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STABILITY OF SARDINE (*SARDINELLA* SP.) OIL SOFT GEL THROUGH SALT SOLUTION AND CITRIC ACID DEGUMMING METHOD

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ABSTRACT

The stability of sardine (*Sardinella* sp.) oil soft gel during storage was analyzed through salt and citric acid degumming process. Soft gel was capsulated with 0.05% mix tocopherol and stored for 16 days at 40°C according to schaal oven test method. The stability of soft gel during storage was determined by its FFA, PV, AV, Totox and clarity value. Sardine oil soft gel prepared through salt degumming treatment met the IFOS standard for FFA, PV, AV and Totox value up to 16th, 12nd, 14th and 10th day of storage respectively. Soft gel produced through citric acid degumming treatment reached the IFOS limit for FFA, PV, AV and Totox value in order on the 10th, 2nd, 2nd and 4th day of storage. Salt degumming treatment performed better results in maintaining fish oil soft gel stability compared to citric acid degumming. One-day storage using *Schaal Oven Test* method equals to 15 days storage at room temperature.

Keyword: degumming, oxidation parameter, sardine oil, soft gel, stability

INTRODUCTION

Crude sardine (*Sardinella* sp.) oil purification has been carried out through several methods, including neutralization, degumming (the removal of gum), bleaching (bleaching of color) and deodorization (Young, 1978). In this study, neutralization was carried out by alkali using sodium hydroxide (NaOH) due to the efficiency and economical issue. Degumming was conducted by using salt and citric acid. The utilization of a salt solution aimed to draw mucus contained in fish oil (Yulianti *et al.,* 2012) while the citric acid used to convert phosphatides that could not be hydrated into hydrated phosphatides so that it could be separated from the oil (Kulkarni *et al.,* 2014). Bleaching using Magnesol XL was aimed to improve the color of fish oil and decreases the peroxide and heavy metals value (Suseno *et al.,* 2011). Refined sardine oil would have a high commercial value when it is produced in the form of capsules, for example soft capsule (soft gel) as food supplement. Fish oil in the form of soft gel could be easily digested and absorbed quickly by the body. In addition, soft gel protected from light or contaminants so that the product less oxidized and less savory (Reddy *et al.,* 2013).

The stability of oil soft gel needed to be maintained due to its prone quality against oxidation which notably caused by temperature used during storage. Storage is a crucial factor of rancidity during processing and marketing of fats, oils and fat-containing foods. The method of determining the oxidative stability, especially the mechanism of antioxidants effectiveness to inhibit rancidity in a product is important. Eastman (2010) stated that the analysis of the storage which was performed under normal conditions provided the most realistic determination of stability. However, this process was usually take too much time. Simple storage method with a faster process namely *Schaal oven* method had been developed. This method is one of the methods for measuring the acceleration of the resistance to oxidation (Gretel, 2003) by using oven as a storage set.

The purpose of this study was to evaluate the stability of sardine oil soft gel obtained through salt solution and citric acid degumming by using *Schall oven* method.

MATERIALS AND METHODS

Materials and Equipment: Materials used in this study were soft gels of sardine oil, distilled water, potassium hydroxide (KOH; Merck), glacial acetic acid (CH_3COOH; Merck), chloroform (Merck), sodium thiosulfate ($Na_2S_2O_3$; Merck), iso-octane (Merck), n-hexane (Merck), p-anisidine solution (Sigma Aldrich), phenolphthalein indicator, starch and alcohol 95%. Tools used were burette (Iwaki Pyrex), UV-VIS spectrophotometer (Agilent 845-3), digital scales (Quattro) and oven.

Procedure of Analysis

Salt solution degumming*:* Salt solution deguming consisted of 4 purification stages namely two degumming processes, neutralization and bleaching step. Crude sardines oil was degummed with 5% (v/v) distilled water, stirred by magnetic stirrer for 15 min. The obtained oil was then degummed with

3% (b/v) NaCl solution (oil: NaCl solution, 1: 3 v/v) and stirred for 20 min. Neutralization was conducted by adding NaOH 22°Be (Baume degree) then stirred for 15 min meanwhile bleaching was performed by adding Magnesol XL 5% (w/v) as adsorbent and stirred for 20 min. Each stage of purification was done by heating the oil up to a temperature of 50°C and centrifuged it for 10 min.

Citric acid degumming: The degumming was carried out by adding 2% citric acid into the sardine oil and stirred it for 15 min. The oil was then neutralized by alkali NaOH 16°Be and stirred for 10 min. The semi-refined oil was decantated for 10 min to separate the soap stock. The last step was bleaching using magnesol XL 5% and stirred for 20 min. Each purification stage was done by heating the oil to a temperature of 70°C and centrifuged for 10 min.

