The effect of iron powder as oxygen absorber active packaging on fish oil total oxidation value

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Abstract. The high content of unsaturated fatty acids causes fish oil can be easily damaged by oxidation. Direct oxygen contact with food is the initial stage of food damage by oxygen. The use of active packaging aims to extend the shelf life, maintain the sensory and quality of the packaged material. Oxygen absorber commonly used in the market is iron powder. This study aims to determine the potential use of iron powder as an active oxygen absorbent material in the packaging in reducing the total value of oxidation of fish oil during the storage period. This study used a completely randomized design with 6 treatments. Fish oil stored in a Polyethylene Terephthalate packaging bottle and placed in a refrigerator at 23°C for 21 days. The addition of iron powder on fish oil packs is 2.7 grams, 5.4 grams, 8.1 grams, and 10.7 grams with negative controls in the form of pure fish oil and positive control in the form of packaged fish oil. The results showed that the addition of 10.7 grams of iron powder as an oxygen absorber to the active packaging of fish oil had a greater ability to reduce the total value of fish oil oxidation during storage (P <0.05).

1. Introduction

The use of packaging in food products initially only aims to wrap and protect food from external contamination [1]. Food packaging continues to grow as a response to consumer needs and the trend of the food industry to food products that use less preservative, fresh, long shelf life and maintained quality [2].

Active packaging aims to extend the shelf life and maintain or even improve the quality of packaged food products [3]. Active packaging is added to help improve packaging capabilities in maintaining quality, safety and sensory aspects of foodstuffs [4]. Active ingredients added to the packaging material, on the packaging surface, in multilayer structures or into special elements which will later be included in food packaging such as sachets, labels and bottle caps [2]. According to Mills [5], oxygen is one of the main factors causing damage to food. The high content of unsaturated fatty acids (Polyunsaturated Fatty Acid) causes fish oil to be easily damaged by oxidation, resulting in the decreased shelf life of fish oil products [6]. Damage to fish oil products due to direct contact with oxygen is expected to be reduced using oxygen absorber based packaging on fish oil packaging.
The active ingredient that is commonly applied as an oxygen absorber on active packaging is iron powder [7]. The application of 0.3 grams of iron powder reacted with metal halide and alkaline earth metal can absorb the entire remaining oxygen contained in a 500 cc capacity with oxygen content up to 0.0041 moles [8]. Based on this theory, it is necessary to measure the total oxidation value in fish oil, which in the packaging is added to the substance of iron powder as an oxygen-absorbing active ingredient.

2. Material and methods

2.1 Research materials

This research was conducted at the Chemistry and Analysis Laboratory and Microbiology Laboratory of the Faculty of Fisheries and Marine Airlangga University in July to August 2018. The materials used were samples of Scott's Emulsion packaged fish oil produced by PT. Combiphar, packaged iron powder (Fresh One®) produced by PT. Sigmaco Saksama Image, pure fish oil obtained from PT. Blambangan Foodpackers Indonesia. The tools used include plastic Polyethylene Terephthalate (PET) bottles, analytic balance (pioneer ohaus) with an accuracy of 0.0001 grams, burette (Duran Schott), fume hood (FH-120G type), refrigerators (GEA Pharmaceutical), and spectrophotometer (Human x-ma 1200).

2.2 Experimental design

This study used a completely randomized design (CRD), 6 treatments with each treatment having 7 replications. Treatment A is packaged fish oil without the addition of iron powder on the packaging, Treatment B is pure fish oil without the addition of iron powder on the packaging, Treatment C is pure fish oil with the addition of 2.7 grams of iron powder on the packaging, Treatment D is pure fish oil with addition 5.4 grams of iron powder on the packaging, Treatment E is pure fish oil with the addition of 8.1 grams of iron powder on the packaging, and Treatment F is pure fish oil with the addition of 10.8 grams of iron powder on the packaging. The treatment unit for fish oil is each contained in a 200 ml dark Polyethylene Terephthalate (PET) bottle and placed in the refrigerator at 23°C for 21 days.

