INTRODUCTION

Human milk fat contains 32.0%-52.0% saturated fatty acid, 30.0%-50.0% monounsaturated fatty acid, and 2.5%-13.8% polyunsaturated fatty acid (Monaco et al., 2016). The primary fatty acids contained in human milk fat are 30.0%-35.0% oleic acid, 20.0%-30.0% palmitic acid, 7.0%-14.0% linoleic acid, and 5.7%-8.0% stearic acid. Besides these fatty acids, the human milk fat also contains long-chain polyunsaturated fatty acids (LCPUFA) and they include docosahexaenoic acid, eicosapentaenoic acid, and arachidonic acid contained in with a concentration of less than 1.0% (Ferreira-Dias and Tecelão, 2014; Wei et al., 2019). In human milk fat, about 60.0%-70.0% of palmitic acid is distributed at the sn-2 position and unsaturated fatty acids (oleic acid, linoleic acid, docosahexaenoic acid, eicosapentaenoic acid, and arachidonic acid) at the sn-1,3 positions (He et al., 2017; Wei et al. 2019).

Human milk fat substitutes (HMFSs) are structured lipids with the distribution of fatty acids similar to human milk fat (Sahin et al., 2005a; 2005b), commonly used as fat in infant formulas. HMFS has a similar function as human milk fat, increasing the permeation of calcium and fat, making the stool softer, and reducing obstipation (Zou et al., 2017). HMFSs are classified into four types, which include sn-2 palmitate (β-palmitate), LCPUFA, medium-chain fatty acid (MCFA), and milk fat globule membrane supplements. Sn-2 palmitate is the most common type of HMFS synthesised and it contains 1,3-dioleoyl-2-palmitoylglycerol (OPO), one of the primary triacylglycerols in human milk fat (Wei et al., 2019).

In general, sn-2 palmitate is synthesised using oils and fats containing palmitic acid at the sn-2 position including, tripalmitin (Ilyasoglu et al., 2011;
2013; Liu et al., 2015; 2017; Tecelão et al., 2010; Wang et al., 2016; Yüksel and Yeşilçubuk, 2012; Zheng et al., 2017), lard (Qin et al., 2014; Wang et al., 2010; Yang et al., 2003; Zhang et al., 2016), catfish oil (Zou et al., 2016a; 2016b), oil from Nannochloropsis oculata (He et al., 2017), butterfat oil (Ronse et al., 2005) and palm oil fractions (Ghosh et al., 2016; Karabulut et al., 2007; Nakagaita and Akoh, 2013). Also, palmitic acid (Robles et al., 2011; Turan et al., 2013) and ethyl palmitate (Turan et al., 2013) are used as palmitic acyl donors to enhance palmitic acid at the sn-2 position of oil containing high unsaturated fatty acids.

Palm oil fractions are the best substrates for HMFS synthesis among the vegetable oils synthesis (Mat Dian et al., 2017). However, palm oil fractions contain high palmitic acid at the sn-1,3 positions (Laseken et al., 2017). The presence of palm oil fractions in infant formula would contribute to the physiological function of the body such as reduction of intestinal permeation of fat, palmitic acid, calcium and lower bone mass (Chen et al., 2019; Koo et al., 2006). Thus, palm oil fractions need to be modified so that more palmitic acid will be at the sn-2 position, hence resembling human milk fat. This article aims to scientifically update the usage of palm oil fractions for sn-2 palmitate synthesis as HMFS. This article will cover the potential of palm oil fractions as substrates, lipases as biocatalysts, reactors and methods synthesis, and fractionation to increase OPO content in HMFS. Prospects of palm-based sn-2 palmitate synthesis were also discussed.

