. All chemicals were analytic grade from Merck.

2.2 Catfish oil extraction

Catfish oil extraction by dry rendering method was conducted referring to Suseno *et al.* (2020) with modifications. The fresh catfish was cleaned and separated from the viscera. Then, a cleaned catfish was cut and heated using an oven at 90°C for 2 hrs. The oil and liquid that came out from oven heating were separated by a filter cloth and collected in an Erlenmeyer flask. The heated catfish was pressed by the manual press and the yield which contains crude catfish oil and other residue was immediately refined in the next procedure.

2.3 Refining of crude catfish oil

The refining procedure was conducted according to Ayu *et al*. (2019) with modifications. The crude catfish oil was centrifugated for 10 mins at 1000 rpm. The upper layer was heated at 65°C and 250 rpm for 1 min. Then, 3% of citric acid and 3% of the sample were added to the oil. The pH was tested to ensure the pH value was below 7. Next, the mixture was held at room temperature for 5 mins. 9.5% NaOH was added to about 0.5-0.7% from the sample weight at 65°C and 250 rpm for 20 mins. The pH was re-tested to ensure the pH value was at the neutral point. The mixture was held at room temperature for 5 mins. The active carbon was added to about 7% of the sample weight to the mixture and stirred at 250 rpm and 65°C for 20 mins. Then, the mixture was held at room temperature for 5 mins and centrifugated at 1000 rpm for 10 mins. The top layer (refined fish oil) was stored at 5°C until further procedures.

2.4 Catfish oil and rice bran oil blending

Two kinds of oils (catfish oil and rice bran oil) were blended following the method by Purnamayati *et al.* (2019) with modifications. The formulation ratio of catfish oil: rice bran oil was: 100:0, 90:10, 80:20, 70:30, and 60:40. The mixture of catfish oil and rice bran oil was stirred by a magnetic stirrer at 400 rpm for 30 mins at room temperature. The mixture was stored in a transparent plastic bottle for 6 weeks and analyzed in week 0, week 2, week 4 and week 6.

2.5 Characterization of physical and chemical quality

Characterization of physical and chemical quality of the mixture of catfish oil and rice bran oil involving several analyses: specific gravity (AOCS Cc 10c-95, 2017), slip melting point (AOCS Cc-3-25, 1997), color through Lovibond Tintometer (AOCS, 1989), free fatty acid content (AOAC Cd 8-53, 1996), peroxide value (AOAC Ca 5a-40, 1996), thiobarbituric acid reactive substances (Zeb and Ullah, 2016) and total phenolic content by Folin-Ciocalteu method (Senter *et al*., 1989).

2.6 Statistical analysis

This research used a completely randomized design (CRD) with one factor, that is the ratio of catfish oil: rice bran oil (100:0, 90:10, 80:20, 70:30, 60:40) and analyzed at weeks 0, 2, 4 and 6. Data were analyzed using IBM SPSS Statistic ver. 23 by the one-way analysis of variance method and continued by Duncan's Multiple Range Test (DMRT) ($p<0.05$) if there is a significant difference result.

3. Results and discussion

3.1 Physical characteristics of the catfish oil: rice bran oil mixture

This study result showed that the addition of rice bran oil during 6 weeks of room temperature storage affected the slip melting point results as presented in Table 1. The higher the addition of rice bran oil, the lower the slip melting point of the mixture. Samples M01, M21, M41, and M61 which contain full of catfish oil has a higher slip melting point while samples M05, M25, M45, and M65 with a higher rice bran oil ratio has a lower slip melting point. This result is in accordance with Aslam *et al*. (2020), who showed the addition of vegetable oil which is rich in unsaturated fatty acids to other types of fat/oil would decrease the slip melting point. Blending two types of fat/oil will affect the slip melting point because of the difference in the atom carbon number and the degree of saturation. The results of this study showed that the increase in storage time resulted in an increase in the slip melting point. Data analysis from week 0 has the lowest slip melting point results, while data from week 6 has the highest slip melting point results. This happens as a result of unsaturated fatty acids having a lower melting point because the cis double bonds' presence triggers bend forming in the hydrocarbon chain and cause weak interaction among atoms so that the melting point will be lower than the saturated one (Siram *et ali.,* 2019). The storage time is directly affected to a degree of saturation that naturally the longer storage time will reduce the unsaturated fatty acids (Gupta *et al*., 2022). The slip melting point of catfish oil in this study was in the temperature range of 31-46°C and according to Lestari *et al*. (2020), the slip melting point of catfish oil was 37°C at the extraction temperature of 80°C.

