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Enzymatic Interesterification of Katsuwonus palamis fish oil from North Sulawesi with lauric acid.

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Abstract

Over the last decade, there has been considerable interest in healthier foods, one of which has been to convert fatty acid components to unsaturated components. This is one of the products known as Lipid-Specific Structure (LSS). The synthesis of lipids structured using the transesterification process is beneficial in improving the functional properties and nutritional value of fats and oils, as expected in some processed products. Enzymatic transesterification of fish oil from North Sulawesi (Katsuoparamis) extracted using a wet recovery process was investigated. The first experiment was carried out using different temperatures i.e. 30o, 40o, 50o and 60o C for 12 hours time of reactions, while the second experiment was carried out using the best reaction temperature obtained from first experiment with different times of reaction namely 0 (early time), 6, 12, 24 and 48 hours respectively. Those samples were then analysed using Gas Chromatography to determine lauric acid incorporation within the skipjack fish oil. The fatty acids in the acidolysis fish oil analysed by changing fatty acid into Fatty Acid Methyl Ester (FAME) before injected into the Gas Chromatography apparatus. The best reaction time was found at 50oC which was shown by 39.79% lauric acid was incorporated within the fish oil, whilst the best time of reaction was obtained at 24 hours where 48.18% as the highest percentage of lauric acid incorporated into the skipjack fish oil. Fatty acids rearrangement due to enzymatic interesterification was observed at sample after interesterification with lauric acid and catalyzed by specific Mucor miehei lipase enzyme as showed by high lauric acid (14.94%) and methyl laurate (25.50%) contents. Therefore it can be concluded that interesterification of skipjack oil with lauric acid will be best if carried out at 50o C for 24 hours.

Keywords: Skipjack fish oil, Interesterification Enzymatic, Lauric acid.

Introduction

Internationally, the concept of structured lipids was developed for nutritional and pharmacological applications, but certain structured lipids were primarily aimed at their functional properties through transesterification. Structured lipids are triacylglycerols that have been modified by changing their fatty acid composition and / or their position within the glycerol skeleton through chemical and enzymatic reactions (Haumann,1997;Akoh,1998 and Hamam and Shahidi, 2004). Akoh (1998) defined that structured lipids are triacylglycerol containing mixture of short and or medium chains of fatty acids and long chain fatty acids within same molecule of glycerol for its functional properties (Batubara and Kartika, 2021; Batubara and Istanto, 2021). According to Xu (2000), Akoh (2002) and Hamam and Shahidi (2004) structured lipids are alkoxy by chemical or enzymatic reactions such as direct reactions between fatty acids and glycerol, or by transferring acyl groups between acids and esters known as acid degradation, and between alcohols and esters. It can be produced by exchanging groups. Alcohol decomposition.

Mu et al. (1998) stated that interesterification lipid with specific 1 – 3 Sn lipase enzyme were quite high catalytic efficiency, specificity and selectivity as it could improve the nutrition quality of lipid by incorporation the required fatty acids at certain position as expected. Some workers have successfully producing structured PUFA rich fish oil by incorporating caprylic acid via lipase acidylosis reaction (Shimada et al.,1997; Akoh and Mousatta,1998 and Kawashima et al.,2001).This method had been successfully used for modification of plant oil fatty acid in producing structured lipid as reported by Lee and Akoh, 1998; Reena et al.,2001 and Xu et al.,2002.

Fish oil is known as a source of polyunsaturated fatty acids (PUFAs), especially in the form of docosahexaenoic acid (DHA; C22: 6) and eicosapentaenoic acid (EPA; C20: 5), and is used as a dietary supplement. Will be. Tuna oil also contains 14.64% DHA and 3.64% EPA, making this fish oil an excellent source of omega 3 fatty acids, (Elisabeth, 1997 and Irimescu et al., 2001) and can be functional food (Rompies *et al.,* 2021; Permatasari *et al*., 2021). Although an intensive studies had been made on enzymatic interesterification of fish oil, however there is limited information on the enzymatic interesterification of skipjack fish (*Katsuwonus palamis*) oil from North Sulawesi, hence the aim of this study was to find out the best temperature as well as time of interesterification for North Sulawesi skipjack fish oil with lauric acid.