2.3 Acid value analysis

Acidity values were analyzed based on the AOCS Ca 5a-40 method [9]. Determination of the degree of acidity is carried out by titrating hydroxide (KOH) against the sample using the principle amount of potassium hydroxide (KOH) needed (mg) to neutralize 1 gram of fat.

\[
\text{Acid Value} = \frac{V \times N \times K}{10 \times G}
\]

Information: 
V: KOH titration volume (ml) 
N: KOH normality (meq/l) 
K: KOH molecular weight (56.1) 
G: Sample weight (g)

2.4 Free fatty acid value analysis

Free fatty acids percentage was analyzed by the AOCS Ca 5a-4 method [9] by weighing 10 grams of oil then adding 25 ml of 96% alcohol, and heated in a water bath for 10 minutes at 40°C. Phenolphtalein is used as the indicator and titrated with 0.1 N KOH until a pink color appears [9].

\[
\% \text{FFA} = \frac{A \times N \times M}{10 \times G}
\]

Information: 
A: KOH titration volume (ml) 
N: KOH normality (meq/l) 
M: Molecular weight of dominant fatty acid (oleic acid) = 282.5 
G: Sample weight (g)
2.5 Total oxidation value analysis
Measurement of Total Oxidation Value (TOTOX) is analyzed with the Cd 18-90 AOCS method [9] with an equation.

\[
\text{Total Oxidation Value} = (2PV + p-AV)
\]

Information:  
PV : Peroxide Value (mEq/kg)  
PAV: p-Anisidine Value (mEq/kg)

Peroxide Values were analyzed using the AOCS Cd-8b-90 method [9], which determined peroxide numbers using the principle of titration of iodine released from potassium iodide compounds by peroxide using a standard solution of thiosulfate as titrant and starch solution as indicator. The equation of Peroxide Value analysis:

\[
\text{Peroxide Value} = \frac{S \times M \times 1000}{\text{Sample Weight (g)}}
\]

Information:  
S: Amount of sodium thiosulfate (ml)  
M: Sodium thiosulfate concentration (0,01 N)

Measurement of p-Anisidine Value was analyzed by the AOCS Cd 18-90 method [9]. Testing of p-Anisidine Value is by measuring two absorbance values from two different test solutions.

\[
\text{p-Anisidin Value} = \frac{25 \times (1,2 \times A2 - A1)}{G}
\]

Information:  
A1: Absorbance value of solution 1  
A2: Absorbance value of solution 2  
G: Sample mass in solution 1 (1 g)

2.6 Total plate count analysis
Total Plate Count analysis is a quantitative method used to determine the number of microbes in a sample. Total Plate Count is determined by calculating the number of microbes in a product that grows on the agar medium with the specified temperature and incubation time [10]. Calculation of Total Plate Count using the common media Plate Count Agar. The number of colonies that grow is calculated by the number of microbial Colony Forming Units.

\[
N = \frac{\sum C}{[(1 \times n_1) + (0,1 \times n_2)] x (d)}
\]

Information:  
N : Total number of colonies (ml/gram)  
\(\sum C\): Total number of colonies in all plates  
n_1: Number of plates in the first dilution  
n_2: Number of plates in the second dilution  
d: Dilution rate o from the first calculated plates

3. Result and discussion
The quality of samples fish oil was measured before being given the treatment of adding the iron powder to the packaging are shown in Table 1. Analysis of the Peroxide Value, p-Anisidine Value, and Total Oxidation Value are shown in Table 2.
Table 1. Quality of Samples Fish Oil

| Fish Oil | Peroxide Value | p-Anisidine Value | Total Oxidation Value | Acid Value | Free Fatty Acid Value |
|----------|----------------|-------------------|-----------------------|------------|-----------------------|
| Pure     | 13.32±0.11     | 17.63±0.19        | 44.26±0.29            | 0.11±0.01  | 0.54±0.05             |
| Packaged | 2.10±0.20      | 3.38±0.30         | 7.58±0.43             | 0.11±0.01  | 0.54±0.06             |

Table 2. Peroxide Value, p-Anisidine Value and Total Oxidation Value

| Treatments | Peroxide Value | p-Anisidine Value | Total Oxidation Value |
|------------|----------------|-------------------|-----------------------|
| A          | 3.38±0.36      | 3.97±0.47         | 10.74±0.44            |
| B          | 15.32±0.30     | 53.24±0.49        | 83.88±0.82            |
| C          | 21.79±0.28     | 29.50±0.27        | 73.17±0.56            |
| D          | 27.88±0.26     | 23.01±0.38        | 78.78±0.40            |
| E          | 5.20±0.47      | 18.28±0.43        | 28.69±0.90            |
| F          | 11.54±0.26     | 17.74±0.45        | 40.81±0.72            |

*) Notation indicated by different superscript letters on the table shows significant differences (p <0.05)

Homogeneity test on the analysis of Peroxide Value (0.089>0.05), p-Anisidine Value (0.855>0.05), and Total Oxidation Value (0.146>0.05) showed that the groups of data tested were homogeneous and the population variance was homogenous. The results of the analysis using variance showed that the addition of iron powder had a significant effect on the Peroxide Value, p-Anisidine Value, and the Total Oxidation Value in fish oil. Data analysis of Acid Value and Free Fatty Acid Percentage are shown in Table 3.

