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CAPRYLINS: PROPERTIES, APPLICATIONS, MANUFACTURING AND RELATED ISSUES

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ABSTRACT
Caprylins are esters of glycerol and caprylic acid that find applications in food, pharmaceutical and cosmetic fields. They have low melting points (10^oC – 30^oC), high boiling points (233^oC – 395^oC), octanol-water partitioning coefficient (Log P) higher than 1.9, being insoluble in water and soluble in organic solvents such as n-hexane and chloroform. Several studies indicate a positive role played by caprylins on the human metabolism – as an alternative to long chain fatty acid lipids- due their low accumulation in adipose tissue, easy intracellular oxidation and high formation of ketone bodies. Therefore, they may be useful for patients that present neurological disorders and/or dietary requirements. Monocaprylin and dicaprylin are considered GRAS emulsifiers (Generally Recognized as Safe) by FDA, being largely applied on foods with no major concerns. They also can act as antimicrobial agents for food conservation purposes. There are several manufacturing processes applicable to caprylins, including the direct esterification of glycerol and caprylic acid catalyzed by immobilized lipase, method that has been receiving special attention during the last two decades. Caprylins can be identified and/or quantified through several analytical techniques (including titration by sodium hydroxide, gas and liquid chromatography coupled to a variety of detection methods, thin layer chromatography and differential scanning calorimetry Despite the existing information on caprylins, the amount of scientific material available about these compounds is much lower than the observed on the long chain glycerides. The development of more efficient manufacturing methods, lowering the production costs and increasing market availability, plays a key role for encouraging further research on clinical, pharmaceutical and technological applications.

Key-words: Monocaprylin, dicaprylin, tricaprylin, caprylins manufacturing, medium chain glycerides.

Abbreviations: GRAS- Generally Recognized as Safe; FDA- Food and Drug Administration; MAG-monoacylglycerol; DAG- Diacylglycerol; TAG- Triacylglycerol; MCT- Medium-chain triglyceride; LCT- Long-chain triglyceride; MCFA- Medium Chain Fatty Acid; HPV- High Production Volume; MCG- Medium Chain Glyceride; FFA- Free Fatty Acid; LDH- layered double hydroxide; GC- gas chromatography ; HPLC- high performance liquid chromatography ; AV- Acid Value; HTGC- High Temperature Gas Chromatography ; FID-Flame Ionization Detector ; MS-Mass Spectrometer; NP- Normal-phase; RP- Reversed-phase; TLC- Thin Layer Chromatography ; HPTLC - High Performance Thin Layer Chromatography ; DSC- Differential Scanning Calorimetry.

1. INTRODUCTION

Esters of glycerol and fatty acids are known as glycerides or acylglycerols. Glycerides may contain one, two or three fatty acids esterified to the glycerol molecule. Therefore, they can be classified as mono- (MAG), di- (DAG) and triacylglycerols (TAG) [dataset] (Indrasena, 2006). They are valuable chemical compounds that find application in pharmaceuticals, food and cosmetic production [dataset] (Kotwal et al. 2011). The specific properties of a glyceride depend on a number of characteristics such as fatty acid chain length and degree of unsaturation.

Natural fats and oils are mixtures comprised of more than 95% of TAG from which fatty acids can be derived from hydrolysis, therefore the composition of fats and oils is frequently given in terms of fatty acid composition [dataset] (Gelardi et al, 2016; Peyronel, 2018). Natural fatty acids are medium to long-chain carboxylic acids with an even number of carbon atoms between 4 and 26 [dataset] (Gelardi et al, 2016). Medium-chain triglycerides (MCT) and long chain triglycerides (LCT) present very different properties due their chain length difference.

The natural availability of LCTs in dietary fats is remarkable higher than the one presented by MCTs [dataset] (Marten et al. 2006; Peyronel, 2018). Therefore, researches on possible health, technology and other applications trend to be more focused on LCTs too. Caprylins, glycerides constituted by 8 carbon-length fatty acids, are MCTs that illustrate well this panorama: gathering data about them can be pretty challenging.

There are different manufacturing routes available for both LCTs and MCTs, being the obtainment processes for LTCs currently better stablished. There are numerous variables that can influence the process course and outcome, including but not limited to: molar ratio; catalysts; temperature; duration of the reaction and presence of solvents. The development of greener processes is a growing trend and enzyme catalyzed reactions are key aspects in this scenario [dataset](Rajendran et al 2009; Pappalardo et al, 2017).

The development of more efficient obtainment processes is essential to encourage more studies about MCTs, including caprylins. Besides, adequate methods for separation, identification and quantification of caprylins are fundamental to access the method development.

Therefore, it is worth to summarize the available information on caprylins, extending the review to other MCTs when necessary and making appropriate comparisons to LCTs. This review will provide valuable and recent information about chemical and physical properties, industrial and health applications, manufacturing methods and characterization of caprylins. Hopefully, this material will assist and encourage the scientific community to pursuit the deepening of knowledge about these compounds, which present great potential in many fields.

2. GENERAL PROPERTIES OF ACYLGLYCEROLS

Triglycerides are considered neutral lipids, which is the most abundant class of lipids: fats and vegetable oils are constituted mainly by a mixture of TAG and contain only traces of MAG and DAG [dataset] (Regitano-D'arce, 2006). Besides, storage fat in animal tissues consists entirely of triacylglycerols [dataset] (Indrasena, 2006).

The amphiphilic structure presented by MAG and DAG is responsible for the extensive use of these lipids in several fields: In the pharmaceutical industry, they are often used as binders in tablets [dataset] (Kaewthong et al, 2005), while in the food industry, MAG is one of the most important emulsifiers for several foods, such as baked goods, ice cream and margarine [dataset] (Fregolente et al, 2010). Frequently, when the target effect is emulsification, a mixture of MAG and DAG is used, since it is cheaper and offers proper performance [dataset] (Fregolente et al, 2010).

Diacylglycerols are also known for their benefits in obesity and weight-related disorders. [dataset] Lo et al (2008) conducted a survey of literature that has shown the effects of DAG on the reduction in the accumulation of body fat in both animals and humans, probably due the faster metabolic pathway they are submitted to. Therefore, DAGs can also be used as a substitute for TAG in the composition of some foods.

