# TROPICAL OILS AS RAW MATERIAL FOR SYNTHESIZING HUMAN MILK FAT ANALOG

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Coconut and oil palm are important trees in the world, which provide food for millions of people. The main product obtained from coconut and palm oil is oil. Coconut oil is obtained from coconut fruits, by extracting the endosperm (coconut meat). On the other hand, the fruit of oil palm can produce two kinds of oil which are crude palm oil and palm kernel oil. Crude palm oil is extracted from mesocarp, whereas palm kernel oil extracted from its kernel.

Coconut oil can be extracted through various methods, either by dry or wet processing. The dry process is the most widely used form of extraction. In this process, the oil is extracted from copra (dried coconut meat) and then it must be refined, bleached and deodorized. The other process is wet processing which entails the extraction of the cream from fresh nature nuts. This process is more desirable, due to the free of usage of chemical solvent. Thus, it is more enviromental friendly than the solvent extraction. The wet method is much simpler, which can be conducted at farmer or farmers group level. Meanwhile the extraction of Palm oil and palm kernel oil are using dry process in industrial scale.

**Tropicals oils such as palm stearin and coconut oil could be utilized as the main raw materials for synthesizing HMF analog. The resulted HMF analog was found to be rich in medium chain fatty acids. The existence of palmitic in the sn-2 position was similar to human milk fat, and the MCFA derived from lauric acid in the sn-1 and sn-3 positions was expected to provide fatty acid energy that can be readily utilized by the body and not stored in the fat muscle.**

Coconut oil has unique properties, because it is rich in saturated fatty acids especially medium chain fatty acids (MCFA) ranging from 58.5 to 62.32% in which lauric acid is the major component. In the processing of crude palm oil some products can be obtained and the main products are palm oil and palm stearin. Palm olein is the liquid fraction obtained from fractionation of palm oil and the solid fraction is called palm stearin. Palm oil has a balanced fatty acid composition in which the level of saturated fatty acids is almost equal to that of unsaturated fatty acids. Palmitic acid (43.5%) and oleic acid (39.80%), are the major components of the fatty acids. The major fatty acid in palm

**Coconut (left) and Oil Palm (right) as Sources of Tropical Oils**

**Table 1. Fatty acid composition (%) of palm stearin and coconut oil**

| Fatty acids | Palm Stearin[a] | Coconut Oil[b] |
|---|---|---|
| C8:0 | ND | 7.41±0.38 |
| C10:0 | ND | 6.28±0.14 |
| C12:0 | ND | 48.24±0.07 |
| C14:0 | ND | 19.26±0.09 |
| C16:0 | 59.41±1.10 | 9.29±0.02 |
| C18:0 | 4.99±1.16 | 2.44±0.06 |
| C18:1 | 29.59±0.05 | 5.83±0.30 |
| C18:2 | 6.00±0.11 | 1.26±0.10 |

*Source: Karouw (2013)*

stearin is palmitic acid, followed by oleic acid.

Palmitic acid is the main fatty acid in human milk fat, reaching to 44.80%, it is predominantly located in the sn-2 position of triglycerides. Meanwhile infant formulas contain palmitic acid which is predominantly located in sn-1,3 positions. The role of palmitic acid in the sn-2 position of the glycerol backbone is to ease the digestion and absorption of the fats in the infant intestine. Long chain saturated fatty acids, like palmitic acid, esterified to sn-1,3 positions during the digestion, can form insoluble fatty acid complexes with calcium rendering it unavailable.

In recent years, there have been considerable researches conducted on structured lipids containing fatty acid profile which is similar to that of human milk or Human Milk Fat analog (HMF analog). Generally, tripalmitin or lard oil was used as sources of palmitic acid in sn-2 position. Palmitic acid is the major fatty acid in palm stearin (49.6-58.8%), in which 58.3% are mainly located in the sn-2 position. Thus, palm stearin is good source of 2-monopalmitin which could be hydrolyzed enzimatically using specific 1,3 lipase such as pancreatic lipase. HMF analog having high percentage of palmitic acid in the sn-2 and high lauric acid in the sn-1,3 positions could be synthesized by enzymatic interesterification of 2-monoglyceride and medium chain fatty acid (MCFA).

It was found that the MCFA, when included in the diet, prevented obesity and reduced body weight. MCFA was proved to increase body endogenous oxidation by changing the composition of the adipose tissue pool through altered endogenous availability. The capability of Medium Chain Triglyceride (MCT) to increase endogenous fat oxidation could have implications in the reduction of adipose tissue mass by increasing adipose tissue mobilization. Medium chain fatty acids (MCFA) contained in Virgin Coconut Oil (VCO) amounting to 46.6-48.0% could be used for inter-esterification reaction. This article presents the potential of coconut oil and palm stearin as raw materials to synthesize human milk fat analog (HMF analog).

## Fatty acid Composition of Palm Stearin and Coconut Oil

Palmitic acid is the major fatty acid in palm stearin (59.41%), followed by oleic acid (29.59%) (Table 1). As shown in Table 1, lauric acid is the main fatty acids in coconut oil. These are similar to the result of Marina et al. (2009), who reported that the lauric acid of coconut oil was about 46.64-48.03%. Based on the fatty acids profile of palm stearin and coconut oil, therefore, these two oils were considered as a good source of 2-monopalmitin and medium chain fatty acids, respectively.

