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Comparative *in vitro* intestinal digestion of 1,3-diglyceride and 1-monoglyceride rich oils and their mixtures

Diana Martin [a,b,*], Maria I. Moran-Valero [a,b], Luis Vázquez [a,b], Guillermo Reglero [a,b,c], Carlos F. Torres [a,b]

[a] Departamento de Producción y Caracterización de Nuevos Alimentos, Instituto de Investigación en Ciencias de la Alimentación (CIAL) (CSIC–UAM), 28049 Madrid, Spain
[b] Sección Departamental de Ciencias de la Alimentación, Facultad de Ciencias, Universidad Autónoma de Madrid, 28049 Madrid, Spain
[c] IMDEA-Food Institute, CEI UAM+CSIC, 28049 Madrid, Spain

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ABSTRACT

Intestinal *in vitro* digestion of 1,3-diolein (DO), 1-monoolein (MO), DO:MO (1:1) rich oils, and triolein (TO), was performed to study the rate and extent of hydrolysis as well as their bioaccessibility in detail, with special emphasis on 1,3-DO and 1-MO forms, as potential bioactive lipids with additional technological functions such as self-emulsifying lipids. The importance of *in vitro* conditions on non-desirable acylmigration was also shown. The rate of *in vitro* intestinal lipolysis was in increasing order TO < DO < DO:MO < MO. At the end of digestion, DO:MO was hydrolyzed to absorbable products at the same level than the MO sample. The degree of lipolysis positively correlated with the level of 1-MG and negatively with the level of 2-MG. Either DO, MO or DO:MO produced higher level of 1-MG than TO. DO:MO produced the highest level of 1-MG and a high ratio of 1-MG to 2-MG.

Most hydrolysis products (>95%) of DO, MO and DO:MO were found within the micellar phase fraction during digestion, suggesting a high bioaccessibility. A positive correlation between the degree of lipolysis and the number of mixed micelles formed at the end of digestion was found.

As summary, the obtained results would enhance the selection of glycerides to formulate ingredients with different purposes. Thus, in case a final high level of 1-MO would be desired to take advantage of the bioactivity of 1-MO, oils under the form of DO or DO:MO might be superior to MO. In case a high 1-MO level together with a low 2-MO level would be desired at the same time, mixtures of DO:MO or MO would be preferred. In case a higher self-emulsifying ability would be desired, the preferred forms would be MO and DO:MO. Finally, in case all the potential functionalities would be desired at the same time, namely the highest bioactivity, together with a high self-emulsifying ability, the mixture DO:MO might be suggested as an interesting product, with the additional economical advantage.

1. Introduction

In recent years, partial glycerides such as diglycerides (DG) or monoglycerides (MG) are achieving increasing popularity from different perspectives (Feltes, de Oliveira, Block, & Ninow, 2013; Wang, Jin, Wang, Huang, & Wang, 2013; Zhao, Liu, Deng, Wang, & Tan, 2011). On the one hand, the most popular function of these lipids is related to their amphiphilic nature and surface-active properties, being well-known as emulsifier ingredients in the food, pharmaceutical and cosmetic industries. On the other hand, partial glycerides, as self-emulsifying lipid delivery systems, are attractive lipids in the formulation of potential vehicles of drugs and bioactive compounds of poor solubilization, in order to favor their bioaccessibility at intestinal level, or to protect against their degradation under gastrointestinal conditions; and ultimately, to reach the most efficient bioactivity of compounds (Pouton & Porter, 2008).

On the other hand, based on the development of advanced tools to obtain partial glycerides, these lipids have been remarked as valuable precursors for the production of diverse structured lipids (Pfeffer et al., 2007; Wang et al., 2013). The production of partial glycerides esterified with fatty acids of bioactive interest is also now possible (Devi et al., 2008; Feltes et al., 2012; Vadivel, Whitten, & Makriyannis, 2011; Wang et al., 2013).