Sardine Oil Softgel and Stability Test*:* Refined oil which was purified through salt solution and citric acid degumming were then capsulated as soft gel by adding 0.05% of mixed tocopherol as an antioxidant. Further stability of capsulated soft gel was evaluated according to the *Schaal Oven Test* methods at a temperature of 40°C for 16 days (equal to 240 days in room temperature storage). Physicochemical properties of refined oil were analyzed by several parameters such as free fatty acid (FFA) (AOCS, 1998), peroxide value (AOCS, 1995), p-*anisidine value* (p-AV) (Watson, 1994), total oxidation (tot ox) value (Perrin, 1996) and clarity value (AOAC, 1995; with modification). All analysis was carried out in every two days with the total of 9 data for each parameter.

RESULTS AND DISCUSSION

Soft gel Stability: Stability analysis during storage was conducted to determine the product's shelf life. *Schaal oven test* was used as the acceleration method where storage at 40°C for 16 days treatment was equal to storage at room temperature for 240 days. In this method one day is equivalent to 15 days of normal storage condition.

Free fatty acid (FFA) value*:* Free fatty acids comprise of hydrolyzed fat which are not bound to triglycerides. The content of free fatty acids is influenced by several factors, including temperature, acidity, water and the process of hydrolysis by the enzyme during the storage process (Hernandez and Kamal, 2013). Figure 1 showed a graph of the increase in the value of free fatty acid fish oil soft gel sardines stored for 16 days at 40°C.

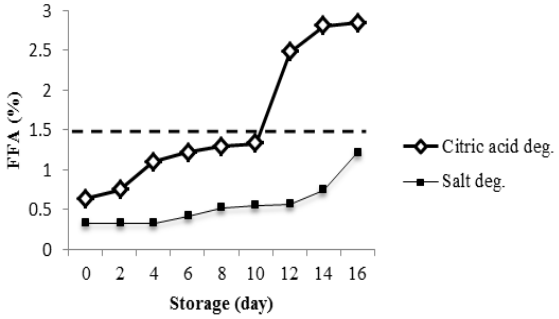


Figure 1. FFA value of sardine oil softgel during storage

In salt degumming phase, FFA value notably increased up to 1.22% at the 16th day of storage. Nevertheless, the changes were still under the acceptable limit of IFOS standard of 1.5%. Kusharto *et al.,* (2015) in his study on capsulated catfish oil stability with *Schaal Oven Test* reported a FFA value of 1.55% which lasted for 5 weeks to reach IFOS standard (2014).

As for the citric acid degumming phase, FFA value met the IFOS standard (2014) on the 10th day of storage or equal to 150 days storage at room temperature. This phenomenon implied that on 12nd to 16th day, fish oil soft gel was no longer could be consumed. High FFA concentration in the body inhibits glucose uptake by muscle and can lead to the risk of metabolic syndrome (Rask-Madsen and Kahn, 2012). Increasing FFA value was expected from the high-temperature used which accelerated the oxidation processes.

Peroxide Value (PV): Free radicals and peroxides react with oxygen to form peroxide radicals by taking hydrogen from other unsaturated molecules (Estiasih, 2009). PV value profile of fish oil soft gel during storage can be seen in Figure 2.

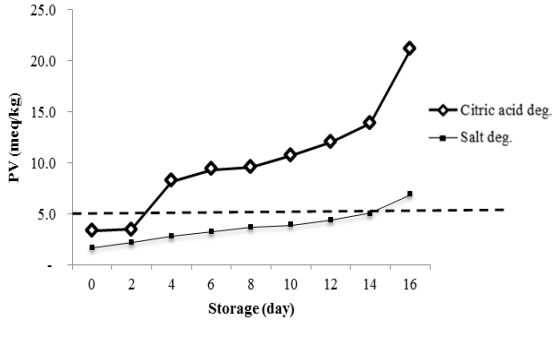
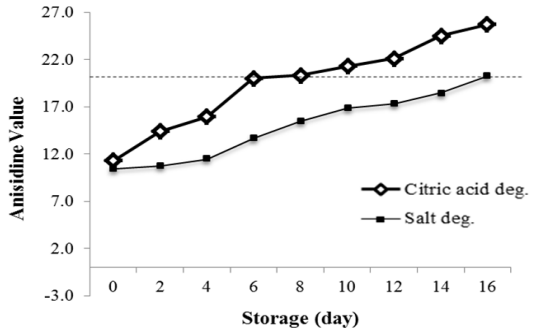


Figure 2: Peroxide value of fish oil softgel during storage (40°C)

The PV of fish oil soft gel with salt degumming stage still met the IFOS standard (2014) until 12nd day of storage. At the 14th day, PV exceeded allowable standard value of the 5.04 meq/kg. As for the citric acid degumming stage, allowable

