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酶法合成 *sn*-2 棕榈酸甘油酯的研究进展

韦伟 [a], 孙聪 [a,c], 王小三 [a], 金青哲 [a], Xuebing Xu [d], Casimir C. Akoh [e], 王兴国 [a,b,*]

[a] *International Joint Research Laboratory for Lipid Nutrition and Safety, Collaborative Innovation Center of Food Safety and Quality Control in Jiangsu Province, School of Food Science and Technology, Jiangnan University, Wuxi 214122, China*
[b] *Beijing Advanced Innovation Center for Food Nutrition and Human Health, Beijing Technology and Business University (BTBU), Beijing 100048, China*
[c] *College of Food Science and Technology, Henan University of Technology, Zhengzhou 450001, China*
[d] *Wilmar (Shanghai) Biotechnology Research and Development Center Co., Ltd., Shanghai 200137, China*
[e] *Department of Food Science and Technology, The University of Georgia, Athens, GA 30602, USA*

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摘要

人乳脂（human milk fat, HMF）是婴儿重要的能量和营养来源，甘油三酯（TAG）在人乳脂中的含量约为98%，其具有独特的分子结构。HMF在甘油碳骨架*sn*-2位的棕榈酸（PA）含量超过70%，而在*sn*-1,3位高度富集多不饱和脂肪酸。研究表明，HMF中特定的TAG结构在婴儿生长过程中起重要作用。*Sn*-2棕榈酸甘油酯是一种结构TAG，主要由1,3-二油酸-2-棕榈酸甘油三酯组成，将其添加到婴幼儿配方奶粉中可以模拟HMF的TAG结构。本文综述了过去25年中酶法合成*sn*-2棕榈酸甘油酯的研究进展及其在实验室条件下的制备流程，重点分析了结构TAG合成过程中所用的商业化*sn*-1,3位选择性脂肪酶、TAG结构分析的常用方法，以及一些市售*sn*-2棕榈酸甘油酯产品。另外，本文还对酶法合成*sn*-2棕榈酸甘油酯的前景进行了展望。

1. 引言

Sn-2棕榈酸甘油酯是结构甘油三酯（TAG）中的一种，通常用作人乳替代脂。结构TAG是一种非天然油脂，它是在天然TAG的基础上通过人工改性获得的一种油脂[1]。为了模拟人乳脂中的TAG结构，人们研发了*sn*-2棕榈酸甘油酯。

人乳是婴儿的最佳食物，脂肪是人乳中的一种重要成分。尽管人乳脂（HMF）在人乳中的含量仅为3%~5%，但可为全母乳喂养的婴儿提供约50%的能量。HMF是最复杂的天然脂质之一，它含有98%~99%的TAG、0.26%~0.80%的磷脂、0.25%~0.34%的固醇（主要是胆固醇），以及各种微量成分，如甘油单酯（MAG）、甘油二酯（DAG）、游离脂肪酸（FFA）等[2]。目前，已经鉴定出的HMF中的TAG结构多达400余种[3]。

目前学者们一致认为油脂的功能和营养特性与其所含的TAG种类密切相关。如图1所示，TAG分子由一个经过三个脂肪酸酯化的甘油碳骨架组成。TAG的种类不仅取决于其脂肪酸组成，还取决于脂肪酸在甘油碳骨架上的位置分布。

甘油碳骨架上的位置采用立体特异性编号（stereospecific numbering, *sn*）系统进行命名，其中R′、R″和

* Corresponding author.
E-mail address: xingguow@jiangnan.edu.cn (X. Wang)

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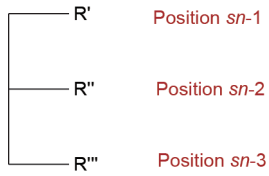