2.1. Medium Chain Fatty Acids

Saturated fatty acids that have 6 to 10 carbons are considered medium chain fatty acids (MCFA). Typical MCFAs are hexanoic acid (C6:0), octanoic acid (C8:0) and decanoic acid (C10:0), commonly named caproic, caprylic and capric acids, respectively (Table 1). Sometimes, dodecanoic acid (12:0, commonly named lauric acid) is included [dataset] (Marten et al. 2006; Lu et al, 2017).

Table 1

Physical-chemical properties of medium chain saturated fatty acids and caprylins.

Compound (name)	Boiling Point (^oC)	Melting Point (^oC)	Refractive index (n_{D20})	Density (g/cm^3)	Solubility (g/100g water)	Solubility in solvents	*Log P
Hexanoic acid (caproic acid)[a]	205	- 3	1.4163	0.926	1.08	Ethanol, ether	1.84
Heptanoic acid (enanthic acid)[a]	223	- 7	1.4216	0.918	0.24	Ethanol, ether, DMF, DMSO	2.37
Octanoic acid (caprylic acid)[a]	240	17	1.4280	0.910	0.07	Ethanol, ether, CS_2, $CHCl_3$	2.90
Nonanoic acid (pelargonic acid)[a]	253	12	1.4330	0.948	Insoluble	Ethanol, ether, $CHCl_3$	3.43
Decanoic acid (capric acid)[a]	270	31	1.4288	0.878	0.01	Ethanol, ether, benzene, CS_2, $CHCl_3$	3.96
Monocaprylin[b,c]	340	30	1.4650	1.000	0.12	n-hexane	1.91
1,2-dicaprylin[b,c]	395	20	1.4600	1.000	insoluble	n-hexane	5.84
1,3-dicaprylin[b,c]	391	20	1.4600	1.000	Insoluble	n-hexane	5.25
Tricaprylin[c,d]	233	10	1.4482	0.954	Insoluble	Ether, benzene, ligroin, $CHCl_3$	9.33

*Octanol/water partitioning coefficient.

[a] Data obtained from Merck Index [dataset] (O'Neil,2013)

[b] Data obtained from Dictionary of Food compounds [dataset] (Yannai, 2012)

[c] Data obtained from [dataset] Silva et al, 2015.

[d] Data Obtained from CRC Handbook of Chemistry and Physics [dataset] (Lide, 2007)

Dietary fats are mainly constituted by fatty acids with a chain length of 14 carbons and more [dataset] (Marten et al. 2006). However, MCFAs can be found in some natural sources, such as in the milk of some mammals, including the human and bovine milk. In fact, three of the MCFA (caproic, caprilyc and capric) have been named after goats, once they contribute to 15% of the total content in goat milk in comparison to 5% in cow milk [dataset] (Kompan, Komprej, 2012). Coconut oil is also a source of these fatty acids, especially lauric acid, which contributes to 50% of the oil composition [dataset] (Trevizan, Kessler, 2009). The caprylic acid can also be found in the seeds oils of some species belonging to the *Cuphea genus*, with a remarkable 75% content reported for the *C. avigera var. pulcherrima* specie, commonly found in Mexico [dataset] (Graham et al, 2016).

There are several methods available for MCFAs' manufacturing, including the distillation of natural fatty acids. Some specific manufacturing methods are: the crude fermentation of butyric acid to obtain caproic acid [dataset] (NCBI, 2018a); oxidation of octanol and preparation from 1-heptene to obtain caprylic acid [dataset] (NCBI, 2018b) and the oxidation of decanol to obtain capric acid [dataset] (NCBI, 2018c). These three fatty acids are included in the 2007 OECD High Production Volume Chemicals list, the most updated OECD list, which contains chemicals which are produced or imported at levels greater than 1,000 tonnes per year in at least one-member country/region (eight countries in addition to European Union's HPV list) [dataset] (OECD, 2009).

Compared to long chain fatty acids, MCFAs have smaller molecular size and higher solubility in the body fluid. These properties contribute to the differentiated metabolism presented by glycerides composed of medium chain fatty acids.

3. CAPRYLINS

3.1. Physical And Chemical Properties

Medium chain glycerides (MCG) are those derived from MCFAs. The acylglycerols that contain one, two or three caprylic acid molecules esterified to a glycerol molecule are generally named caprylins (Figure 1).

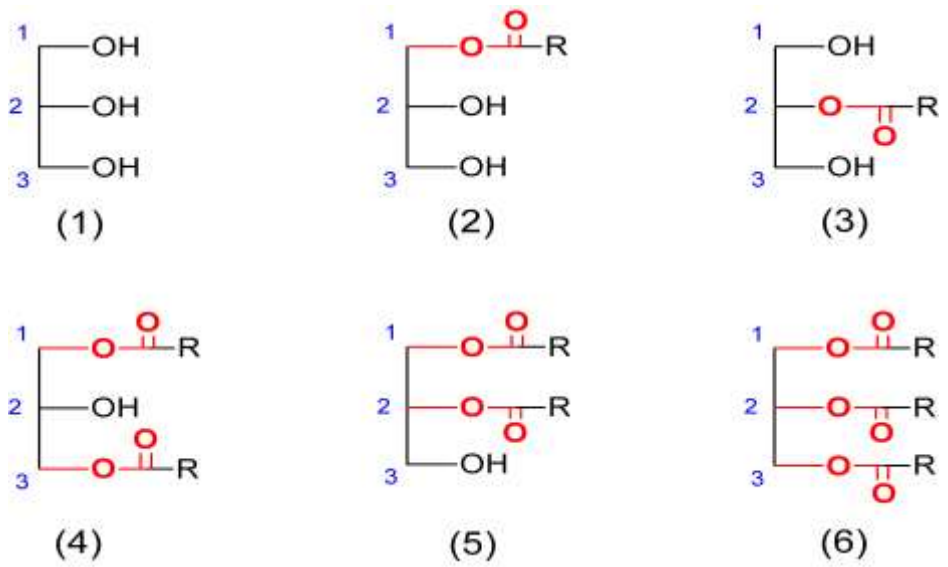

Figure 1. Molecular structures of caprylins: (2) 1-monocaprylin; (3) 2-monocaprylin; (4) 1,3-dicaprylin; (5) 2,3-dicaprylin; (6) tricaprylin; and glycerol (1). Symbols: R represents the radical heptyl [$-(CH_2)_6-CH_3$] and the ester bonds are indicated in red.