### TABLE 1. FATTY ACIDS OF PALM OIL FRACTIONS

| Fatty acids (%) | Palm oil* | Palm olein* | Palm stearin* | Soft palm stearin** | Hard palm stearin** | Palm kernel oil* |
|----------------|-----------|-------------|---------------|---------------------|---------------------|-----------------|
| C6:0           | ND        | ND          | ND            | ND                  | ND                  | ND              |
| C8:0           | ND        | ND          | ND            | ND                  | ND                  | ND-0.8          |
| C10:0          | ND        | ND          | ND            | ND                  | ND                  | 2.4-6.2         |
| C12:0          | ND-0.5    | 0.1-0.5     | 0.1-0.5       | 0.01-0.25           | 0.14-0.32           | 45.0-55.0       |
| C14:0          | 0.5-2.0   | 0.5-1.5     | 1.0-2.0       | 0.97-1.21           | 1.06-1.36           | 14.0-18.0       |
| C16:0          | 39.3-47.5 | 38.0-43.5   | 48.0-74.0     | 50.94-54.84         | 76.36-81.04         | 6.5-10.0        |
| C16:1          | ND-0.6    | ND-0.6      | ND-0.2        | ND-0.76             | ND-0.9             | ND-0.2          |
| C17:0          | ND-0.2    | ND-0.2      | ND-0.2        | ND                  | ND                  | ND              |
| C17:1          | ND        | ND-0.1      | ND-0.1        | ND                  | ND                  | ND              |
| C18:0          | 3.5-6.0   | 3.5-5.0     | 3.9-6.0       | 4.44-5.46           | 3.61-4.87           | 1.0-3.0         |
| C18:1          | 36.0-44.0 | 39.8-46.0   | 15.5-36.0     | 30.82-34.06         | 11.62-13.66         | 12.0-19.0       |
| C18:2          | 9.0-12.0  | 10.0-13.5   | 3.0-10.0      | 6.91-8.71           | 1.95-3.33           | 1.0-3.5         |
| C18:3          | ND-0.5    | ND-0.6      | ND-0.5        | ND-0.21             | ND-0.9             | ND-0.2          |
| C20:0          | ND-1.0    | ND-0.6      | ND-1.0        | ND-0.18             | ND-0.12            | ND-0.2          |
| C20:1          | ND-0.4    | ND-0.4      | ND-0.4        | ND-0.06             | ND-0.09            | ND-0.2          |
| C20:2          | ND        | ND          | ND            | ND                  | ND                  | ND              |
| C22:0          | ND-0.2    | ND-0.2      | ND-0.2        | ND                  | ND                  | ND              |
| Iodine value (IV) | 50-56 | 56 | 48 | 39.90-43.14 | 14.77-19.33 | 14.1-21.0 |

Note: ND - not detected.
Source: *Codex Alimentarius (2001), **Hasibuan and Siahaan (2013).
triaclylglycerol with oleic acid at the sn-2 position (Table 2). When triacylglycerol containing oleic acid at the sn-2 position is acidolysed with oleic acid using a specific lipase of sn-1,3 will produce triolein (Wang et al., 2020), which is not a sn-2 palmitate product. Table 3 shows that palm stearin has palmitic acid content at the sn-2 position ranging from 23.0%-70.1%. Thus, the palmitic acid content at the sn-2 position in palm stearin needs to be increased to produce a good substrate for HMFS synthesis (Hasibuan et al., 2021b).

Technologies for improving the positioning of palmitic acid at the sn-2 position of palm stearin are solvent fractionation (Ghosh et al., 2016; Lee et al., 2010; Wang et al., 2010; Zou et al., 2012a), enzymatic interesterification (Jiménez et al., 2010a; 2010b) or chemical interesterification (Zou et al., 2011; Zou et al., 2012b). Palm stearin is fractionated using acetone as a solvent to produce a tripalmitin-rich triacylglycerol (92.0%) (Lee et al., 2010) and palmitic acid (88.57%-92.30%) (Ghosh et al., 2016; Wang et al., 2019; Zou et al., 2012a). In general, the palm stearin solvent fractionation process condition is carried out at an acetone ratio of 5-9 and a fractionation temperature of 20°C-40°C for 3-24 hr.

Enzymatic interesterification between palm stearin (60.0% palmitic acid and 23.0% palmitic acid at the sn-2 position) with palmitic acid using Novozyme 435 produces a product with 68.0%-75.0% palmitic acid at the sn-2 position (Jiménez et al., 2010a; 2010b). Meanwhile, chemical interesterification of palm stearin (41.7% palmitic acid at the sn-2 position) resulted in a product with 58.0% palmitic acid at the sn-2 position (Zou et al.,