图1. TAG分子的结构。R′、R″和R‴分别为酯化在甘油碳骨架*sn*-1、*sn*-2和*sn*-3位的脂肪酸。

R‴分别是酯化在*sn*-1、*sn*-2和*sn*-3位的脂肪酸。HMF是具有独特TAG组成的天然脂类，其特征在于脂肪酸在甘油碳骨架上的分布规律。HMF的三种主要脂肪酸是油酸（OA，18:1，*n*–9，约占33%）、棕榈酸（PA，16:0，约占24%）和亚油酸（LA，18:2，*n*–6，约占15%）[4]。在人乳中，约70%的PA分布在甘油碳骨架的*sn*-2位[5]。*Sn*-1和*sn*-3位主要为不饱和脂肪酸（UFA），如OA和LA [6,7]。长链多不饱和脂肪酸（LCPUFA）也多数酯化在*sn*-2位，如二十碳五烯酸（EPA，20:5，*n*–3）和二十二碳六烯酸（DHA，22:6，*n*–3）[8]。HMF中含量最高的TAG是1,3-二油酸-2-棕榈酸甘油酯（OPO，约占16%~29%）和1-油酸-2-棕榈酸-3-亚油酸甘油酯（OPL，约占13%~20%）[3,6,9,10]。

与HMF相比，婴儿配方奶粉中通常使用的植物油具有不同的脂肪酸分布。在植物油中，PA主要（>80%）被酯化在*sn*-1和*sn*-3位。因此，经过食用油加工工艺（如油脂分提和油脂调配）和化学酯交换作用的天然植物油无法达到与HMF相同的TAG组成。

HMF中独特的TAG结构在婴儿生长中具有重要的作用。TAG的消化主要发生在婴儿的胃和小肠中，该消化过程主要通过脂肪酶水解反应进行。由于胃和胰脂肪酶具有*sn*-1,3位选择性，因此TAG水解后生成了*sn*-2 MAG和FFA [11]。HMF中的长链饱和脂肪酸（主要是PA）通常以*sn*-2 MAG的形式被吸收。在婴儿的胃肠道微环境中，游离的PA与钙和镁结合后形成了不溶性钙皂，然后经粪便排出[12–14]。因此，目前的研究重点之一是对婴儿配方奶粉脂肪中的TAG进行改性（通过添加*sn*-2棕榈酸甘油酯），以提高脂肪酸的吸收率，并减轻由棕榈酸钙引起的症状[4,15,16]。

由于PA在甘油碳骨架上的不同位置导致了脂质吸收的差异性，因此人们对*sn*-2棕榈酸甘油酯来源的开发和商业化产生了极大的兴趣。油脂改性方法，即改变脂肪酸在甘油碳骨架上的位置，可以改善油脂的营养品质[17]。而基于*sn*-1,3位选择性脂肪酶的酶法技术使这种脂质改性成为可能。*Sn*-1,3位选择性脂肪酶催化的TAG和酰基供体的酯交换反应可以实现对甘油碳骨架上*sn*-1和*sn*-3位脂肪酸的改性，从而产生具有特定结构的TAG。除*sn*-2棕榈酸甘油酯外，研究人员利用该技术还开发了多种结构TAG，如类可可脂（CBE）、中/长链甘油三酯（MLCT）等。

在过去的20年，大量关于酶法合成*sn*-2棕榈酸甘油酯的文章和专利已经被发表。有几种*sn*-2棕榈酸甘油酯产品已经被作为营养补充剂加入到了婴幼儿配方食品中。目前已经有一些关于位置选择性脂肪酶及其在*sn*-2棕榈酸甘油酯中应用的文章[1,4,18–20]。但是，这些文章中很少提及有关TAG结构分析的详细信息，而这些信息对TAG的功能评估至关重要。因此，本文综述了在过去20年中酶法合成*sn*-2棕榈酸甘油酯所取得的进展，并着重分析了TAG异构体。另外，我们在实验室和工业规模上对*sn*-2棕榈酸甘油酯的未来发展趋势进行了展望。

2. 用于合成 *sn*-2 棕榈酸甘油酯的脂肪酶

脂肪酶（EC 3.1.1.3，TAG水解酶）是脂质改性中最常用的生物催化剂之一[21]。油脂是脂肪酶的天然底物。酶促反应具有几个公认的优势，即温和的反应条件、较少的环境污染，以及可用于生产更天然的产品。然而，酶法改性TAG有一个更特殊的特点，即脂肪酶能够对TAG特定位置的脂肪酸进行改性[1]。使用不同的脂肪酶可以合成具有不同分子结构的TAG，这是化学酯交换方法所无法达到的，而且这种方法可以被用于大规模的食品加工[1,22]。