Comparing to triglycerides containing mainly saturated long-chain fatty acids, MCTs have a lower melting point (Table 1), are liquid at room temperature and have less chemical potential energy. All these properties affect the way MCFAs are absorbed and metabolized [dataset] (Marten et al., 2006).

From Figure 1 we observe that 1-monocaprylin and 1,2-dicaprylin have one asymmetric carbon atom (C2 of glycerol backbone), which could lead to a molecular dissymmetry and, as consequence, to the existence of enantiomers. Searching throughout the literature it can be found some information about enantiomers (2R and 2S) related to 1,2-dicaprylin [dataset] (Piyatheerawong, et al., 2005), but nothing about the possible enantiomers of 1-monocaprylin. Probably the enantiomers for these compounds are irrelevant for their envisaged application in industry and health. The antimicrobial capacity of caprylins is probably a result of the Log P value (Table 1) – the lipophilic character enable the caprylins molecules to diffuse easily through the lipid layer of external (cell wall and cytoplasm membrane) and internal (cytoplasm organelles) cell structures causing their disarrangement - than any intrinsic molecular dissymmetry of them. Another point to be enhanced is the isomerism which can easily be seen regarding monocaprylin (the octanoate ester is positioned at C1 or C2 of glycerol backbone) and dicaprylin (the octanoate esters are positioned at C1 and C2 or C1 and C3 of glycerol backbone). Similarly to the stereoisomerism, the isomerism is not a subject of interest if considered the minimum information available on the literature.

3.2. Industrial And Health Applications

Long chain triglycerides (LCT) have been substituted by medium chain triglycerides (MCT), once they originate smaller molecules that go through a faster metabolic path, thus reducing their adipose tissue uptake [dataset] (Mumme, Stonehouse, 2015). Besides, they also provide quick delivery of energy via oxidation, thus been beneficial for some patients, infants and individuals that require special diets [dataset] (Lu et al, 2017). Additionally, the metabolism of MCTs generates ketone bodies, which may be beneficial for a variety of neurological disorders, as suggested by several studies [dataset] (Farah, 2014).

In a retrospective cohort study, [dataset] Maynard and Gelblum (2013) studied the efficacy of tricaprylin in patients with mild to moderate Alzheimer's disease. The results indicate that the tricaprylin's addition to the usual pharmacotherapy for Alzheimer's disease was associated with the stabilization or improvement of the disease and of the ability to perform daily activities for the majority of patients after approximately 19 months of administration.

Medium chain mono- and diglycerides are capable of enhancing the absorption of drugs through the intestinal mucosa, due their ability of interacting with membrane lipids and proteins, increasing membrane permeability [dataset] (Hosmer et al, 2009). Therefore, they are common tools for enhancing the oral bioavailability of poorly soluble drugs, being often included in lipid formulations that can also contain long chain lipids and other surfactants and/or co-solvents [dataset] (Lee et al. 2013)

In food and cosmetic industry, MCGs are considered safe and environmental friendly (Deleu, Paquot, 2004). In fact, the Food and Drug Administration considers that mono- and diglycerides are generally recognized as safe (GRAS) and that can be used in food with no limitation other than current good manufacturing practices [dataset] (FDA, 2018).

Caprylins can potentially be used in foods in order to prevent contamination by pathogenic microorganisms. Studies have shown that caprylic acid and monocaprylin are both capable of inactivating childhood pathogens such as *Herpes simplex* virus-1), Respiratory syncytial virus, *Haemophilus influenza*, and Group B *Streptococci* in human and bovine milk and infant formula at 37°C [dataset] (Mohan Nair et al., 2004). [dataset] Chang, Redondo-Solano and Thippareddi (2010) evaluated the efficacy of using caprylic acid and monocaprylin to reduce *E.coli* O157:H7 and *Salmonella spp* on alfafa seeds, which have been implicated, along with other seed sprouts, in several human illness outbreaks in the United States. The results indicated that monocaprylin was more effective than caprylic acid in reducing the bacteria's population and that monocaprylin at a concentration of 75mM can be used as an alternative sanitizer to reduce *E.coli* O157:H7 and *Salmonella spp* on alfafa seeds without compromising the seed germination rates. Monocaprylin at 50mM concentration, in combination with 1% acetic acid, also proved capable of reducing *Listeria monocytogenes* activity in vacuum-packaged pork-beef frankfurters kept at 4°C and that at 45 or 50°C resulted in a significant growth inhibition of this bacterium [dataset] (Garcia et al, 2007)

Monocaprylin has also proved being capable of inhibiting microbial activity when applied on textile. [dataset] Vltavská and coworkers (2012) demonstrated that modified textile materials with 15% monocaprylin ethanolic solution suppressed the growth of pathogenic yeast (*Candida albicans* and *Candida parapsilosis*), as well as Gram-positive (*Staphylococcus aureus*) and Gram-negative (*Escherichia coli, Klebsiella pneumoniae*) bacteria. These results indicate a possible industrial application, since monocaprylin acts on bacteria and moulds that may occur during storage and use of textiles.

3.3. Manufacturing

There are three main routes used for production of glycerides: the glycerolysis of TAG; the direct esterification of glycerol and fatty acids and the partial hydrolysis of TAG. These three methods can be either chemically or enzymatically catalyzed, producing a mixture of MAG, DAG or TAG, in addition to unreacted reagents [dataset] (Fregolente et al, 2010; Hu et al 2013). The proportion of products depends on the catalyst and other reactions variables, such as temperature and molar rate.

The commercial synthesis of glycerides is carried out by glycerolysis and direct esterification [dataset] (Mostafa et al 2013). The increasing availability of glycerol, a by-product generated during biodiesel production, plays an important role in this matter [dataset](Chakraborty, Mandal, 2015).