The addition of rice bran oil during 6 weeks of room temperature storage affected the specific gravity of the oil mixture. The higher the addition of rice bran oil, the greater the specific gravity. The results also showed that the longer the storage time, the greater the specific gravity. Sample M01 has the lowest specific gravity (0.905 g/mL) and sample M65 has the highest specific gravity (0.933 g/mL). It is presumed that there is a

Table 1. The physical characteristics of the catfish oil: rice bran oil mixture in each analysis week.

Sample	Slip Melting Point (°C)	Specific gravity (g/mL)	Color	
			Red	Yellow
M01	31.0-42.0	0.905 ± 0.002^{a}	-6.625 ± 0.982^{h}	21.720 ± 0.173^{o}
M02	31.0-42.0	0.907 ± 0.001^{b}	-6.570 ± 0.242^{h}	21.335 ± 0.618^{o}
M03	30.0-41.0	0.908 ± 0.004^{bc}	-7.335 ± 0.121^{fg}	20.660 ± 0.162^{n}
M04	29.0-41.0	0.909 ± 0.001^{c}	-7.600 ± 0.092^{ef}	8.700 ± 0.000^{f}
M05	28.0-38.0	0.913 ± 0.002^{d}	-8.620 ± 0.300^{b}	7.845 ± 0.029^{de}
M21	32.0-43.0	0.918 ± 0.004^{ef}	-6.640 ± 0.023^{h}	12.800 ± 0.254^{i}
M22	31.0-42.0	0.921 ± 0.002^{gh}	-7.615 ± 0.017^{e}	9.785 ± 0.248^{g}
M23	31.0-41.0	0.921 ± 0.001^{gh}	-8.085 ± 0.375^{d}	9.075 ± 0.064^{f}
M24	29.0-41.0	0.922 ± 0.001^{h}	-8.380 ± 0.058^{bc}	8.065 ± 0.064^{e}
M25	28.0-38.0	0.930 ± 0.001^{j}	-8.655 ± 0.064^{b}	7.000 ± 0.115^{c}
M41	32.0-43.0	0.917 ± 0.001^{e}	-7.215 ± 0.214^{g}	18.430 ± 0.404^{m}
M42	32.0-42.0	0.917 ± 0.000^{ef}	-7.510 ± 0.242^{ef}	16.585 ± 0.225^{l}
M43	31.0-41.0	0.918 ± 0.000^{ef}	-8.165 ± 0.006^{cd}	16.485 ± 0.064^{l}
M44	29.0-41.0	0.921 ± 0.000^{gh}	-8.470 ± 0.023^{b}	7.675 ± 0.468^{de}
M45	28.0-38.0	0.935 ± 0.001^{k}	-9.220 ± 0.358^{a}	7.440 ± 0.808^{d}
M61	33.0-46.0	0.919 ± 0.000^{fg}	3.920 ± 0.069^{l}	15.720 ± 0.196^{k}
M62	32.0-45.0	0.926 ± 0.001^{i}	2.630 ± 0.173^{k}	15.020 ± 0.185^{j}
M63	31.0 45.0	0.927 ± 0.001^{i}	2.195 ± 0.052^{j}	11.750 ± 0.042^{h}
M64	30.0-42.0	0.929 ± 0.001^{j}	2.060 ± 0.046^{j}	6.240 ± 0.069^{b}
M65	29.0-41.0	0.933 ± 0.002^{k}	1.640 ± 0.196^{i}	5.400 ± 0.023^{a}

Values are presented as mean±SD. Values with different superscripts are statistically significantly different ($p<0.05$).
Sample code: M is the mixture of CFO and RBO; 2nd digit is the storage week where 0 = week 0, 2 = week 2, 4 = week 4 and 6 = week 6; 3rd digit is the ratio CFO: RBO where 1 = 100:0, 2 = 90:10, 3 = 80:20, 4 = 70:30, and 5 = 60:40.

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difference in the specific gravity of catfish oil and rice bran oil so the number of carbons will increase along with the specific gravity. The presence of oxidation also causes the decrease in oil quality during room temperature storage and leads to an increase in oil specific gravity (Kumar *et al*., 2020).