One of the most novel areas of interest is focused on the natural bioactivity of specific forms of partial glycerides such as 1,3-DG or 1(3)-MG. The 1,3-DG has been reported as a hypocaloric lipid that decreases weight, body fat and serum triglycerides (TG), or increases energy expenditure; but the mechanism of these effects is still under intense research (Dhara, Dhar, & Ghosh, 2013; Jandacek, 2007; Kristensen, Jørgensen, & Mu, 2012; Lo, Tan, Long, Yusoff, & Lai, 2008; Meng, Zou, Shi, Duan, & Mao, 2004; Murase, Aoki, Wakisaka, Hase, & Tokimitsu,

* Corresponding author at: Instituto de Investigación en Ciencias de la Alimentación (CIAL), Campus de la Universidad Autónoma de Madrid, 28049 Madrid, Spain. Tel.: +34 910017930.
E-mail address: diana.martin@uam.es (D. Martin).

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2002; Rudkowska et al., 2005; Yanai, Yoshida, Hirowatari, & Tada, 2012; Yanai et al., 2010). The most popular mechanism has been related to the metabolism of 1,3-DG within the enterocyte. The intestinal hydrolysis of typical TG or 1,2-DG yields free fatty acids (FFA) and 2-MG as major digestion products catalyzed by pancreatic lipase at the intestinal lumen. Once inside the enterocyte, these lipid products are reesterified to TG by the 2-MG pathway of specific acyltransferases, and the new TG are included in chylomicrons for their systemic distribution. On the contrary, 1,3-DG are hydrolyzed to FFA, 1(3)-MG or glycerol during digestion, and these 1(3)-MG and glycerol are not so easily recognized as substrate of acyltransferases, so the alternative glycerol-3-phosphate pathway of the enterocyte seems to be the main metabolism of 1(3)-MG. This pathway is less efficient in the reassembly of TG, so less chylomicrons are formed, and residual lipid substrates seem to be redirected to the portal vein accompanied by an increase in β-oxidation of fatty acids (Jandacek, 2007; Lo et al., 2008; Matsuo & Tokimitsu, 2001; Murase et al., 2002; Murata, Hara, & Ide, 1994).

Furthermore, concerning the specific form of 1(3)-MG, besides the particular intestinal metabolism, other interesting bioactivities have been reported. Chaibi, Ababouch, and Busta (1996) already showed the antimicrobial activity of monoolein, monomyristin, monolaurin and monolinolein. Cho, Hong, and Lee (2010) showed MG as antioxidant, anti-atherosclerotic and inhibitor of protein glycation, monoolein being stronger than monopalmitin. On the other hand, the use of MG as a regulator in postprandial insulin level has been patented (Takeno, Shimotoyodome, & Meguro, 2009). Recently, Shimotoyodome et al. (2012) reported that dietary 1-monoolein stimulated fat utilization, and attenuated induced obesity, postprandial lipemia and insulin response in mice.

Therefore, partial glycerides such as 1,3-DG or 1(3)-MG for formulation of ingredients and foods might be an interesting opportunity to develop either a lipid delivery system as carrier of bioactives, and at the same time, taking advantage of the bioactivity of the own lipid delivery system by itself. In fact, according to these evidences, the methods for production of 1(3)-MG- and 1,3-DG rich oils are under intense research, mainly due to the low levels of these lipid forms naturally found in oils (Duan, Du, & Liu, 2013; Feltes et al., 2013; Meng, Xu, Zhou, Wu, & Yang, 2014; Morita & Soni, 2009; Wang et al., 2013; Zhao et al., 2011).

Taking into account the self-emulsifying properties of these lipids in the intestinal lumen, as well as their bioactivities related to intestinal metabolism, the study in detail of the digestion process of these lipids is relevant. Specifically, detailed information on the digestion process of the particular bioactive 1(3)-MG and 1,3-DG is limited (Kondo, Hase, Murase, & Tokimitsu, 2003; Murase et al., 2002; Osaki et al., 2005). In general, we consider that the ratio of 1-MG/2-MG released after digestion of partial glycerides, or mixtures of partial glycerides, should be addressed based on the hypocaloric mechanism of 1,3-DG. In this sense, it could be interesting to approach strategies to produce a high level of 1(3)-MG, together with a low level of 2-MG as possible at the same time, after physiological digestion. In this scenario, the probability of TG reesterification and formation of chylomicrons might be even lower, because the importance of the glycerol-3-phosphate pathway depends on the insufficiency of 2-MG more than on the sufficiency of 1-MG (Chung & Tappenden, 2006; Trotter & Storch, 1993).