3.3.1. Hydrolysis

Hydrolysis is the reaction of a molecule of water with one molecule of MAG, DAG or TAG. Therefore, the possible products of hydrolysis are diacylglycerols, monoacylglycerols, glycerol and free fatty acids (Figure 2)

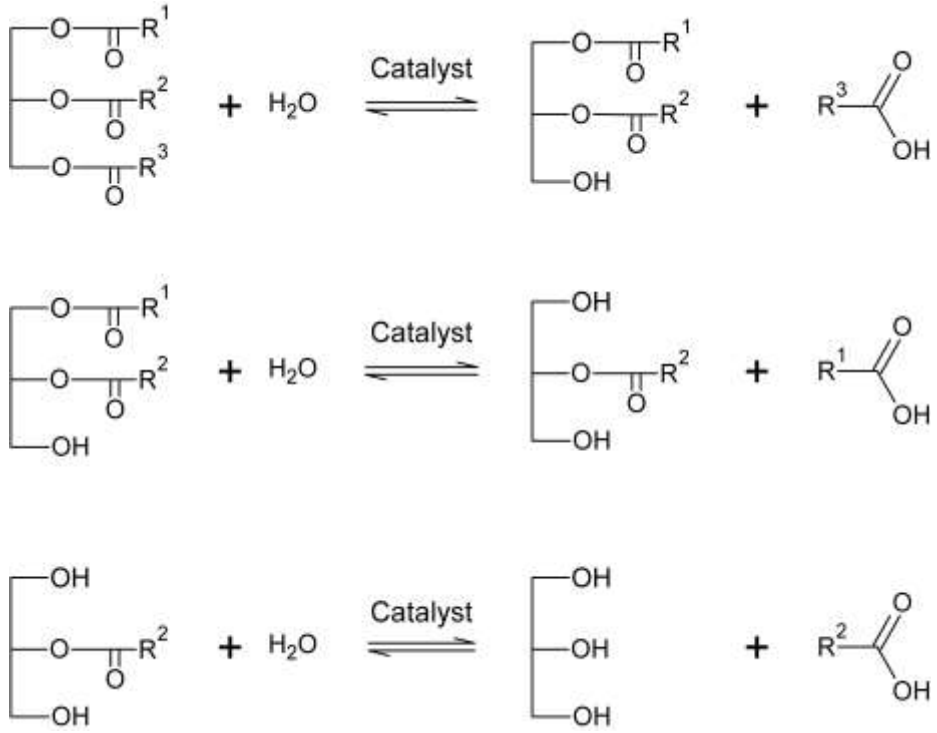

Figure 2. Complete hydrolysis of a triacylglycerol molecule. The reverse reaction represents the esterification process.

The hydrolysis of TAG and fats is an important reaction route to produce FFAs and glycerol, which are important raw materials for several industrial applications [dataset] (Bornscheuer, 1995; Rodrigues, Fernadez-Lafuente, 2010). Hydrolysis can be carried out using either chemical or enzymatic catalysts and can even happen as an un-catalyzed reaction, that happens when fats and water reacts at suitable conditions [dataset] (Salimon et al. 2011). In fact, the usual method of hydrolysis of oils to fatty acids and glycerol applies high temperature and pressure, which can lead to polymerization of the oil and by-product formation, resulting in a dark colored fatty acid [dataset] (Rodrigues, Fernadez-Lafuente, 2010).

Partial hydrolysis can be used to obtain mono and diglycerides. However, a strictly control is necessary to avoid the complete hydrolysis into glycerol and fatty acids. Additionally, hydrolysis of triglycerides possesses a low production capacity [dataset] (Bornscheuer, 1995; Hu et al., 2013).

There are only few studies regarding the synthesis of MAG and DAG through partial hydrolysis of TAG, and they usually are about LCTs, due their greater availability in natural oils. [dataset] Matos et al. (2011) obtained a DAG yield of 30% by partial hydrolysis of palm oil under microwave irradiation. In order to achieve better yields, the authors also investigated the use of packed bed reactor under conventional heating continuous flow regime. DAG production achieved the best results (128g of DAG) with 0.1mL/min flow rate and 24h. [dataset] Ghattas and coworkers (2014) investigated lipase catalyzed monoolein production by triglycerides hydrolysis and were able to reach a 78% monoglyceride yield under 37°C in a reaction time of 15min.

3.3.2. Glycerolysis

Glycerolysis of TAG is a complex reaction, since mono and diglycerides also react in forward and reverse reactions, resulting in multiple possible routes. (Figure 3) It's also important to consider the acyl migrations between 1(3)-MAG and 2-MAG and between 1,3-DAG and 1,2 (2,3)- DAG, especially when only one of the isomers is the desired product [dataset] (Zhong, Xu, Cheong, 2014). (Figure 3)