### Table 2. Triacylglycerols Composition of Palm Oil Fractions

| Triacylglycerols (%) | Palm oil* | Palm olein* | Palm stearin* | Soft palm stearin** | Hard palm stearin** | Palm kernel oil* |
|----------------------|-----------|-------------|---------------|---------------------|---------------------|-----------------|
| CCLa                 |           |             |               |                     |                     | 6.8             |
| CLaLa                |           |             |               |                     |                     | 9.9             |
| LaLaLa               |           |             |               |                     |                     | 21.2            |
| LaLaM                |           |             |               |                     |                     | 17.0            |
| LaLaO                |           |             |               |                     |                     | 5.3             |
| LaMM                 |           |             |               |                     |                     | 8.8             |
| PLL                  |           |             |               |                     |                     |                 |
| MMM                  | 0.4       | 0.6         | 0.2           | ND                  | ND                  |                 |
| LaLaP                |           |             |               |                     |                     | 1.2             |
| LaMO                 |           |             |               |                     |                     | 4.6             |
| MPL                  | 2.4       | 3.7         | 1.0           | 0.1                 | ND                  |                 |
| LaMP                 |           |             |               |                     |                     | 4.6             |
| LaOO                 |           |             |               |                     |                     | 3.8             |
| LaPO                 |           |             |               |                     |                     | 4.3             |
| LaPP+MMO             | 0.9       | 8.9         | 0.1           | 0.8                 | ND                  |                 |
| OOL                  | 1.8       | 2.6         | 0.8           | ND                  | ND                  | 0.7             |
| MMP                  | 2.0       |             |               |                     |                     |                 |
| MOO                  | 10.1      | 15.8        | 5.3           | 6.9                 | 0.9                 |                 |
| PPL                  | 9.8       | 11.2        | 7.8           | 8.1                 | 3.8                 | 0.6             |
| MPP                  | 0.6       | ND          | 2.3           | ND                  | ND                  |                 |
| OOO                  | 4.1       | 5.6         | 1.8           | 4.1                 | 4.5                 | 1.4             |
| POO                  | 24.2      | 36.3        | 12.0          | 18.0                | 3.0                 | 1.9             |
| PPO                  | 31.1      | 17.1        | 29.8          | 32.0                | 26.4                | 1.1             |
| PPP                  | 5.9       | 0.1         | 29.2          | 20.6                | 51.0                | 0.1             |
| SOO                  | 2.3       | 3.6         | 0.8           | ND                  | ND                  | 0.4             |
| PSO                  | 5.1       | 2.5         | 3.8           | 4.9                 | 2.9                 | 0.4             |
| PSS                  | 0.9       | ND          | 5.2           | 3.5                 | 7.4                 |                 |
| SSO                  | 0.5       | ND          | ND            | ND                  | ND                  |                 |

Note: ND - not detected; L - lauric acid; M-myristic acid; O - oleic acid; P - palmitic acid; S - stearic acid.
Source: *Tan and Man (2002); ** Ibrahim et al. (2006).
2012b). In other studies, Zou et al. (2011) reported chemical interesterification of palm stearin (56.8% palmitic acid at the sn-2 position) and manufactured a product with 69.8% palmitic acid at the sn-2 position.

Palm oil fractions can also produce palmitic acid and oleic acid as palmitic and oleic acyl donors for the sn-2 palmitate synthesis. Both are manufactured by hydrolysis of palm oil fractions, then separated from other fatty acids (Esteban et al., 2011; Jimenez et al., 2010a). Besides palmitic acid, ethyl palmitate can also be used as a palmitic acyl donor (Pina-Rodriguez and Akoh, 2009; Turan et al., 2013), produced through the esterification of palmitic acid with ethanol. Likewise, ethyl oleate is an oleic acyl donor (Lee et al., 2010). Palmitic acyl donors are used as a substrate for improving palmitic acid at the sn-2 position of oil and fat. Meanwhile, acyl oleic donors are used to increase sn-1,3 oleic acid of oils and fats that contain high tripalmitin.

| Fatty acids (%) | Total | sn-2 |
|-----------------|-------|------|
| C12:0           | ND - 0.9 | ND - 0.3 |
| C14:0           | 1.3 - 1.7 | ND - 1.0 |
| C16:0           | 68.8 - 70.1 | 23.0 - 70.1 |
| C18:0           | 4.8 - 5.2 | 0.7 - 2.9 |
| C18:1           | 18.7 - 29.0 | 30.9 - 65.2 |
| C18:2           | 3.9 - 7.5 | 8.3 - 12.6 |
| C18:3           | ND - 0.3 | ND - 0.1 |

Source: Jimenez et al. (2010a, 2010b); Zou et al. (2011; 2012b).