1990年，研究人员开始研究脂肪酶的三维结构和催化机理，随后，他们发现脂肪酶是一种丝氨酸蛋白酶。脂肪酶的丝氨酸羟基对底物的羰基碳产生亲核攻击，从而导致作为中间体的酰基酶的形成[17]。作为亲核试剂，酰基酶在水溶液中被水解[1,23]。

尽管越来越多的微生物脂肪酶已经被成功地用作实验室中的生物催化剂，但只有少量的脂肪酶被用作商业性开发。表1列出了生产结构TAG的常用的商业化脂肪酶。迄今为止，在合成*sn*-2棕榈酸甘油酯中使用最广泛的脂肪酶是Lipozyme TL IM和Lipozyme RM IM。表 1提供了合成*sn*-2棕榈酸甘油酯所使用的脂肪酶的详细信息。

脂肪酶的位置选择性在酶法合成特定结构的TAG过程中非常重要。然而，目前，关于脂肪酶位置选择性的一般机制仍不太明确。由于大多数脂肪酶对甘油碳骨架

表1 常用的商业化*sn*-1,3位选择性脂肪酶

Abbreviation	Source organism	Immobilization materials	Company
Lipozyme TL IM	*Thermomyces lanuginosa*	Silica gel	Novozymes (Denmark)
Lipozyme RM IM	*Rhizomucor miehei*	Ion-exchange resin	Novozymes (Denmark)
Lipozyme 435	*Candida antarctica*	Macroporous anionic resin	Novozymes (Denmark)
NS40086	*Aspergillus oryzae*	Macroporous acrylic resin	Novozymes (Denmark)
Lipase DF-15	*Rhizopus oryzae*	–	Amano (Japan)

的*sn*-1和*sn*-3位具有选择性，因此其被称为*sn*-1,3位选择性脂肪酶。具有*sn*-1,3位选择性的脂肪酶包括胰脂肪酶、胃脂肪酶和微生物脂肪酶，如*Penicillium camembertii*、*Rhizopus arrhizus*、*Penicillium roquefortii*、*Rhizomucor miehei*脂肪酶（RML）等。脂肪酶对甘油碳骨架的位置选择性取决于底物和脂肪酶的种类[24,25]。RML的*sn*-1,3位选择性可通过底物与脂肪酶结合位点的对接进行解释[26]。有研究结果表明，只有几种脂肪酶显示出*sn*-3位选择性（如兔胃脂肪酶）和*sn*-2位选择性（如南极假丝酵母脂肪酶A）[27]。但是，脂肪酶的位置选择性可能会因反应条件而改变[28]。Xu [1]、Bornscheuer [29]、Adlercreutz [23]及其他研究人员对一些常用脂肪酶的位置选择性进行过大量研究，因此，本文将不详细介绍这些脂肪酶。

脂肪酶的稳定性是另一个重要特性，脂肪酶的长期保存过程稳定性、可重复使用性、有机溶剂的耐受性等特性显著影响着工业应用的成本。脂肪酶的稳定性，尤其是热稳定性，对于它的存储和运输非常重要。固定化是提高脂肪酶稳定性的一种有效且常用的方法，该方法可使脂肪酶在高温反应下比游离酶更稳定，而且可被重复利用[30]。大多数有关酶法合成结构TAG的研究都采用了固定化脂肪酶，即商业化产品或在实验室中被固定的脂肪酶。蛋白质工程的定向进化是提高酶稳定性的有效方法[31,32]。其他加工方法，如高静水压法[33]和氧阴离子残基的疏水性改性法[34]也可以增加某些脂肪酶的稳定性和活性。