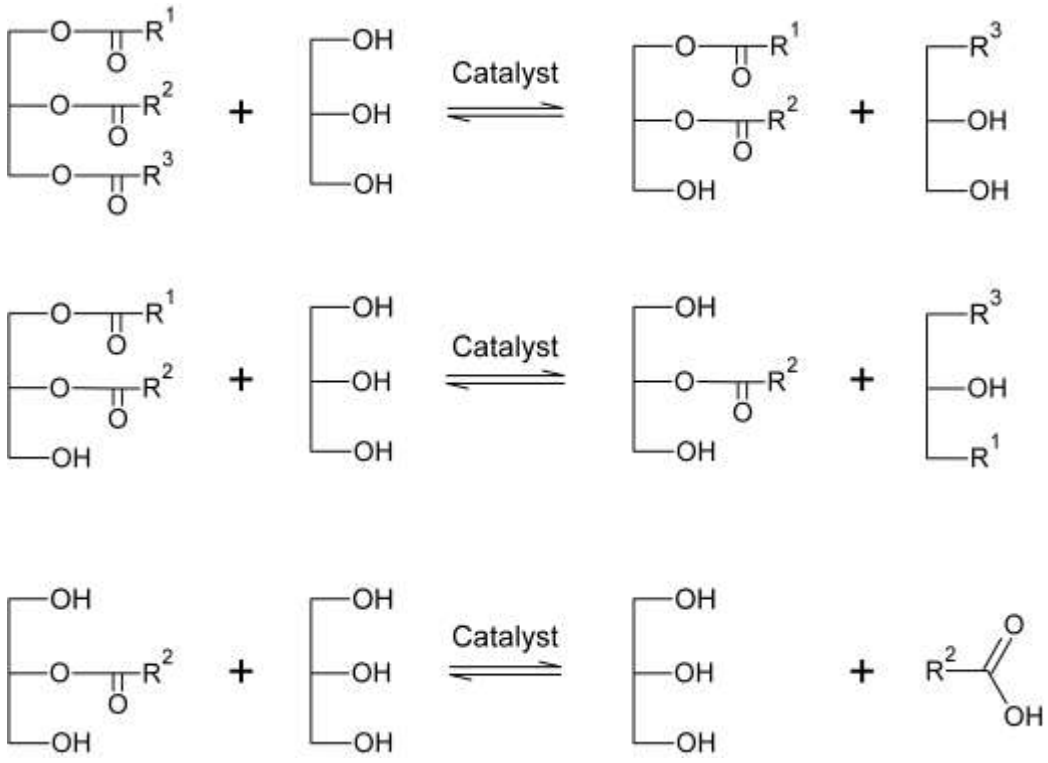

Figure 3. Glycerolysis of a triacylglycerol molecule into diacylglycerol and monoacylglycerol. This scheme represents only the main reaction route; however, multiple parallel routes can also occur.

Currently, glycerolysis of TAG is the primary reaction route for the production of MAG and DAG [dataset] (Cai et al, 2016) and it is usually conducted employing alkaline catalysts at high temperatures (250°C) [dataset] (Zhong et al, 2014; SOLAESA et al., 2016). Despite its wide application on industrial scale, the process presents several drawbacks, such as being high energy consuming, requiring several methods of separation and purification of the products and the formation of byproducts [dataset] (De Lima et al, 2013; Kapoor,

Gupta, 2012). Besides, the application of vacuum or molecular sieves is often required in order to promote dehydration of the reaction system, thus avoiding the competitive hydrolysis [dataset] (Hu et al, 2013).

The chemical glycerolysis of TAG generates a mixture of 35-60% MAG, 35-50% DAG, 1-20% TAG, 1-10% of FFA and their alkali metal salts [dataset] (Hu et al, 2013). Many approaches to improve the contents of specific glycerides, at the lowest cost possible, have been investigated. For instance, it's possible to selectively precipitate MAG, aiming to shift the equilibrium towards its formation. The design of heterogeneous catalysts could also enhance MAG and/or DAG contents [dataset] (Zhong et al, 2014).

Among all strategies studied to obtain milder and less energy consuming conditions, researches involving enzyme catalyzed reactions are particularly popular. Most of these concern the glycerolysis of long chain triglycerides, the most abundant ones in vegetable oils. Also, the milder conditions are suitable for heat-sensitive oils such as fish oils, extensively studied due their high content in polyunsaturated fatty acids [dataset] (Solaesa et al, 2016)

There are very few studies concerning the glycerolysis of medium chain glycerides. [dataset] Hu et al (2013) performed a comparative study on esterification and glycerolysis production of medium chain DAG, both lipase catalyzed. Esterification in batch reactor achieved the best conversion of caprylic acid (94.42%), but a lower DAG content (45.45%) than the one observed in the glycerolysis in t-butanol, that yielded 53.17% DAG, with a triacylglycerol conversion of 74.21% under the optimum conditions.

[dataset] De Lima and coworkers (2013) performed the hydrolysis and glycerolysis of tricaprylin using miniemulsions droplets as environment, at slightly elevated temperatures in aqueous dispersion without any organic solvent. The authors evaluated two lipases that exhibited different regiospecificities in the studied conditions, thus enabling the potential application of their findings in the obtainment of specific isomers.

3.3.3. Direct Esterification

Esterification is basically the inverse of hydrolysis reaction (Figure 2), in which the fatty acid reacts with glycerol, MAG or DAG, generating glycerides and water as products. The equilibrium between hydrolysis and esterification is usually controlled by the water content of reaction medium.

The direct esterification allows greater accuracy in the obtainment of designed glycerides, by previously selecting only the desired fatty acids [dataset] (Mostafa et al, 2013). Besides, it's an especially useful method when the TAG that would be a raw material for glycerolysis is not naturally abundant.

The commercial esterification of glycerol and fatty acids is often carried out using homogeneous acids catalysts, such as sulphuric and sulfonic acids [dataset] (Kotwal et al, 2011; Mostafa et al, 2013). There are, however, several issues related to this kind of catalysts, such as corrosiveness, exothermic behavior when in contact with water, environmental hazards and the requirement for laborious and costly steps of separation and purification of the products [dataset] (Wong et al, 2000; Kotwal et al, 2011; Chakraborty, Mandal, 2015)

The difficulty in catalyst recovery is another disadvantage presented by the homogenous acids. As a consequence, several solid catalysts have been reported, including zeolites, ion-exchange resins and modified silica materials. [dataset] Kotwal et al (2011) assessed the use of a solid Fe-Zn double-metal cyanide complex as a catalyst for the esterification between glycerol and C12:0, C14:0, C18:0 and C:18:1 fatty acids, getting an acid conversion of 75.0%, 75.9%, 62.2% and 63.4% at 180°C and with an acid:glycerol molar ratio of 1:1. At the end of each run, the catalyst was separated by centrifugation and reused in the next cycle without any kind of pre-treatment, presenting a similar catalytic activity. [dataset] Hamerski and Corazza (2014) reported the esterification of lauric acid and glycerol in a solvent-free system using a LDH (layered double hydroxide, Mg-Al-CO_3) as catalyst, reaching a 99% glycerol conversion at 180°C and 60 minutes of reaction.

Despite existing efforts to develop chemical catalysts alternatives, the studies are not focused in the obtainment of medium chain glycerides. A rare report regarding the chemical catalyzed esterification of medium chain glycerides was performed by [dataset] Deshmane et al (2008). The authors studied the effects of cavitation on esterification reaction between C8-C10 fatty acids and glycerol with sulfuric acid as a catalyst. According to the authors, cavitation allowed a conversion of 98.5% in a 6 hours reaction, under similar conditions of other reactions conducted without cavitation and that took 24 hours for a conversion of about 90%. Another interesting point raised is that cavitation makes the synthesis possible at much less severe conditions.