Table 3. Acid Value and Free Fatty Acid Value

| Treatments | Acid Value (mg KOH/gram fish oil) | Free Fatty Acid (%) |
|------------|-----------------------------------|---------------------|
| A          | 0.10±0.003                        | 0.46±0.02           |
| B          | 0.10±0.004                        | 0.52±0.02           |
| C          | 0.10±0.0005                       | 0.49±0.02           |
| D          | 0.09±0.0005                       | 0.45±0.02           |
| E          | 0.08±0.0004                       | 0.39±0.02           |
| F          | 0.07±0.0004                       | 0.37±0.02           |

*) Notation indicated by different superscript letters on the table shows significant differences (p <0.05)

The homogeneity test on the analysis of Acid Value (0.253>0.05) and Free Fatty Acid Value (0.505>0.05) showed that the groups of data tested were homogeneous, and the population variance was homogenous. The results of the analysis using variance showed that the addition of iron powder had a significant effect on Acid Value and Free Fatty Acid Value. Total Plate Count in pure fish oil and packaged fish oil after storage treatment has a mean value that is not significant and tends to be the same.
Table 4. Total Plate Count Analysis

| Treatments | Replication 1 | Replication 2 | Replication 3 | Total | Average |
|------------|--------------|--------------|--------------|-------|---------|
| A          | 4.72         | 4.85         | 4.88         | 14.46 | 4.82    |
| B          | 4.84         | 4.78         | 4.63         | 14.25 | 4.75    |
| C          | 4.83         | 4.66         | 4.79         | 14.28 | 4.76    |
| D          | 4.87         | 4.74         | 4.64         | 14.25 | 4.75    |
| E          | 4.88         | 4.77         | 4.68         | 14.33 | 4.78    |
| F          | 4.70         | 4.68         | 4.85         | 14.23 | 4.74    |
| Average    | 85.80        |              |              |       | 28.60   |

*) Total Plate Numbers Unit in CFU / ml

Based on the results of the analysis Total Plate Count. The treatment of the addition of iron powder on the packaging does not affect the Total Plate Count of fish oil because of the value of F-count (0.2562) <F-table (3.1059).

Based on the research conducted after the storage process and the treatment of iron powder addition on fish oil packaging, it is known that peroxide value has fluctuating values. The initial peroxide value of pure fish oil (13.32±0.11 meq/kg) is higher than the packaged fish oil peroxide value (2.10±0.20meq/kg). Treatments E and F have lower peroxide value compared to treatments C. D. and B (controls). Table 2 showed that the amount of iron powder added to fish oil packaging is not linear with fish oil peroxide value decrease. Lower peroxide value numbers do not always indicate that the oxidation process is running at an early stage. but it can also be caused by products are oxidized by fat which has decomposed into other compounds at an advanced level [11]. According to Samples [12]. in the early stages of the oxidation process, there will be a continuous increase in peroxide value numbers until it reaches the maximum condition. After achieving maximum conditions, this will trigger an increase in the reaction speed of the secondary production of the oxidation process. so that the peroxide level will decrease.

Numbers of pure fish oil p-Anisidine value and packaged fish oil p-anisidine value increased after the storage period. Table 2 showed p-Anisidine value decreases inversely with the amount of iron powder added to each treatment. The p-Anisidine number in treatments C. D. E. and F has a lower number of p-Anisidine than treatment B as control of pure fish oil stored without the addition of fish oil during the storage period. From the results obtained. It is known that a bigger amount of iron powder added causes the value of the p-Anisidine number to decrease. The number of p-Anisidine is measured by compound decomposes peroxide number. The decomposition of hydroperoxide components forms malonaldehyde compounds as a result of secondary oxidation [13]. Based on the research carried out the total oxidation value of fish oil treated with the addition of iron powder with different concentrations on the packaging has a fluctuating value because the dominant total oxidation value is fluence by peroxide numbers whose it is values are also volatile. However, packaged fish oil total oxidation value. Which has antioxidant content continues to experience an increase in value compared to its initial quality. This shows that even though antioxidants have been added. Fish oil still can oxidation during the storage period.