PV only remained until the 4th day or equal to 60 days storage at room temperature. This high PV was expected from the different initial PV value at the 0 day, which was higher in the sample with citric acid degumming treatment (3.22 meq/kg). The high storage temperature also accelerated its oxidation rate so that on the 4th day PV start exceeding IFOS standard (2014). Oxidation rate is also affected by storage conditions (Kilcast and Subramaniam, 2011). The longer the oil is stored, the higher PV will increase until a certain point before dropping back. Sardine oil quality can be maintained for longer shelf life by using antioxidant such as tocopherol. α-tocopherol inhibits oxidetion by bounding peroxide radicals to stop propagation chain, at the same time it reacts with free radicals to inhibit hydroperoxide decomposition and reduces aldehydes formation (Ketaren 2012).

p-Anisidine Value (AnV): The results of analysis found the AnV changes during storage in the presence of temperature treatment. Increasing AnV of fish oil soft gel during storage at 40°C was presented in Figure 3.



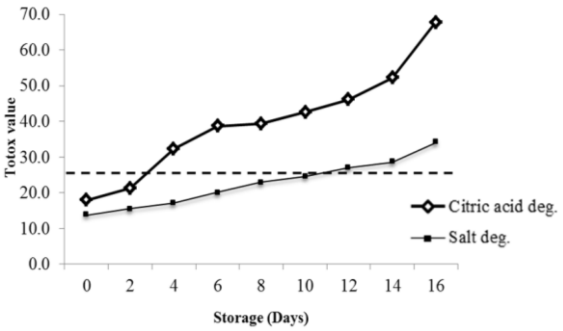
IFOS standard (≤ 20.0)

Figure 3: Anisidine value of fish oil softgel during storage (40ºC)

AnV of fish oil soft gel with salt degumming treatment has exceeded the IFOS maximum threshold of 20.32 on the last day of storage. Kusharto *et al.* (2015) studied the stability of encapsulated catfish oil where anisidine produced p-value of 11.66 in the presence of antioxidant. In the degumming citric acid treatment, allowable AnV only maintained up to the 4th day. The low AnV from 0 to 4th day was expected from lower hydro-peroxide decomposition in the first 4 days and was constantly increasing in the following days.

Total Oxidation (Totox) Value: Total oxidation expressed the number of primary and secondary oxidation in fish oil. Totox value was obtained by adding two times PV with one AnV. Total oxidetion value changes of fish oil soft gel during storage can be seen in Figure 4.

Totox value in salt degumming treatment started exceeding (27.03) the IFOS limit (26.0) on the 12nd day. As for the citric acid degumming treatment, the results showed that Totox value only met the IFOS standard up to the 2nd day or equal to 30 days of storage at room temperature. Starting from the 4th day, Totox value was no longer in acceptable limit. This is presumably due to the high decomposition of hydroperoxide into compounds of secondary oxidation since the 0 day of storage. Hence, a total of oxidation on 4th day easily increased. Totox value of salt degumming that lasts up to the 12th day was allegedly helped by the presence of antioxidants. Antioxidants has ability to inhibit or stop the chain reaction of free radicals that oxidize fat (Ketaren, 2012).



IFOS standard (≤ 26.0)

Figure 4: Totox value of fish oil softgel during storage (40ºC)

Clarity: Clarity is one important factor in determine the quality of fish oil which is represented by the percentage of transmission. The higher transmission value (approaching 100%) the better clarity of fish oil (Suseno *et al.*, 2014). The clarity value of fish oil soft gel changes during storage was shown in Figure 5.

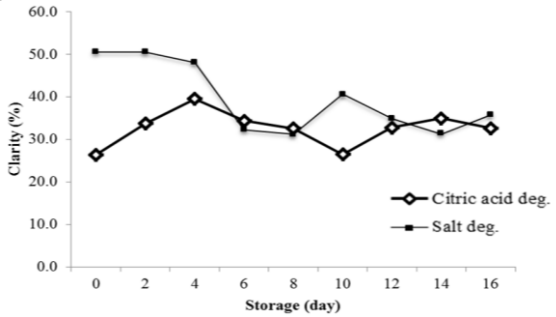


Figure 5. Clarity value of fish oil softgel during storage (40ºC)

Based on the analysis of clarity during storage, clarity grade changes of fish oil soft gel with salt degumming treatment were tending to decrease. Clarity value decreased from 50.51% (0 day) to 35. 82% (16th day). This decline changes were allegedly caused by oil breakdown process during storage. The color changes in fish oil caused by free fatty acids reaction forming a colored compound (Estiasih, 2009). In the citric acid degumming

treatment, fish oil's clarity lied under 50% from 0 day until the end of storage. This case implied that fish oil from the current treatment has poor clarity. This phenomenon was presumably caused by the presence of oil degradation products and other left-over materials remaining which affected the clarity level of oil (Boran *et al*., 2006).

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