On the other hand, enzyme catalyzed esterification is a much more explored field, being possible to find more studies regarding the obtainment of medium chain glycerides, even though long chain glycerides remain being the main focus of most researches.

3.3.4. Chemical versus enzymatic catalysis.

Academic and industrial communities have been demonstrating a great interest in the development of more sustainable technologies and processes. Enzyme catalyzed routes are a popular approach to meet this modern concern, facilitating the suitability of processes to increasingly demanding legislations.

Enzyme catalyzed reactions are an eco-friendly alternative, since they require mild conditions of temperature and pressure, unlike the traditional industrial processes [dataset] (Rajendran et al. 2009; Pappalardo et al, 2017). Additionally, enzymes are inherently specific, minimizing unwanted side reactions and thus enabling the obtainment of high purity products [dataset] (Hasan et al, 2006; More et al, 2017). The regioselectivity of some enzymes is particularly useful when high yields of 1,3-DAG is desired, since chemical methods lack positional selectivity [dataset] (Rosu et al, 1999). Finally, enzymatic catalysis usually has lower waste treatment costs, since the effluents are less aggressive to the environment [dataset] (Rajendran et al, 2009; Hasan et al, 2006; Rodrigues, Fernadez-Lafuente, 2010).

Despite their great potential, enzymes present some drawbacks that severely lower their industrial application, such as stability and catalytic efficiency issues, high cost and recovery difficulties [dataset] (Zhao et al, 2017). A variety of approaches have been studied to overcome these issues, including screening of enzymes from natural sources, genetic modification and immobilization [dataset] (Choi et al, 2015).

3.3.5. Lipases

Lipases (triacylglycerol acylhydrolase, EC.3.1.1.3) play a major role in the biotechnological field, due their ability of catalyzing not only the hydrolysis of carboxylic ester bonds but also a variety of other reactions, including esterification, alcoholysis, acidolysis and interesterification [dataset] (Rodrigues, Fernadez-LAFUENTE, 2010; Gofferjé et al., 2014; More et al. 2017).

Lipases can be found in animal, plant or microbial sources and their properties may differ greatly depending on their origins [dataset] (Hasan et al, 2006; Amini et al, 2017). Microbial enzymes are usually the ones of choice due the possibility of obtaining high yields and the easier genetic manipulation [dataset] (Hasan et al, 2006).

Lipases are enzymes that do not require any cofactor, thus being capable of remaining dissolved in oil water Interface under natural conditions [dataset] (Rajendran et al 2009). In fact, it has been long known that they have little activity towards molecularly dissolved substrates in aqueous solution, but demonstrates high activity when the substrate concentration is high enough to form micelles or emulsion droplets [dataset] (Reis et al, 2009).

In order to shift the equilibrium towards esterification, there must be only traces of water in reaction medium, making the water removal imperative in the obtainment of satisfactory yields [dataset] (Rajendran et al, 2009; Pappalardo, et al, 2017).

The lipase-catalyzed esterification can be conducted either on organic non-polar solvents or in solvent-free systems. [dataset] (Pappalardo et al, 2017; Freitas et al, 2007). Immobilization is often applied to enhance the lipase activity in organic solvents [dataset] (Foukis et al, 2017) and to facilitate its recovery, thus performing an important role in minimizing its expensive nature [dataset] (Amini et al, 2017).

3.3.6. Direct esterification catalyzed by lipases

It's notable the greater availability of papers regarding the obtainment of medium chain glycerides via direct esterification catalyzed by lipases. Many of them approach specifically the obtainment of caprylins, fact that is not observed in the chemical catalyzed esterification. However, it's important to reemphasize that the main focus of most scientific papers are still the long chain triglycerides. Hopefully, as new caprylins applications are developed, the demand for improved obtainment routes will increase.

[dataset] Kim and Rhee (1991) first studied the solvent free enzymatic synthesis of medium-chain glycerides by using capric acid and glycerol as substrates, Lipozyme RM IM as catalyst, in a batch reactor system. Since then, other studies came up using different reactors, enzymes and parameters.

[dataset] Nandi et al (2005) hydrolyzed the neutral glycerides present in coconut and palm kernel fatty acid distillates and obtained fractions rich in MCFAs. Then, these fractions were esterified with Lipozyme RM IM to produce MCGs, getting high fatty acids conversion of 96.1% and 95.9% from coconut and palm kernel distillates, respectively.

The highest dicaprylin yield reported so far was obtained by [dataset] Rosu, and coworkers (1999), that optimized the reaction parameters for the production of 1,3-dicaprylin and reached the maximal yield of 98%. Despite the excellent results, the evaluation of scale-up feasibility is still necessary.

[dataset] Langone e Sant'Anna (1999), on the other hand, evaluated the specific obtainment of tricaprylin, tricaprin, trilaurin and trimyristin in solvent-free media by lipase catalyzed esterification reactions. Under the optimal conditions, appreciable levels of all triglycerides were achieved, except for tricaprylin, which was obtained with a highest yield of 20%.

Most of enzymatic esterification reactions require long periods of time for reasonable yields. In order to overcome this issue, [dataset] Wan et al (2012) studied the enzymatic synthesis of partial caprylins in an up-flow column reactor, obtaining a caprylic acid conversion of 90.2% in a 4 hours reaction. As a comparison, the authors performed the same reaction in a batch reactor, under similar conditions, obtaining a lower 88% caprylic acid conversion in a longer reaction time of 9h. In addition, the up-flow column reactor presented a good operating stability, with a conversion decrement of only 6.5% after 30 runs.

More recently, [dataset] More et al (2017) studied an ultrasound treatment in the initial stages of the reaction. The authors concluded that, with a maximum tricaprylin yield of 94.8% in 7h reaction time, the ultrasound pretreatment greatly influence the rate of reaction and the yield.

It has also been reported the usage of 1,3-dialkylimidazolium-based ionic liquids as media for the enzymatic synthesis of tricaprylin, for comparison purposes with the conventional organic solvent hexane. Even though a higher reaction time (24 hours) was required, a yield of 92.4% was reached up, proving the feasibility of ionic liquids as media for lipase catalyzed esterification [dataset] (Pan et al 2013).