3. TAG 的分析

天然油脂中TAG的组成通常以脂肪酸表示，包括脂肪酸含量和脂肪酸组成[2]。由于TAG的结构对脂肪消化[11]、脂肪代谢[35]和整体健康的改善有显著影响，因此人们对TAG的组成（如脂肪酸在甘油碳骨架上的分布）的研究兴趣日益增加。然而，TAG的分析是一项具有挑战性的工作。TAG是一种极其复杂的混合物，它包含与三种相同或不同脂肪酸相连接的甘油分子[36]。近几十年来，有关TAG分析的研究越来越多[36]，这主要是由于先进分析技术的改进，尤其是质谱技术（mass spectrometry）的改进。在研究*sn*-2棕榈酸甘油酯时，TAG的分析非常重要，因为合成产物的不同位置分布将直接影响其功能和营养特性。

甘油碳骨架上脂肪酸的位置分布差异首先可通过胰脂肪酶的水解作用得到验证，胰脂肪酶通过水解作用可以分离出*sn*-2位的脂肪酸。最终，我们可以得到*sn*-1,3位和*sn*-2位脂肪酸组成，但是这种方法无法得到TAG结构的信息。由于该方法成本低且计算过程简单，所以，该方法仍被用于研究*sn*-2棕榈酸甘油酯的合成。该方法的使用通常会丢失TAG结构的真实信息。

应用最广泛且功能最强大的方法是色谱法，如气相色谱（gas chromatography, GC）法和液相色谱（liquid chromatography, LC）法，以及GC、LC与质谱（MS）联用[37]。许多优秀的书籍和研究论文对色谱法在TAG分析中的作用进行过描述，如Buchgraber等[38]有关色谱技术的综述、Fuchs等[39]有关薄层色谱法（TLC）的综述、Ruiz-Samblás等[40]有关高温GC法的综述、Indelicato等[36]的综述以及Christie等[41]的书籍。本文总结了常用的TAG分析方法，尤其针对结构TAG合成的研究，并着重分析了TAG位置异构体。

结构TAG的常用分析方法是高温GC法、非水反相LC与蒸发光散射检测器（NARP LC-ELSD）结合检测法以及银离子液相色谱法。高温GC是指毛细管柱保持在高温（>350 ℃）时的气相色谱。TAG的分离是根据其不饱和程度进行的。但是，常用的检测器，即火焰离子检测器无法提供TAG异构体的信息。因此，TAG的鉴定是基于相比于标准样品的保留时间进行的。对于结构TAG的分析，我们通常将此方法与*sn*-2位脂肪酸组成分析相结合。

LC是TAG分析中广泛使用的一种方法，根据两种色谱相的相对极性，该方法可被分为两种，即正相LC（NP LC）法和非水反相LC（NARP LC）法。NARP LC

使用了梯度洗脱和各种流动相系统[36]。洗脱顺序取决于碳原子数（CN）和双键数（DB），这取决于碳原子当量（ECN），即CN−2 × DB。在优化的色谱条件下，具有相同ECN的TAG可被分离出来[42–44]。该方法的样品制备简单，且设备相对便宜。因此，这种方法使用最为广泛[42,43,45–95]。但是，这种方法不能分离TAG位置异构体。

TAG分析中最常用的NP-LC是银离子正相液相色谱法。由于固定相的银离子与双键的π电子之间形成了弱络合物，所以银离子LC法可被用于分离TAG异构体[96]。分离后的TAG根据DB数的不同可被分成不同的组。银离子正相液相色谱法可被用于测量每个TAG异构体的含量[44,45]。

高分辨率^{13}C核磁共振（NMR）法也可以提供附着在TAG上以及在TAG特定*sn*-2位的脂肪酸信息，该信息可被用于分析TAG上脂肪酸的位置选择性[44]。最近报道的LC法，包括配备四极杆飞行时间MS的超高效液相色谱[3]法、二维GC[97]法和银离子大气压化学电离（APCI）MS [98]，可被用于有效分离天然油脂中的TAG，以及对TAG进行立体定向分析，将来，该方法还可被用于结构TAG的分析。