Based on the research conducted, acid numbers and free fatty acid levels decreased inversely with the amount of iron powder added to each treatment. The acid numbers and free fatty acid levels in treatments C. D. E also F have a lower value than treatment B as a negative control of pure fish oil stored without the addition of iron powder on the packaging. Table 3 showed that the greater the amount of iron powder added causes the value of the free fatty acid level to decrease. According to Christie et al. [14], the level of free fatty acid is an indicator of the oxidation process in oils that produce other organic acid components. The decrease in free fatty acid levels is due to the free fatty acid component undergoing an autooxidation...
process at the termination stage. The termination phase describes free fatty acids into aldehydes and ketones.

The free fatty acid level is a tertiary product of the oxidation process, which shows the occurrence of oxidative and hydrolytic rancidity. The level of free fatty acids is a fatty acid that is not bound as triglycerides. Free fatty acids are produced from oxidation or hydrolysis. The results of the reaction from the hydrolysis process are glycerol and free fatty acids. Factors that influence the hydrolysis process include water acidity and catalyst (enzyme). Increased levels of free fatty acids cause a decline in taste and odor in fish oil [15].

Based on the test of the total plate count in Table 4 it is known that there is no significant difference in the number of bacteria in fish oil. According to Rorong et al. [16], the form of damage to oil is mainly caused by the interaction of oxygen in the air against fat. Fat decomposition by microbes can only occur when there are water and nitrogen compounds while oil oxidation by oxygen in the air (autooxidation) occurs spontaneously with the speed of the oxidation process based on the type of fat [16].

4. Conclusion

The addition of iron powder to fish oil packaging has a significant effect on fish oil total oxidation value during the storage period as evidenced by the secondary oxidation product indicator in the form of p-anisidine, which decreases with an increasing amount of iron powder added to fish oil packaging. The addition of 10.8 grams of iron powder on fish oil packaging can inhibit the addition of p-anisidine numbers to point 17.74 mEq/kg for 21 days of storage. However. Further research is needed to periodically measure peroxide numbers because of its fluctuating value. Periodic measurement of peroxide numbers aims to determine the cycle of up and down peroxide numbers through the maximum and minimum values of peroxide value during the storage period.

5. References

[1] Nofrida R 2013 Institute Pertanian Bogor 1-3
[2] Widiastuti D W 2016 Food Pack 1-28
[3] Ahvenainen R 2003 Active and Intelligent Packaging: Novel Food Packaging Techniques (Abington: Woodhead Publishing p 5-21
[4] Day B P F 2008 Active Packaging of Food. Smart Packaging Technologies for Fast Moving Consumer Goods (England: John Willey & Sons Limited) p 75-96
[5] Mills A 2005 The Royal Society of Chemistry 34, 1003-1011.
[6] Boran G, Karacam H, and Boran M 2006 Food Chem. 98(4), 693-698
[7] Rozana 2013 Sekolah Pascasarjana Institut Pertanian Bogor.
[8] Labuza T P and Breene W M 1988 Applications of “Active Packaging” for Improvement of Shelf-Life and Nutritional Quality of Fresh and Extended Shelf-Life Foods (Minnesota: Department of Food Science and Nutrition University of Minnesota) p 1-69
[9] American Oil Chemists Society 1998 Official Methods and Recommended Practices of the American Oil Chemist Society AOCAS Champaign IL.
[10] Standar Nasional Indonesia 2008 Metode Pengujian Cemaran Mikroba Dalam Daging. Telur dan Susu. Serta Hasil Olahannya (SNL 2897:2008) p 1-31
[11] Dewi E N, Ibrahim R, and Yuaniva N 2011 Jurnal Saintek Perikanan 6(1), 6-12
[12] Sampels S 2013 Journal of Agricultural and Food Chemistry.
[13] Kolakowska A 2003 Lipid Oxidation in Food System: Chemical and Functional Properties of Food Lipids (Boca Raton: CRC Press) p 133-166
[14] Christie T M, Ma’ruf W F, and Susanto E 2015 Jurnal Pengolahan dan Bioteknologi Hasil Perikanan 5(1) 2442-4145
[15] Suseno S H, Nurjanah, and Faradiba T 2013 Jurnal Pengolahan Hasil Perikanan Indonesia 2(16), 142-149
[16] Rorong J, Aritonang H, and Ranti F P 2008 Chem Prog 1(1), 9-18

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