[dataset] Guebara et al (2018) attained caprylins at a yield of 88% by esterification of glycerol with caprylic acid at 1:2 ratio catalyzed by immobilized lipase (Lipozyme®) in a batch reactor coupled or not to a microfiltration membrane. The reaction conditions used were 50°C, agitation of 250 rpm for 6h under pressure of 8 mbar. The authors showed that by using the reactor coupled to a microfiltration membrane it was possible reuse the catalyst at least three times, being the loss of catalytic activity lower than 10%.

4. CHARACTERIZATION OF CAPRYLINS

Due to quality, trade and authenticity control issues there is a growing interest in lipid analysis [dataset] (La Nasa et al, 2018). Different methods may be used, from acid value to rapidly assess reactional yields to sophisticated analytical methods that can provide information at a molecular level. Regarding medium chain glycerides, the most observed techniques in scientific papers are gas chromatography (GC) and high performance liquid chromatography (HPLC). This section covers both the most widely used methods for separating, identifying and quantifying caprylins and other medium chain glycerides, as well as not so common but feasible methods reported by some authors.

4.1. Determination Of Acid Value (AV)

This titration method is described in the AOCS Official Method Cd 3d-63 [dataset] (AOCS, 2009) and is largely applied, with or without modifications, for quantification of free fatty acids.

As a fast and simple method that only requires materials commonly found in laboratories, the AV is a valuable tool to assess reaction yields in which fatty acids are the reactant or the product. [dataset] Wong et al (2000), [dataset] Deshmane et al, (2008), [dataset] Fang et al (2008) and [dataset] More et al (2017) are some of the authors that successfully applied Acid Value to assess the obtainment of medium chain glycerides.

[dataset] Vitolo et al (2017) specifically evaluated the feasibility on use of AV as an assessment tool of the lipase catalyzed esterification between glycerol and caprylic acid. The authors observed that it was possible to determine the acidity of glycerol and caprylic acid blends and, therefore, that AV could be considered a rough parameter for controlling this reaction at production scale.

4.2. Spectrophotometric Methods

The determination of the free fatty acid content can also be accomplished by spectrophotometric assays, even though they are not extensively applied, probably by the AV value popularity, a very simple and accessible method.

[dataset] Kwon and Rhee (1986) developed a colorimetric method to determine free fatty acids produced by lipase from triacylglycerols by observing the color developed using a cupric acetate-pyridine as a color development reagent. The authors evaluated the sensitivity and reproducibility of the method for medium chain and long chain fatty acids, obtaining good results for all the studied compounds.

[dataset] Walde (1990) developed another colorimetric method suitable for the determination of free fatty acids by using phenol red as fatty acid indicator, which is solubilized in reversed micelles formed by sodium bis-(2-ethylhexyl) sulfosuccinate. The results

showed that the method could be extended to a continuous determination of fatty acids released during lipase catalyzed hydrolysis of triglyceride substrates in reverse micelles.

4.3. Gas Chromatography

The profiling and quantification of free fatty acids by gas chromatography (GC) usually requires de derivatization of acidic moieties by methylation or silylation reactions in order to increase the volatility and decrease polarity of their molecules. Derivatization of mono, di and triglycerides aiming to increase their volatility is also a common practice [dataset] (Isidorov et al, 2007), even though the analysis of intact glyceride molecules is frequently observed in papers.

Performing gas chromatography in underivatized TAG may be more challenging due its non-volatile nature. High Temperature Gas Chromatography (HTGC) is a way around this issue, but it can be limited by the decomposition of TAG at high temperatures [dataset] (La Nasa et al, 2018).

Among the existing detectors used in gas chromatography, certainly the most reported in lipid analysis are the Flame Ionization Detector (FID) and Mass Spectrometer (MS) [dataset] (Moldoveanu, Chang, 2011). A proper separation, identification and quantification of medium chain fatty acids and glycerides have been reported by several authors, by applying a variety of GC methodologies, which were invariable coupled with one of those two detectors [dataset] (Langone,Sant'anna, 1999; Nandi, Gangopadhyay, Ghosh, 2005; Isidorov et al, 2007; Hu, Chen, XIA, 2013).

Several authors have successfully applied GC-FID or GC-MS in MCG analysis, achieving a proper separation, identification and quantification of fatty acids, mono-, di- and triglycerides.

GC is a well-stablished and highly sensitive technique that provides results with high accuracy and high reproducibility [dataset] (Fisk et al, 2014). Nevertheless, the technique presents some drawbacks that can influence the choice for other analytical methods. The main disadvantages include the complex and time-consuming sample pretreatments that can lead to sample contamination and the use of very sophisticated instrumentations, leading to high costs for a single analysis and requiring the analyst to be an expert in the use of several analytical instrumental set-ups [dataset] (Mingrone et al, 1993; La Nasa et al, 2018).

4.4. High Pressure Liquid Chromatography

HPLC is a separation process based on the selective interaction of solutes between a solid, stationary phase and a liquid, mobile phase. There are two separation modes frequently applied in lipid analysis: normal-phase (NP), characterized by the reversible adsorption of solutes to polar groups of the solid adsorbent and reversed-phase (RP), based on the partition of solutes that occurs between the mobile phase and hydrophobic ligands attached to the stationary phase. Therefore, in RP-HPLC the mobile phase has more polarity than the stationary phase, while in NP-HPLC the opposite situation is observed [dataset] (Buszewska-Forajta et al.,2018)

The profiling and semi-quantitative analyses of glycerides are more commonly performed in RP-HPLC, interfaced with UV spectrophotometric, evaporative light scattering detection, charged aerosol detector (CAD) or MS detection. The quantification of fatty acids in the same chromatographic run is possible by mass spectrometers operating in negative mode, even though it's frequently necessary to perform derivatization reactions, e.g. derivatization with 2-bromoacetophenone, to increase the retention time and enable the use of spectrophotometric detectors. [dataset] (Moldoveanu, Chang, 2011; La Nasa et al, 2018).

The pre-treatment requirements in addition to the occasional HPLC limitation caused by the small efficiency of liquid chromatographic columns frequently result in the use of two different analytical procedures to characterize free fatty acids and glycerides [dataset] (Isidorov et al, 2007; La Nasa el al, 2018). [dataset] Fang, Sun and Xia (2008) evaluated the obtainment of caprylins by combining the measurement of acid value and Refractive Index HPLC to assess the glycerides' composition. [dataset] De Lima et al (2013), on the other hand, were able to quantify caprylic acid, monocaprylin, dicaprylin and tricaprylin in a single run by using a HPLC system with an UV detector set up to perform the reading at a wavelength of λ=220 nm. Additionally, the authors didn't perform any kind of derivatization reaction.