**LIPASE FOR PALM-BASED sn-2 PALMITATE SYNTHESIS: TYPES AND SOURCES**

Lipase (triacylglycerol hydrolase, EC 3.1.1.3) is a biocatalyst that naturally acts on carboxylate ester bonds to catalyse the hydrolysis of triacylglycerol (Araújo et al., 2016). This substrate is insoluble in water, and the reaction usually occurs at the organic-water interface, where lipase works best (Adlercreutz, 2013). In non-aqueous media, lipase catalyses esterification, acidolysis, alcoholysis, and interesterification (Araújo et al., 2016; Rodrigues and Fernandez-Lafuente, 2010; Speranza and Macedo, 2012). Lipase has serine-histidine-aspartate catalytic active sites, which is responsible for its catalytic activity (Fernandez-Lafuente, 2010; Ortiz et al., 2019; Rodrigues and Fernandez-Lafuente, 2010). Lipase shows variable stability against the extreme pH conditions, the appearance of organic solvents, and ionic liquids (Kapoor and Gupta, 2012).

Lipase is an excellent biocatalyst for synthesising structured lipids, and triacylglycerol with fatty acids at a specific position (Iwasaki and Yamane, 2000). HMFS is a structured lipid produced using various types of enzymes, substrates, and acyl donors (Soumanou et al., 2013). The lipases commonly used for HMFS synthesis are Novozyme 435, Lipozyme RM IM, and Lipozyme TL IM (Table 4).

Lipozyme TL IM is obtained from Thermomyces lanuginose and is immobilised using silica. Lipozyme TL IM can maintain activity at 55°C-60°C (Fernandez-Lafuente, 2010) and shows positional specificity at the sn-1,3 (Soumanou et al., 2013). Lipozyme RM IM is derived from Rhizomucor miehei and is immobilised using Duolite ES 562. Lipozyme RM IM is highly specific in the choice of substrate, stereospecific, regioselective, active and stable (Rodrigues and Fernandez-Lafuente, 2010; Zou et al., 2014). Lipozyme RM IM also shows positional specificity at the sn-1,3 (Soumanou et al., 2013). Novozyme 435 is generated from Candida antarctica lipase B and is immobilised using acrylic resin. Novozyme 435 is one of the most stable commercial lipases commonly used for various reactions (Ortiz et al., 2019). Novozyme 435 can be used at 60°C-70°C (Soumanou et al., 2013). When the substrate is triacylglycerol, Novozyme 435 does not show positional specificity (Jiménez et al., 2010a; 2010b; Soumanou et al., 2013).

**METHODS FOR PALM-BASED sn-2 PALMITATE SYNTHESIS**

Interestereification is an accepted oil and fat modification technique by redistributing the fatty acid groups between and within the triacylglycerol. After the interestereification of the substrate, the product has a distinct chemical composition and improved physical characteristics (Pacheco et al., 2015). Enzymatic interestereification can be carried out non-specifically and specifically (Silva et al., 2012). Non-specific enzymatic interestereification is a random process similar to chemical interestereification. Meanwhile, specific enzymatic interestereification is an acyl exchange process to a particular position, mainly at the sn-1,3 position using regioselective lipase (Gibon et al., 2009).