Analysis of color by the L* a* b* color system explained that the Aa* (positive) indicates red, while Aa* (negative) indicates green values. The Ab* (positive) indicates a yellow value, while Ab* (negative) indicates blue values (Ly *et al*., 2020). The yellowness analysis of this study exhibited the positive results of all the samples which indicated the yellow value of the oil mixture color. Samples M01, M21, M41, and M61 showed higher results than others each week. The higher the rice bran oil addition, the lower the Ab* result. The redness analysis of this study exhibited that weeks 0, 2, and 4 have negative Aa* results which indicated green values of the oil mixture color. During week 6 storage, the Aa* analysis had a positive result that indicated the red values of the oil mixture. Samples M01, M21, and M41 had lower results than others in each week, while sample M61 showed a higher result than others in each week. Both redness and yellowness in this study were significantly affected by the addition of rice bran oil. According to Sayyad and Ghomi (2017), several factors affect the color result such as the degradation of hemoglobin as well as the release of haem pigment in greater amounts during extraction.

3.2 Chemical characteristics of the catfish oil: rice bran oil mixture

This study showed that the addition of rice bran oil significantly affected the peroxide value, free fatty acid (FFA) content, thiobarbituric acid (TBA) value, and total phenolic content (TPC) results as presented in Table 2. The peroxide value was not detected in week 0 of storage. Samples M21, M41 and M61 showed higher peroxide values than samples M25, M45 and M65 in each analysis week. The peroxide value seemed to decrease along with the addition of rice bran oil but increased along with the length of storage time. It is presumed that rice bran oil addition succeeds in inhibiting oil oxidation. The oxidation may occur during the storage through initiation and propagation of the lipid oxidation stage and contributes to the peroxides forming that allows the peroxide value to the maximum level. The triglyceride free radical may react with the oxygen and generate the peroxy radicals which can react with another triglyceride leads to a hydroperoxide and new free radical forming. These unstable hydroperoxides may break down into secondary oxidation products that produce rancidity and unpleasant flavors (Ayala *et al*., 2014). The antioxidant activity found in rice bran oil is expected to inhibit oxidation which is indicated by

Table 2. The chemical characteristics of the catfish oil: rice bran oil mixture in each analysis week.

Sample	Peroxide value (mEq/kg)	Free fatty acid content (%)	Thiobarbituric acid (mg malonaldehyde/kg)	Total phenolic content (%)
M01	0±0^{a}	0.689±0.019^{c}	0.185±0.003^{c}	1.622±0.029^{g}
M02	0±0^{a}	0.598±0.032^{b}	0.171±0.008^{c}	4.870±0.058^{j}
M03	0±0^{a}	0.481±0.004^{a}	0.114±0.001^{b}	7.538±0.065^{n}
M04	0±0^{a}	0.454±0.005^{a}	0.067±0.002^{a}	10.186±0.007^{p}
M05	3.838±0.032^{e}	1.033±0.019^{h}	0.655±0.017^{i}	0.035±0.000^{a}
M21	3.489±0.303^{d}	1.018±0.006^{h}	0.611±0.007^{h}	1.515±0.050^{f}
M22	3.294±0.250^{d}	0.890±0.065^{fg}	0.552±0.014^{g}	4.333±0.101^{i}
M23	2.862±0.001^{c}	0.821±0.050^{e}	0.406±0.019^{e}	7.300±0.051^{m}
M24	1.935±0.007^{b}	0.732±0.062^{cd}	0.303±0.012^{d}	10.080±0.043^{o}
M25	7.644±0.084^{k}	1.374±0.012^{k}	0.763±0.008^{k}	0.022±0.000^{a}
M41	6.655±0.043^{i}	1.255±0.100^{j}	0.713±0.009^{j}	1.397±0.029^{e}
M42	5.479±0.131^{g}	1.097±0.005^{i}	0.694±0.001^{j}	4.146±0.043^{h}
M43	4.801±0.215^{f}	0.935±0.061^{g}	0.463±0.002^{f}	5.926±0.223^{k}
M44	3.807±0.094^{e}	0.829±0.019^{ef}	0.450±0.006^{f}	6.751±0.021^{l}
M45	10.046±0.388^{l}	1.405±0.003^{k}	1.358±0.064^{m}	0.010±0.000^{a}
M61	7.324±0.470^{j}	1.278±0.084^{j}	0.895±0.046^{l}	0.210±0.014^{b}
M62	6.700±0.026^{i}	1.102±0.005^{i}	0.885±0.044^{l}	0.316±0.022^{c}
M63	6.247±0.147^{h}	1.069±0.045^{hi}	0.652±0.033^{i}	0.672±0.029^{d}
M64	5.532±0.158^{g}	0.843±0.001^{ef}	0.627±0.043^{hi}	1.428±0.022^{ef}
M65	29.0-41.0	0.933±0.002^{k}		