## Synthesis of Human Milk Fat Analog

Human milk fat analog (HMF analog) is a structured lipid which has the fatty acids profile and distribution similar to that of human milk fat. Generally, HMF analog is obtained through enzymatic reaction by using specific 1,3 lipase. This lipase selectively cleaves the fatty acids in sn-1 and sn-3 position.

HMF analog is synthesized using palm stearin as the source of palmitic acid and coconut oil. The HMF analog is synthesized through two main reactions. The first step is preparation of 2-monoglyceride from palm stearin and the second step is intereseterification of 2-monoglyceride and fatty acid methyl ester of coconut oil.

### Preparation of 2-monoglyceride

Palm stearin was hydrolized through enzymatic reaction with lipase from *Rhizomucor miehei* and pancreatic lipase. Lipase from *R. miehei* and pancreas have different capability to hydrolize palm stearin to obtain 2-monoglyceride at various ratios of substrate: phospate buffer during the same hydrolysis

**Table 2. Fatty acids profile of human milk fat analog and human milk fat**

| Fatty acid | % Fatty acid | |
|---|---|---|
| | HMF analog[a] | Human milk fat[b] |
| C10:0 | 4.49±0.43 | 2.35 |
| C12:0 | 39.37±0.92 | 13.82 |
| C14:0 | 16.06±0.39 | 12.12 |
| C16:0 | 24.33±1.59 | 23.02 |
| C18:0 | 5.37±0.69 | 4.75 |
| C18:1 | 8.98±0.74 | 21.85 |
| C18:2 | 1.40±0.11 | ND |
| Total MCFA | 43.56 | 16.17 |

*Noted:*
*[a] Karouw (2013).*
*[b] Human milk fat from breastfeeding mother in Philippine (Yuhas et al., 2006)*

reaction time. The highest monoglyceride fraction was obtained from the following substrate ratios: phospate buffer 10:1 by *Rhizomucor miehei* lipase (9.14%) and phospate buffer 10:4 by pancreatic lipase (15.36%). At similar substrate ratio, phosphate buffer, the monoglyceride fraction obtained by pancreatic lipase was higher than by lipase from *R. miehei* which were 13.12% and 9.14%, respectively.

Enzymes have specific activity to catalyze the reaction, therefore the amount of water was required by enzymes to maintain their catalytic ability depending on the type of substrate and enzyme. The highest monoglyceride fraction was obtained from substrate ratios as the following: phospate buffer 10:4 at 42 hours of incubation by pancreatic lipase (40,45%) and phospate buffer 10:1 at 18 hours of incubation by *Rhizomucor miehei* lipase (21,59%).

## Synthesis of HMF analog

The HMF analog was synthesized through enzymatic interesterification reaction by using lipase from *R. miehei* as byocatalist. All of the enzymatic esterification reactions were carried out in a waterbath shaker operating at 120 strokes/minute. The HMF analog markedly increased during the first 6 hours of reaction and continuously increased up 60.24% at 12 hours. This result indicates that the esterification reaction started at the early stage of reaction (6 hours), however, after 12 hours, the reaction was dominated by hydrolysis reaction of HMF analog to diglyceride and hydrolysis of diglyceride to monoglyceride. The diglyceride decreased until 18 hours and then increased slightly to reach approximately 7.57% at 24 hours.

The diglyceride may be formed through hydrolysis of triglyceride or esterification of mono-glyceride. The HMF analog content increased with the raise of enzyme concentration. The HMF analog contents were 48.21, 53.06, 59.38 and 62.25% at enzyme concentration of 2.5, 5.0, 7.5, and 10.0% by weight of total substrate, respectively. At the same duration of reaction, the rising of enzyme concentration resulted in increasing product concentration.

## Fatty Acid Profile of HMF Analog

The human milk fat analog derived from interesterification process of 2-monoglyceride of palm stearin and coconut oil, was found to be rich in medium chain fatty acid. The fatty acid profiles of the HMF analog compared to fatty acid of human milk fat are presented in Table 2. The palmitic acid content, thought to be located in the sn-2 position of the HMF analog is comparable to that of human milk fat.

However, oleic acid, which is also thought to be in the sn-2 position, was noticeably lower than that in the human milk fat. The MCFA (lauric acid from coconut oil) was successfully incorporated into the triglyceride. It was reported that human milk fat analog synthesized from tripalmitin and Neobee (the mixture of medium chain fatty acids) contained MCFA of 23.4 g/100g.

## Conclusion

Tropicals oils such as palm stearin and coconut oil could be utilized as the main raw materials for synthesizing HMF analog. The resulted HMF analog was found to be rich in medium chain fatty acids. The existence of palmitic in the sn-2 position was similar to human milk fat, and the MCFA derived from lauric acid in the sn-1 and sn-3 positions was expected to provide fatty acid energy that can be readily utilized by the body and not stored in the fat muscle.

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