4. 酶法反应方式

本文概述了1997—2018年间发表的关于酶法合成*sn*-2棕榈酸甘油酯的研究，如表2所示。总体上，酶法合成*sn*-2棕榈酸甘油酯存在三种反应方式，即酸解反应、酯交换反应和醇解反应。其中酸解反应是最常见的方法，其次是酯交换反应。

4.1. 酸解反应

Sn-1,3位选择性脂肪酶催化酸解反应的典型方案如图2所示。酸解反应通常是通过*sn*-2位富含PA的TAG与FFA或FFA混合物在*sn*-1,3位选择性脂肪酶作用下进行的。三棕榈酸甘油酯（PPP）通常在实验室中被用作底物，因为其具有较高的纯度。但是，由于PPP价格昂贵，所以便宜的天然油脂（如棕榈硬脂、棕榈油、黄油和猪油等）常被用作PPP的替代品。FFA的来源一般是OA、LA、γ-亚麻酸（GLA）、FFA植物混合油（如大豆油、菜籽油、向日葵油、棕榈仁油、椰子油和榛子油等）、鱼油或富含LCPUFA的单细胞油。

如表2所示，在过去的20年中，大多数研究采用的都是酸解反应，合成产物中*sn*-2位的PA含量通常超过60%。Esteban [72]等使用了几种*sn*-1,3位选择性脂肪酶（包括脂肪酶DF、Lipozyme RM IM、Palatse 20000L、Lipozyme TL IM和脂肪酶QLC）来催化OA与富含PA的TAG的酸解反应。结果表明，脂肪酶DF可以在较短的反应时间（1 h）内实现较高的OA转化率（50.4%），从而在*sn*-2位上保持较高的PA含量（68.6%）。在对各种因素进行优化之后，最终得到的结构TAG在*sn*-1,3和*sn*-2位分别含有67.2%的OA和67.8%的PA。

最近我们团队也进行了*sn*-2棕榈酸甘油酯的合成

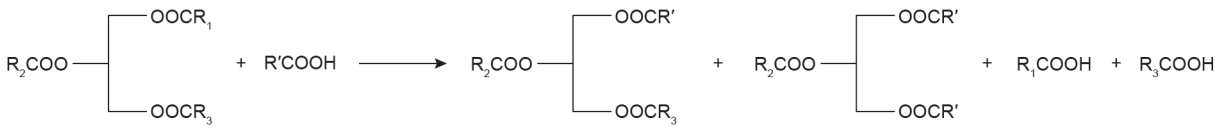

图2. *Sn*-1,3位选择性脂肪酶催化酸解反应方案。

表2 有关酶法合成*sn*-2棕榈酸甘油酯的25年间的文献研究

Lipase	Substrate	Products	Reaction conditions	Reactor	TAGs analysis	Reference
Immobilized *Candida lipolytica* lipase	PPP and OA	46.5% of OPO	50 °C, *n*-hexane	Vial	Silver-ion LC	2017 [45]
Novozym 435, Lipozyme 435, Lipozyme TL IM, Lipozyme RM IM	*Nannochloropsis oculata* and FFAs from *Isochrysis galbana*	59.38%–68.13% PA in the *sn*-2 position, 13.92%–17.12% *n*-3 PUFA	50/60 °C	—	—	2017 [46]
Lipozyme RM IM	Lard and selected oils	39.2% PA in the *sn*-2 position, 24.78% OPO	50 °C	PBR	NARP LC	2016 [47]
Lipozyme RM IM	Catfish oil and FFA from sesame oil	67.7% PA in the *sn*-2 position, 23.2% LA	40 °C	25 mL RBF	NARP LC	2016 [48]