Materials and Methods

Materials.

The fish oil used as a sample was from Katsuwonus palamis in Manado, North Sulawesi, from Astawan (1998), the specific 1.3 Mucor miehei lipase enzyme (optimal pH 8.0) and 70^{o}C (Novo Nordisk Denmark). And pure lauric acid ($CH_3(CH_2)_{10}COOH$) with a molecular weight of 200.32 (Sigma Aldrich) was purchased from a local distributor. All Sigma Aldrich organic solvents (hexane, acetone, petroleum ether, formic acid) and chemical reagents (KOH, anhydride Na2SO4) were purchased from Sigma Aldrich in all analytical grades.

Methods.

Interesterification of skipjack fish oil with lauric acid using microbial lipase enzyme were carried out using the method of Yankah and Akoh (2000) with slightly modification. In the first experiment 1.74 g of skipjack fish oil mixed with 3.32 g lauric acid(fish oil molarity and lauric acid ratio was 1: 5) in erlenmeyer flask added with 0.50 g lipozyme (10% of substrate) and 8.1 ml hexane. This mixture was then incubated in shaking waterbath (120 rpm for 12 hours) at 30^o, 40^o, 50^o and 60^o C respectively. In the second experiment same sample was also prepared as in first experiment except the mixture was incubated in shaking waterbath for 0(early time), 6, 12, 24 and 48 hours respectively at the best temperature obtained from first experiment. Preparation of FAME of sampels and calculation of incoporated lauric acid into skipjack fish oil were following the method as described by Park and Goins (1994), The incorporated lauric acid into skipjack fish oil was determined using Gas Chromatography Hewlett-Packard 5890 series II with HP 5 Column (5% Phenyl Methyl Siloxone) 30 m length,and initial temperature 180^o C for 2 minutes then increased 10^{o}C minutes until reaching 280^o C. Injector temperature 280^o C and FID detector at 300^o C and helium as gas carier with flow rate of 10ml/minute as described by Park and Goins (1994).

Results and discussion.

Enzymatic interesterification at different reaction temperature.

Reaction temperature is one of the most important factor affected the activity of microbial lipase enzyme in the interesterification of fish oil with lauric acid. It was found that the biggest amount of lauric acid (39.79%) could be incorporated into skipjack fish oil was at reactions temperature of 50^{o}C as shown in Table 1.

Table1. Calculation results of incorporation of lauric acid into skipjack fish oil at different reaction temperatures as measured using Gas Chromatography(GC).

Temperature (°C).	Lauric acid area.	Total area	Molecular weight of lauric acid.	Means of molecular weight of lauric acid.	Lauric acid molecules.	Fatty acid molecules.	% incorporation
30	6581784	24634031	200	277.81	0.70	1.87	37.47
40	274027	31016672	200	277.81	0.70	2.02	34.69
50	143304	15610008	200	277.81	0.77	1.94	39.79
60	552081	49059014	200	277.81	0.44	1.58	27.86

It is interesting to note that at reaction temperature of 40°C the incorporation percentage of lauric acid into skipjack fish oil was slightly decreasing compare to the one at reaction temperature of 30°C, and at 50°C the percentage reached the highest.Whilst increasing reaction temperature to 60°C decreasing further to 27.86%. The decreasing percentage of incorporation at 60°C was assumed at this reaction temperature there was a partial hydrolysis of triglyceride into intermediate products namely mono- and di-glyceride. However some workers reported that acidolysis of coconut oil with omega – 3 using lipase from *Mucor miehei* immobile was optimum at 51.1°C, and most of the immobile lipase enzymatic reactions were optimum at 30 – 62°C.(Malcata et al., 1992 and Rao et al.,2002). While Chua et al. (2012) noted that the highest initial reaction rate and highest yield of free fatty acids production in virgin oil using Mucor miehei enzyme could be obtained at 40° for 100 hours.

Enzymatic interesterification at different reaction time.

The optimum temperature (50°C) obtained from first experiment was used for enzymatic interesterification of skipjack fish oil at different reaction times, and it was found that 24 hours reaction time was the optimum reaction time as shown in Table.2.