4.5. Thin Layer Chromatography

Thin layer chromatography (TLC) is an important analytical tool in lipid analysis, since it provides valuable information in a short period of time and at relatively low costs. It's a simple technique that basically requires a solid stationary phase, spread as a thin layer on a plate made of an inert material, and a solvent mobile phase. The components of the mixture are differentially distributed according to their polarity and can be identified by the Rf value, which quantifies the component migration on the plate [dataset] (Shahidi, Wanasundara, 2002; Nikolova-Damyanova, 2005; Hinrichsen, Steinhart, 2006).

TLC has been applied mainly for qualitative purposes, considering that it provides less accurate results than GC or HPLC [dataset] (Hinrichsen, Steinhart, 2006). However, there have been some important developments over the years that have allowed a gradual

increase in the application of the technique for quantitative measurements and the development of its high performance version, the HPTLC [dataset] (Fucks et al., 2011).

The main contributing factors to this TLC improvement are the increasing commercial availability of pre-coated TLC plates and the greater variety of adsorbent materials applied on the plates [dataset] (Fucks et al., 2011). Additionally, almost all steps of TLC analysis can ben automatized, even though the costs involved can impact the positive low cost advantage that this method presents [dataset] (Nikolova-Damyanova, 2005; Hinrichsen, Steinhart, 2006). Last but certainly not least, the quantification of separated analytes, usually carried out by densitometric or fluorimetric measurement can be optimized by using scanners and an appropriate software [dataset] (Nowak et al, 2017).

In glycerides analysis, there are few reports on the use of TLC for quantitative purposes. Generally, the authors use TLC as separation and qualitative tool and complement the analysis with other quantitative methods. [dataset] Wong et al (2000), for example, identified MCG from the esterification reaction by using TLC on precoated silica gel plates and a mobile phase consisting of chloroform/acetone (9.5:0.5. vol/vol). The quantification was later accomplished by GC.

[dataset] Yang and coworkers (2004) determined the content of fatty acids and acylglycerols obtained by glycerolysis of butterfat by scraping their bands of TLC plate, calculating the weights of each lipid species according to their FA composition and expressing the result as the weight percentage after normalized calculation including the four studied compounds.

[dataset] Sek, Porter e Charman (2001) developed a HPTLC assay for the quantification of tricaprylin and triolein, as well as their lipolytic products, bile salts and phospholipids. The medium chain lipid separation was optimized using four developments and the spots were visualized by staining the plate with a solution of ammonium molybdate-perchloric acid (70%, w/w)-hydrochloric acid (1 M)-Milli-Q water (6:40:90:75, w/v/v/v). Then, the compounds were quantified by densitometric measurements performed by a commercial TLC scanner. Finally, the authors validated the developed assay, demonstrating its accuracy and precision. Recently, [dataset] Hares-Jr et al. (2018) applied the software JustTLC® (Sweday, Sweden) for identifying and quantifying 1-monolein, 1,3-diolein, oleic acid and triolein through thin layer chromatography. The procedure consisted on taking samples from reaction vessel, diluted 1:10 (vol/vol) in n-hexane and applied to TLC plates (silica gel 60 TLC Merck, 20 x 20cm, 0.25 mm). On each plate was also applied a standard mixture of oleic acid and mono, di and triolein. The plates were introduced into a chamber containing as mobile phase a mixture of n-hexane, ethyl ether and acetic acid (80:20:1 vol/vol/vol). After the run, the plates were dried and revealed with iodine sublimated for 30 min. After development and revelation, plates were photographed and the digital images analyzed by the software. The authors observed deviation levels of less than 1.5% ($p < 0.05$).

4.6. Differential Scanning Calorimetry

Differential Scanning Calorimetry (DSC) is a very common method applied in the characterization of several materials. This technique is used to measure temperature and heat flow associated with transitions in materials as a function of time and temperature in a controlled atmosphere, providing quantitative and qualitative information about chemical and physical changes involving endothermic and exothermic process or changes in heat capacity [dataset] (Farah et al, 2018).

The thermal-characterization of fats and oils is most frequently accomplished by DSC and provides important information for the identification of these materials [dataset] (Tan, Man, 2002). While the thermal profiles of fats and oils are widely studied, this technique is not frequently applied to assess the progress of hydrolysis and esterification reactions, neither to monitor the following purification processes [dataset] (Silva e al, 2015).

An interesting study was performed by [dataset] Silva and coworkers (2015), in which the melting and crystallization behavior of chromatographic standards of monocaprylin, dicaprylin and tricaprylin, as well as binary mixtures of mono and dicaprylin, were evaluated. The authors concluded that DSC could be a valuable tool to assess the progress of an esterification reaction, even though a complete assessment with other techniques would still be necessary to provide a full picture of the reaction under research.

5. CONCLUSIONS

The applicability of caprylins and caprylic acid has been subject of several researches over the years and their use is already common in some fields, such as the food and cosmetic industries. This is also true for other MCGs and their saturated fatty acids.

Nevertheless, insufficient attention is paid to improving traditional manufacturing routes of MCGs, although the scenario for long chain glycerides is quite different. Either way, the current trend is the development of greener processes, with the enzymatic catalysis playing a key role. For the obtainment of caprylins, a popular approach is the direct esterification catalyzed by lipase, since it allows a more controlled obtainment of the desired products, even though the scale-up remains being an issue to overcome.

An adequate analytical characterization of caprylins is essential to guarantee the compliance with quality, trend and authenticity requirements. There are a number of techniques available to the identification and quantification of these compounds, being the chromatographic methods the better stablished ones. For the simple assessment of reactional yields, the acid value method has proved to be an excellent tool.

This review thoroughly surveyed the available information on caprylins and, to some extent, on other medium chain glycerides. Hopefully, further developments and discoveries will be encouraged, since there is still much research to be done regarding the properties, applicability, obtainment and characterization of caprylins.

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