（续表）

Lipase	Substrate	Products	Reaction conditions	Reactor	TAGs analysis	Reference
Lipase *Candida* sp. 99-125 coupled with -cyclodextrin	Lard and OA	79.51% PA in the *sn*-2 position, 55.3% OPO	40–50 °C	—	HPLC–ELSD	2016 [42]
Candida sp. 99–125 lipase	Monopalmitin and OA	75% PA in the *sn*-2 position, 40% OPO	38 °C	Orbital shaker	GC	2016 [49]
Immobilized *Rhizopus oryzae* lipase and Lipozyme RM IM	PPP and FFA from camelina oil	42.6%-52% *sn*-2 palmitate, 67.7 mol% PA in the *sn*-2 position	60 °C	Stirred batch reactor	GC	2016 [50]
Lipozyme RM IM	Silkworm pupae oil and OA	42.38% ALA in the *sn*-2 position, 20.11% PA, and SA	60 °C	Conical flasks	—	2015 [51]
Lipozyme TL IM	Palm stearin, high oleic sunflower oil, and tricaprin	40% PA in the *sn*-2 position, 21.22% CA	60 °C, *n*-hexane	Labeled Teflon-lined test tube	NARP LC	2015 [52]
Aspergillus oryzae Lipase	Palm stearin with OA	55.08% PA in the *sn*-2 position, 45.65% C52	65 °C	100 mL RBF	GC	2015 [53]
Novozym 435 Lipozyme RM IM	Lard and 2-MAG enriched in OA and LA	~58.7% PA in the *sn*-2 position, 23.1% OPO	37 °C,*n*-hexane	—	NARP LC	2015 [54]
Lipozyme IM-20	PPP and OA	55.2% OPO	Isooctane	—	Silver-ion LC	2015 [55]
Lipozyme RM IM	PPP and OA	35.9% OPO	50 °C, *n*-hexane	—	LCAPCIMS/MS	2015 [56]
Novozym 435	1,3-diolein and PA	94.8% OPO	Dichloromethane, 1-Ethyl-3-(3-dimethylaminopropyl) carbodiimide and 4-dimethylaminopyridine	—	Proton Nuclear Magnetic Resonance (^{1}H NMR)	2015 [57]
Lipozyme RM IM	Catfish oil and high oleic sunflower oil	50%–80% PA in the *sn*-2 position	50 °C	25 mL RBF	NARP LC	2015 [58]
Lipozyme TL IM and Lipozyme RM IM	PPP and OA	~85% PA in the *sn*-2 position, 32% OPO	50/60 °C, *n*hexane/ no solvent	25 mL RBF	NARP LC	2015 [59]
Lipozyme RM IM	Lard and FAs from camellia oil	~70% PA in the *sn*-2 position	45 °C, no solvent	Orbital shaker	—	2014 [60]
Novozym 435	PA-enriched refined olive oil and DHA	55.79 mol% of PA in the *sn*-2 position and 3.54 mol% total DHA	60 °C, *n*-hexane	Screw-capped test tubes	—	2014 [61]
Lipozyme RM IM	Lard and vegetable and single-cell oils	60%–70% PA in the *sn*-2 position	60 °C, no solvent	50 mL RBF	NARP LC	2014 [43]
Lipozyme RM IM	*Cinnamomum camphora*seed oil and OA	78.69% PA in the *sn*-2 position	60 °C, no solvent	50 mL RBF	NARP LC	2014 [62]
Lipozyme TL IM	PPP and FA from olive oil	~60% PA in the *sn*-2 position	65 °C, no solvent	Erlenmeyer flask	NARP LC	2013 [63]
Novozym 435	Hazelnut oil and PA; and ethyl palmitate	63.5 mol% of PA and 71.1% of PA in the *sn*-2 position	65 °C, *n*-hexane	Test tubes[a]	NARP LC	2013 [64]
Novozym 435 Lipozyme TL IM	PPP and FA from olive oil, AA), and DHA	>50% PA in the *sn*-2 position	60 °C, no solvent	Erlenmeyer flasks	NARP LC	2013 [65]
Novozym 435 Lipozyme RM IM	PA-enriched hazelnut oil and DHA, and ARA	57.3% of DHA, 2.7% of ARA, 2.4% of PA, and 66.1 PA in the *sn*-2 position	50/60 °C, no solvent	Screw-cap test tubes	—	2012 [66]
Carica papaya lipase	PPP and OA	≥70% PA in the *sn*-2 position, 22.1 mol% OA	60 °C, no solvent	Closedcylindrical batch reactors	—	2012 [67]