Values are presented as mean±SD. Values with different superscripts are statistically significantly different ($p<0.05$).
Sample code: M is the mixture of CFO and RBO; 2nd digit is the storage week where 0 = week 0, 2 = week 2, 4 = week 4 and 6 = week 6; 3rd digit is the ratio CFO: RBO where 1 = 100:0, 2 = 90:10, 3 = 80:20, 4 = 70:30, and 5 = 60:40.

decreasing the peroxide value. Ali *et al*. (2019) also showed that the addition of rice bran oil to soybean oil significantly slowed the increment of peroxide value. Rice bran extract showed moderate radical scavenging activity at a low concentration (50 µg/mL). This antioxidant potential may occur by donating hydrogen atoms or free electrons to inhibit or slow down the initiation of lipid oxidation (Olanguju *et al*., 2022).

Similarly to peroxide value, FFA content is a result of fat/oil oxidative degradation. Samples M01, M21, M41, and M61 showed a higher FFA content while samples M05, M25, M45, and M65 showed a lower FFA content than others in each analysis week. The longer the storage time, the higher the FFA content. The results also showed that the higher the addition of rice bran oil, the lower the FFA content. The free fatty acid value indicated that oxidation and hydrolysis have occurred in the oil. The result obtained in this study concurred with those reported by Olanguju *et al*. (2022), who reported that the rice bran extract addition to soybean oil effectively decreased the FFA value which indicated the rice bran extract has an anti-hydrolytic effect that could decrease the FFA.

Samples M01, M21, M41, and M61 showed a higher TBA value while samples M05, M25, M45, and M65 showed a lower TBA value than others in each analysis week. These results indicated that rice bran oil addition succeeds in decreasing the TBA value. The data result also showed that the longer the storage time, the higher the TBA value. The TBA also is a result of secondary lipid peroxidation products that usually increase during heating or storage treatment. The results obtained in this study are in agreement with Ali *et al*. (2019), who reported that the addition of rice bran oil effectively decreased the TBA value. This happens as a result of volatilization of secondary lipid peroxidation products as well as their further breakdown which indicates good stability of oxidative.

Samples M01, M21, M41, and M61 showed the lowest TPC than others in each analysis week. The data result from week 6 has the lowest TPC, which means the longer the storage time, the lower the TPC results. These results also explained that the higher the addition of rice bran oil, the higher the TPC results. Total phenolic content results are related to the composition of phenolic compounds on the rice bran in general. Jung *et al*. (2017) reported that the total phenolic content of rice bran was 156.08 mg gallic acid equivalents/g with high radical scavenging activity (71.30%) as well as the γ-oryzanol content as much as 294.77±6.74 mg/100 g. Thus, rice bran is considered to have the potential to be used as a source of antioxidants to inhibit the oil/fat oxidation process.

Fish oil standards in Indonesia are regulated through SNI 8467:2018 about refined fish oil. This regulation is declared that good fish oil characteristics are: <5 mEq/kg of peroxide value, iodine value >120 m/iod, acid value <20 mg KOH/g, anisidine value <20 mEq/kg, and total oxidation <26 APM/g (National Standardization Agency of Indonesia, 2018). International Fish Oil Standards regulations are the peroxide value <5 mEq/kg, p-anisidine value <20 mEq/kg, FFA content <1.5%, and total oxidation <26 mEq/kg (CODEX, 2017). Therefore, all of the oil mixtures of this study have the oxidative value (FFA content, peroxide value, and TBA value) that meets the standard. Based on the effectiveness index by De Garmo analysis showed that the highest score was found in the week 0 treatment (0.81). Therefore, the week 0 treatment was determined as the best treatment with the lowest hydrolytic-oxidative values, the highest total phenolic content, and the best physical characteristics.

4. Conclusion

The addition of rice bran oil into catfish oil significantly affected the physical and chemical quality of catfish oil and was proven through several analyses during 6 weeks of room temperature storage as follows: slip melting point, specific gravity, color, free fatty acid content, peroxide value, thiobarbituric acid value, and total phenolic content. Rice bran oil seemed potentially used as an antioxidant agent that inhibits the catfish oil oxidation at a ratio (CFO: RBO) = 60: 40. This ratio has a great total phenolic content with a low free fatty acid, peroxide value, and thiobarbituric acid. Week 0 has the best characteristic results because there has not been a significant change in the physical and chemical quality of the oil mixture. However, all the analysis results obtained in this study followed the standards and referred references so the oil mixture quality remains good until the 6th week of storage.

Conflict of interest

The authors declare no conflict of interest.

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