Therefore, a deep knowledge of the intestinal digestion of partial glycerides or their mixtures would allow developing efficient strategies to formulate ingredients depending on the main functionality desired, which might be either the natural bioactivity of the lipids, or just the formulation of lipid delivery systems. For both purposes, digestive behavior of DG:MG mixtures would be of interest, which would be also relevant from the economic point of view, since individual production and isolation of pure 1(3)-MG or 1,3-DG rich oils are much more laborious and expensive.

In vitro intestinal models of lipid digestion are interesting approaches for obtaining preliminary and valuable information concerning digestion of lipid species. In fact, most of them have been traditionally developed to test efficient lipid delivery systems of drugs, taking into account the relevant role of digestion on the efficiency of the product formulation (Pouton & Porter, 2008). Due to their utility, their use in the study of dietary lipids for ingredients and foods has increased, becoming a well-accepted analytical tool. However, we consider that the selection of a model that closely simulates *in vivo* conditions is especially essential when performing *in vitro* lipid digestion of novel or unknown lipids, in order to accurately understand obtained results and to avoid misinterpretations due to the methodology used. In this respect, concerning the particular case of partial glycerides, it is important to take into account that the own conditions of the *in vitro* models can lead to non-physiological artifacts. This is because extensive undesirable acyl-migration of fatty acids from sn-2 to sn-1(3) positions of 1,2-DG and 2-MG might take place under some experimental conditions, such as long time exposure to basic or acid solutions, and high temperature. The consequence of extensive acyl-migration might extremely modify the final result of experiments because normal 2-MG should not be hydrolyzed, whereas its isomer 1(3)-MG derived from acylmigration would be extensively digested by the sn-1(3) specific pancreatic lipase. Unfortunately, the extent of acyl-migration is not usually assessed when partial glycerides are studied under *in vitro* models of digestion.

The aim of the present research was to perform a comparative *in vitro* study on the intestinal digestion of triolein (TO), diolein (DO), monoolein (MO) or DO:MO rich oils, in order to evaluate differences in the rate and extent of reaction, lipid products and micellar structures produced, and their potential bioaccessibility, with emphasis on the 1-MG to 2-MG ratio. Furthermore, the importance of the acyl-migration under *in vitro* conditions will be also considered.

2. Materials and methods

2.1. Reagents and materials

Olive oil was used as representative of triolein rich oil (TO). Concentrated diolein oil (DO) and monoolein oil (MO) were obtained by molecular distillation of a commercial mixture of glycerides (MG, DG and TG) from Sigma-Aldrich Chemie GmbH (Steinheim, Germany) according to Vázquez, Fernandez, Martin, Reglero, and Torres (2013). Briefly, a molecular distiller pilot plant from POPE Scientific Inc. (Saukville, USA) was used. The equipment consists of a 2 inch distiller coupled with a mechanical vacuum pump (1×102 Pa) and a diffusion pump (1 Pa) both from Edwards Limited (Crawley, UK). An external condenser and a cryogenic trap were installed immediately downstream of the still. The condensable low molecular weight compounds are collected in the cryogenic trap upstream of the vacuum system. Molecular distillation was performed at 200 °C and a pressure of 0.005 bar at 250 mL/h flow rate.

Trizma, maleic acid, pancreatin from porcine pancreas, bile salts, and phosphatidyl choline from egg yolk were from Sigma-Aldrich Chemie GmbH (Steinheim, Germany). Anhydrous sodium sulfate, sodium chloride, calcium chloride and absolute ethanol were purchased from Panreac (Barcelona, Spain). All solvents used were of HPLC grade from Lab-Scan (Dublin, Ireland).

2.2. In vitro lipid digestion

The *in vitro* lipid digestion model was based on Martin, Morán-Valero, Señoráns, Reglero, and Torres (2011) with brief modifications. Basically, the digestion mixture consisted of 0.5 g of TO dispersed in 17 mL of Trizma-maleate buffer 0.1 M pH 7.5. In case of digestion of DO, MO or DO:MO (1:1 w/w), same moles of fatty acid as those added for TO digestion were utilized. This medium was pre-emulsified by homogenization for 2 min at 3500 rpm. On the other hand, a blend trying to simulate biliary secretion was prepared by mixing 0.1 g of lecithin, 0.25 g of bile salts, 0.02 g of cholesterol, 0.5 mL of 325 mM