In general, sn-2 palmitate synthesis (especially OPO) is fabricated by a one-step reaction including transesterification or acidolysis and two-step reactions such as alcoholysis and esterification or two-step acidolysis (Hasibuan et al., 2021) (Figure 1). Apart from oleic acid (monounsaturated fatty acid, MUFA), fatty acids that are incorporated at the sn-1,3 position of sn-2 palmitate are fatty acids of MCFA (Karouw et al., 2012), LPCUFA (Ghosh et al., 2016; Nagachinta and Akoh, 2012; 2013), MUFA and LPCUFA (Wang et al., 2019; Zou et al., 2012) or MCFA, MUFA and LPCUFA (Hasibuan and Ijah, 2016; Karabulut et al., 2007; Zou et al., 2011; 2012b). Process conditions for palm-based sn-2 palmitate...
| Type of reaction | Lipase     | Substrate                                                                                       | Solvent system          | Mode of operation | Lipase loading (%) | Substrate ratio (mol) | Temperature (°C) | Time (hr) | Characteristics of product                                                                                     | Reference       |
|-----------------|------------|-------------------------------------------------------------------------------------------------|-------------------------|-------------------|--------------------|-----------------------|------------------|-----------|----------------------------------------------------------------------------------------------------------------|-----------------|
| Transesterification | Lipozyme TL IM | Palm oil, palm kernel oil, olive oil, sunflower oil, and marine oil                             | Solvent-free system     | Batch             | 10                 | 4.0:3.5:1.0:1.5:0.2 | 60               | 6         | 23.00% palmitic acid and distributed at sn-2 41.5%                                                            | Karabulut et al. (2007) |
| Transesterification | Lipozyme TL IM | Palm stearin fractionates and ethyl oleate                                                      | Solvent-free system     | Batch             | 10                 | 1.5:5 | 50               | 3         | 31.43% OPO, 80.60% palmitic acid at the sn-2 position, and 64.90% sn-1,3 oleic acid                            | Lee et al. (2010) |
| Transesterification | Lipozyme TL IM | Palm stearin fractionates and PUFA-rich fish oil                                               | Solvent-free system     | Batch             | 10                 | 2:1                  | 60               | 12        | 75.98% palmitic acid at the sn-2 position, 0.27% arachidonic acid, 3.43% eicosapentaenoic acid, 4.25% docosahexaenoic acid, and melting point of 42°C | Ghosh et al. (2016) |
| Transesterification | Novozyme 435 | Palm stearin, palm kernel oil, soybean oil, olive oil, and tuna fish oil                       | Solvent-free system     | Batch             | 10                 | 2.9:3:4:1:5:2:0:2 | 60               | 4         | Fatty acids composition resembles human milk fat, melting point of 28°C                                        | Hasibuan and Ijah (2016) |
| Acldolysis      | Lipozyme RM IM | Palm stearin and a mixture of fatty acid from rapeseed oil, sunflower oil, palm kernel oil, stearic acid, and myristic acid | Solvent-free system     | Batch             | 10.7               | 1:14.6               | 57               | 3.4       | 29.70% palmitic acid and 62.80% palmitic acid at the sn-2 position                                             | Zou et al. (2011) |
| Acldolysis      | Novozyme 435 | Palm olein, docosahexaenoic acid, and arachidonic acid                                          | Hexane                  | Batch             | 10                 | 1:18                 | 60               | 24        | Docosahexaenoic acid +arachidonic acid incorporated in triacylglycerol 25.25% (w/w) and docosahexaenoic acid +arachidonic acid incorporated at sn-2 17.20% (w/w) | Nagachinta and Akoh (2012) |
| Acldolysis      | Novozyme 435 | Palm olein and a mixture of fatty acid (23.23% docosahexaenoic acid, 31.42% gamma-linolenic acid, and 15.12% palmitic acid) | Hexane                  | Batch             | 10                 | 1:2                  | 60               | 22.7      | 35.11% palmitic acid at the sn-2 position, 3.75% docosahexaenoic acid, and 5.03% gamma-linolenic acid          | Nagachinta and Akoh (2013) |
| Type of reaction | Lipase | Substrate | Solvent system | Mode of operation | Lipase loading (%) | Substrate ratio (mol) | Temperature (°C) | Time (hr) | Characteristics of product | Reference |
|-----------------|--------|-----------|----------------|------------------|-------------------|---------------------|----------------|-----------|----------------------------|-----------|
| Acidolysis      | Lipozyme RM1IM | Palm stearin and fatty acid from rapeseed oil | Solvent-free system | Batch | 8 | 1:10 | 60 | 4 | 39.60% palmitic acid and 70.50% distributed at sn-2 | Zou et al. (2012a) |
| Acidolysis      | Lipozyme RM1IM | Palm stearin and a mixture of stearic acid, myristic acid, fatty acid from rapeseed oil, sunflower oil, and palm kernel oil | Solvent-free system | Packed bed reactor | 19.5 | 58 | 2.7 | | 28.80% palmitic acid and 53.20% distributed at sn-2 | Zou et al. (2012b) |
| Acidolysis      | Lipozyme RM1IM | Palm stearin fractionates and fungal oil from Mortierella alpina ALK (1:0.3) and oleic acid | Solvent-free system | Batch | 8 | 1:6 | 60 | 6 | Oleic acid incorporated 53.50% in triacylglycerol, fatty acid at sn-2: 68.70% palmitic acid, 9.80% arachidonic acid, and 7.9% oleic acid | Wang et al. (2019) |
| Acidolysis      | Lipozyme RM1IM immobilised on macroporous acrylic resin | Palm stearin fractionates, oleic acid and linoleic acid 2-monoacylglycerol from alcoholysis of palm stearin, then esterified with lauric fatty acid methyl ester from coconut oil | Solvent-free system | Batch | 8 | 1:6:4 | 60 | 4 | OPO and OPL 69.26%, 87.75% palmitic acid at the sn-2 position | Wang et al. (2020) |
| Alcoholysis and esterification | Lipozyme RM1IM | Hexane | Batch | 10 | 1:3 | 50 | 12 | MCFA 43.86% with lauric acid 39.37%, 24.18% palmitic acid at the sn-2 position | Karouw et al. (2012) |
| Two-step acidolysis | Acidolysis I: Novozyme 435 Acidolysis II: Lipase DF (Rhizopus oryzae) | Acidolysis I: Palm stearin and palmitic acid Acidolysis II: Palmitic acid-rich triacylglycerol from palm stearin and oleic acid | Hexane | Batch stirred tank reactor | Acidolysis I: 9.6 g lipase × h/g triacylglycerol Acidolysis II: 0.4 g lipase × h/g triacylglycerol | Acidolysis I: 1:3 | Acidolysis II: 1:6 | 37 | 67.20% sn-1,3 oleic acid and 67.80% palmitic acid at the sn-2 position | Esteban et al. (2011) |
synthesis are presented in Table 4. Sn-2 palmitate synthesis can be performed in a solvent or solvent-free system. However, the solvent-free system is advisable for HMFS synthesis in terms of food safety and low production costs (Ferreira-Dias et al., 2019).