Table2. Calculation results of incorporation of lauric acid into skipjack fish oil at different

Reaction times (hours).	Lauric acid area.	Total area	Molecular weight of lauric acid.	Means of molecular weight of lauric acid.	Lauric acid molecules.	Fatty acid molecules.	% incorporation.
0	6807027	41056175	200	`277.81	0.27	1.45	23.39
6	11130348	55011545	200	277.81	0.42	1.47	28.45
12	4431292	15600008	200	277.81	0.77	1.94	39.79
24	7751259	25339753	200	277.81	0.06	0.13	48.18
48	12792106	53029603	200	277.81	0.04	0.11	38.99

Data in Table 2 showed that increasing the reaction time up to 24 hours tends to increase the percentage of lauric acid incorporated and further increasing reaction time did not increasing the percentage of incorporation.It assumed that 24 hours of reactions was the optimum reaction time and so called equilibrium had been reached as far as the system condition and reactor were not changed.

The fatty acids composition of either fresh skipjack fish oil, lauric acid and skipjack fish oil after interesterification with 25.5% lauric acid were also determined using GC-MS and the results are presented in Table 3.

Table 3 Fatty acids composition of skipjack fish oil, lauric acid and skipjack fish oil after interesterification with 25.5% lauric acid.

Free Fatty acids.	Skipjack fish oil. (%)	Lauric acid. (%)	Skipjack fish oil after interesterification. (%)
Propionic acid	12.05	1.04	-
Lauric acid	-	96.09	14.94
C13:0	3.08	-	-
Myristic acid	7.75	0.64	-
C15:3	3.03	-	-
Palmitic acid	52.01	0.95	-
Stearic acid	10.47	0.39	-
Oleic acid	5.29	-	-
Pentadecane	-	-	0.98
Dodecane 2,7,1, - trimethyl	-	-	1.18
Ethyl Laurate	-	-	25.50
Ethyl Myristate	-	-	4.64
Ethyl Pentadecanoate	-	-	1.48
Ethyl Palmitate	-	-	14.14
Ethyl-9-Hexadecenoate	-	-	2.38
Ethyl Heptadecanoate	-	-	1.94
Nonadecanoic acid	-	-	1.96
Ethyl Stearate	-	-	7.52
Ethyl Oleate	-	-	9.51
Ethyl Linoleate	-	-	1.15
Eicosatrieonic acid	-	-	1.09
Eicosapentaenoic acid	-	-	5.90

The free fatty acids in skipjack fish oil before interesterification was dominated by palmitic acid (52.01%) followed by propionic acid (12.05%), stearic acid (10.47%), myristic acid (7.75%) and oleic acid (5.29%) and lauric acid purity was also proven by the lauric acid content of 96.09%. while after interesterification the skipjack fish oil contained ethyl laurate 25.50%, lauric acid 14.94%, ethyl palmitate 14.14%, ethyl oleat 9.51%, ethyl stearate 7.52% and eicosapentaenoic acid 5.90%. These results showed that interesterification of skipjack fish oil with lauric acid and *Mucor miehei* lipase enzyme could produced a restructured lipid as expected. According to Thomas et al. (1988) and da Silva et al. (2010) interesterification could extremely rearranged fatty acids and producing new kind of triacylglycerol such as by reducing saturated and unsaturated triacylglycerol and also could increase the amount of either mono- and diunsaturated triacylglycerol of all oil mixture. While Nieto et al. (1999) reported that in their study using sardine oil interesterified using *Mucor miehei* lipase enzyme in laboratory scale resulted a structured triacylglycerol containing medium – fatty acids at position Sn-1 and Sn-3, while long chain polyunsaturated acid from fish oil was at Sn-2 position.

Conclusion.

This study showed that for interesterification enzymatic reactions using *Mucor miehei* lipase enzyme with lauric acid into skipjack fish oil the optimum reaction temperature was 50°C for 24 hours to obtain the highest percentage of incorporation in producing structured lipid. A rearrangement of fatty acids were observed after interesterification procedure and regiospecific study need to be conducted to confirm the position of those fatty acids.

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