**Transesterification.** Transesterification is a reaction between 1) triacylglycerol and triacylglycerol or 2) triacylglycerol and esterified fatty acids (Hassim et al., 2018; Wei et al., 2019). In the first type of reaction, Karabulut et al. (2007) conducted the interesterification of a mixture of palm oil, palm kernel oil, olive oil, sunflower oil, and marine oil using Lipozyme TL IM. The product contained 23.0% palmitic acid and 41.5% palmitic acid at the sn-2 position. Ghosh et al. (2016) reported transesterification between palm stearin fractionated with PUFA-rich fish oil with Lipozyme TL IM produced HMFS with 75.98% palmitic acid at the sn-2 position, 0.27% arachidonic acid, 3.43% eicosapentaenoic acid, and 4.25% docosahexaenoic acid. In the synthesis using the second reaction, Lee et al. (2010) reported transesterification of palm stearin fractions and ethyl oleate using Lipozyme TL IM. The product contained 31.43% OPO, 80.6% palmitic acid at the sn-2 position, and 64.9% oleic acid at the sn-1,3 position.

**Acidolysis.** Acidolysis is a reaction of triacylglycerol and fatty acid (Hassim et al., 2018; Wei et al., 2019). Acidolysis between triacylglycerol contains high palmitic acid at the sn-2 position from palm stearin with MUFA, MCFA, and LCPUFA using Lipozyme RM IM has been reported by Zou et al. (2012a; 2012b) and Wang et al. (2019). Meanwhile, Nagachinta and Akoh (2012) used Novozyme 435 for acidolysis of palm olein with docosahexaenoic acid, and arachidonic acid to produce triacylglycerol with 25.25% (w/w) docosahexaenoic acid+arachidonic acid.
acid incorporation and 17.20% (w/w) docosahexaenoic acid + arachidonic acid at the sn-2 position. Nagachinta and Akoh (2013) also reported Novozyme 435 for acidolysis between palm olein with palmitic acid, docosahexaenoic acid, and gamma linoleic acid to produce triacylglycerol containing palmitic acid at the sn-2 position, docosahexaenoic acid, and gamma linoleic acid 35.11%, 3.75% and 5.03%, respectively.

Two-step process. The two-step process can be carried out by alcoholysis of triacylglycerol using a specific lipase sn-1,3 to produce sn-2 monoacylglycerol and then sn-2 monoacylglycerol esterified with fatty acid (Wei et al., 2019) or esterified fatty acid (Karouw et al., 2012). This method produces high yield and purity (Soumanou et al., 2013). The two-step process can also be conducted using two-step acidolysis, as reported by Esteban et al. (2011). First, palm stearin is acidolysed with palmitic acid using Novozyme 435 to produce triacylglycerol containing high palmitic acid at the sn-2 position. Second, triacylglycerol is acidolysed with oleic acid using lipase D of Rhizopus oryzae. The product had 67.80% palmitic acid at the sn-2 position and 57.20% distributed at the sn-2 position.

REACTORS FOR PALM-BASED sn-2 PALMITATE SYNTHESIS

The challenge in structured lipid synthesis by enzymatic interesterification is that production costs are relatively high, so it is necessary to use continuous processes and cost-effective catalysts (Bourliou et al., 2009; Jala and Kumar, 2018). The selection of reactors is essential to fabricate high product yields. A batch and continuous reactor are used in the enzymatic interesterification for sn-2 palmitate. The batch reactor is usually suitable for operation at a laboratory scale, whereas the continuous system is very appropriate for an industrial scale. The optimal reaction conditions for palm-based sn-2 palmitate synthesis in a batch reactor are enzyme load of 8.0%–10.0% (w/w of the total substrate), temperature 40°C–60°C, and reaction time 3-24 hr.

Continuous enzymatic interesterification is an economical technology for large-scale production because of its minimal costs, ease of operation, and being able to control the fatty acid distribution due to the selectivity and regiospecific of lipases (Silva et al., 2012). The continuous system commonly used in enzymatic interesterification is a packed bed reactor (Soumanou et al., 2013; Zou et al., 2012b). The advantages of a packed bed reactor over batch reactors are due to relatively high enzyme stability, ease of operation on large scales, high reaction rates, and mass transfer, thereby reducing the occurrence of acyl migration (Sen et al., 2016; Zou et al., 2012b).

A packed bed reactor is best applied continuously on an industrial scale to minimise the labour and costs of the processes (Nielsen et al., 2006). Zou et al. (2012b) used a packed bed reactor in acidolysis between interesterified palm stearin with a mixture of stearic acid, myristic acid and fatty acids from rapeseed oil, sunflower oil, and palm kernel oil. The optimum conditions obtained using the response surface methodology approach were a substrate ratio of 9.5 mol/mol with a residence time of 2.7 hr at 58°C. The final product contained 28.8% palmitic acid and 53.2% palmitic acid at the sn-2 position.

ENHANCING OF sn-2 PALMITATE-RICH HMFS THROUGH FRACTIONATION

Karabulut et al. (2007) reported on the enzymatic interesterification of a mixture of palm oil, palm kernel oil, olive oil, sunflower oil, and marine oil was not optimum enough to produce a product with high palmitic acid at the sn-2 position. Fractionation can be applied in HMFS products obtained through enzymatic interesterification to increase triacylglycerol containing high palmitic acid at the sn-2 position (Hasibuan et al., 2021c).

Fractionation of the acidolysis product between butterfat and a mixture of fatty acids from rapeseed oil and soybean oil using acetone as solvent at a ratio of 2.5, temperature of 0°C for 3 hr was reported by Sørensen et al. (2010). The product contained 56.12% palmitic acid at the sn-2 position. This value was higher than the product obtained from butterfat’s acidolysis fractionated first (47.26%). In another study, Lee et al. (2015) reported the OPO content enhanced from 25.2% to 53.3% after fractionation at 22°C for 12 hr of the interesterified palm oil and camellia oil.

FUTURE OUTLOOK: CHALLENGES AND OPPORTUNITIES IN sn-2 PALMITATE SYNTHESIS

Betaprol is commercial sn-2 palmitate developed by Loders Croklaan in 1995 through acidolysis of the tripalmitin-rich palm stearin with oleic acid from high oleic sunflower oil using Lipozyme RM IM as a biocatalyst (Wei et al., 2020). Palm oil fractions will continue to be developed as a substrate for sn-2 palmitate production because of the following advantages; high palmitic acid content, abundant availability, and low price (Hasibuan, 2021a). In addition, palm oil is one vegetable oil that does not contain cholesterol (Gesteiro et al., 2019). Palm stearin as a substrate in the sn-2 palmitate synthesis is very interesting. However, the melting point of palm stearin is relatively high, so in the enzymatic process, it is necessary to add organic solvents (such
as hexane) or be carried out at a sufficiently high reaction temperature (Jimenez et al., 2010a; 2010b).

The addition of solvents can lead to an increase in production costs and potential toxicity. In addition, the use of solvents is not recommended in terms of food safety (Ferreira-Dias and Tecelão, 2014). Thus, the production of HMFS in solvent-free systems is preferred in terms of food safety, cost, environmental friendly and ease of product purification (Ferreira-Dias and Tecelão, 2014; Tecelão et al., 2019). However, the reaction in a solvent-free system needs to be carried out at a high temperature (Hasibuan, 2021a), which affects the lipase stability. For this reason, the reaction in a solvent-free system can use immobilised enzymes, which have higher stability than the original free suspended enzymes. Enzyme immobilisation can prevent denaturation and leakage of enzymes so that the number of batches or the duration of synthesis can be increased (Adlercreutz, 2013).

Several studies reported that the use of commercial immobilised lipases such as lipases of Novozyme 435, Lipozyme TL IM, and Lipozyme RM IM in the synthesis of HMFS show good activity and stability (Hasibuan, 2021a). Jimenez et al. (2010a) reported that lipase Alcaligenes sp. immobilised on diatomaceous earth remained stable for at least 11 times using acidolysis between palm stearin and palmitic acid at a mole ratio of 1:3, reaction temperature of 65°C and reaction time of 24 hr. In addition, Esteban et al. (2011) reported that the lipase Rhizopus oryzae immobilised on Accurel MP1000 remained stable for at least ten times usage in acidolysis between palm stearin high in palmitic acid at the sn-2 position and oleic acid at a mole ratio of 1:6, 50°C for 19 hr. Exploring new biocatalysts with high catalytic activity and operational stability through isolation and genetic engineering is of interest for future research (Wei et al., 2020). Efficient and stable biocatalysts will reduce operating costs (Tecelão et al., 2019).

In contrast to lard, catfish oil, and butterfat oil, palm stearin has high sn-1,3 palmitic acid, requiring a high ratio of fatty acyl donors. The use of a high acyl donor ratio is unattractive because of the difficulty of the separation process (Zou et al., 2016b), so the cost for separation after processing is high (Zhang et al., 2016). Thus, it is necessary to develop specific lipases to synthesise palm-based HMFS using palm stearin with low acyl donor ratios. Faustino et al. (2016) reported that the acidolysis between tripalmitin and fatty acids from camellina oil at a low fatty acid mole ratio of 1:1.2 using lipase Rhizopus oryzae immobilised on Lewatit VPOC 1600 at a reaction temperature of 65°C could use up tripalmitin at 62.7% w/w.

Specialised treatments such as crystallisation fractionation are important to enrich palmitic acid at the sn-2 position. Acyl donors (MUFA, MCFA, and PUFA) will continue to be explored to produce palm-based HMFS resembling human milk fat (Hasibuan et al., 2021c). In addition, palm-based HMFS formulation for infant formula needs to be developed according to the baby’s needs (age and condition) and regulations related to infant formula. Commercial formula is divided into three stages depending on the age of the baby, namely infant formula (0-6 months, stage 1), follow-up formula (6-12 months, stage 2), growth formula (12-36 months, stage 3). The rules for formulas of the various stages may differ (Wei et al., 2019).

The relevant regulations enacted by several authorities to regulate infant formula include the Codex Alimentarius Commission (CAC), the US Food and Drug Administration (FDA), the European Commission (EC), and the National Health Commission of the People’s Republic of China (NHC). CAC, EC and NHC require that ω-3 and ω-6 LCPUFAs be less than 1.0% of total fat acids, trans-fatty acids should be less than 3.0% of total fatty acids, erucic acid should be less than 1.0% of total fatty acids and eicosapentaenoic acid levels should be no more than docosahexaenoic acid levels. Docosahexaenoic acid is recommended as the essential constituent (4.8-12 mg/100 kcal). The EC and NHC recommend that docosahexaenoic acid do not exceed 2.0% and 0.5% of total fatty acids, respectively, and arachidonic acid levels should not exceed 1.0%. The EC recommended that the ω-3 and ω-6 LCPUFAs be less than 1.0% and 2.0% of the total fatty acids, while the CAC allowed the addition of LCPUFAs, respectively. CAC requires no commercial hydrogenated fats and oils to be used as raw materials. The EC also states that sesame seed oil and cottonseed oil are not allowed in infant formula because of potential allergens (Wei et al., 2019).

**CONCLUSION**

Primarily, palm stearin is used as a substrate for sn-2 palmitate synthesis for HMFS. Triacylglycerol rich in palmitic acid at the sn-2 position of palm stearin needs to be increased to be an excellent substrate for the production of HMFS with palmitic acids at the sn-2 position >60%. The selection of enzyme types and loading, substrate types, synthesis methods, and reactor configuration is essential to improve the efficiency of HMFS synthesis. Generally, the optimal reaction conditions for palm-based sn-2 palmitate synthesis in a batch reactor are enzyme load of 8%-10% (w/w of the total substrate), temperature of 40°C-60°C, and reaction time of 3-24 hr. The OPO content in palm-based HMFS produced by enzymatic interesterification can be increased using fractionation. The challenge in HMFS synthesis is
the high production cost. In addition, the resulting product must resemble HMF, hence the complete set of acyl donors from fatty acids such as MUFA, MCFA and PUFA must be present in HMF. The reduction in the production cost of HMFS can be accomplished through the exploration of acyl donors and novel lipase enzymes with high catalytic activity and stability at a low cost.

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