（续表）

Lipase	Substrate	Products	Reaction conditions	Reactor	TAGs analysis	Reference
Lipozyme TL IM	PPP and SDA from hazelnut oil, commercial oil	46.2% PA in the *sn*-2 position	60 °C, *n*-hexane	Flasks with glass stoppers	—	2012 [68]
Lipozyme RM IM	Palm stearin and mixed FA of SA and MA from rapeseed oil, sunflower oil, and palm kernel oil	28.8% PA and 53.2% PA in the *sn*-2 position	58 °C	PBR	NARP LC	2012 [69]
Lipozyme TL IM	Lard and soybean oil	~50% saturated fatty in the *sn*-2 position	60 °C	PBR	GC, Carbon-13 Nuclear Magnetic Resonance (^{13}C-NMR)	2011 [70]
Lipase DF, Palatase 20000L Lipozyme RM IM, Lipozyme TL IM lipase QLC	TAGs rich in PA and OA	68.6% PA in the *sn*-2 position	37 °C, no solvent	Flasks with silicone capped stoppers	—	2011 [71]
Lipozyme RM IM	PPP and FAs from hazelnut oil and Neobee	Rich in medium-chain FA	57 °C, *n*-hexane	Orbital shaking water bath	—	2011 [72]
Lipozyme RM IM	Lard and FAs from camellia oil	43.72% OPO	60/34 °C	A 25 mL conical flask	NARP LC	2011 [73]
Novozym 435 Lipozyme TL IM	PPP and SDA from soybean oil	≥60% PA in the *sn*-2 position, 6.82% SDA	50 °C, *n*-hexane	Teflon-lined test tubes	—	2011 [74]
Lipozyme RM IM	Palm stearin and mixed FAs from rapeseed oil, sunflower oil, palm kernel oil, SA, and MA	29.7% PA in the *sn*-2 position, 62.8% PA	60 °C, no solvent	—	NARP LC	2011 [75]
Novozym 435	PA and palm oil stearin	70.5% PA in the *sn*-2 position, 70.7% PA	37/50 °C, *n*-hexane	Erlenmeyer flasks[a]	—	2010 [76]
Lipozyme TL IM	PPP-rich fraction and ethyl oleate	31.43% OPO, 64.9% PA in the *sn*-2 position, 80.6% PA	50 °C	Erlenmeyer flask[a]	Silver-ion HPLC	2010 [77]
Lipozyme TL IM, RM IM Novozym 435 *C. parapsilosis*	PPP and OA /omega-3 PUFA	—	60 °C, no solvent	PBR	—	2010 [78]
Lipozyme RM IM	Lard and FAs from palm kernel oil, tea seed oil, and soybean oil	Similar to HMF	60 °C, no solvent	Orbital shaker flask	—	2010 [79]
Novozym 435	Ethyl palmitate and amaranth oil	76.6% PA in the *sn*-2 position, 45.5% PA	60 °C, no solvent	Test tubes[a]	—	2009 [80]
Bacillus stearothermophilus MC7	PPP and OA	Conversion exceeded 50%	60 °C, no solvent	—	Silver-ion-Thin layer chromatography (TLC)	2008 [81]
Lipozyme RM IM	PPP, coconut oil, safflower oil, and soybean oil	40.8% PA in the *sn*-2 position, 24.6% PA	55 °C, *n*-hexane	Test tubes[a]	—	2007 [82]
Lipozyme RMIM	FAs and lard	~70% PA in the *sn*-2 position	65 °C	PBR	—	2006 [83]
Lipozyme RM IM	PPP and hazelnut oil FA , and omega-3 FA	76.6% PA in the *sn*-2 position	55 °C, *n*-hexane	Test tubes[a]	—	2006 [84]
Lipozyme RM IM LIP1 (Candida *rugosa* lipase)	PPP and OA/ methyl oleate	49.4% OA (Lipozyme RM IM, methyl oleate)	65/45 °C, *n*-hexane	Test tubes[a]	—	2006 [85]

（续表）

Lipase	Substrate	Products	Reaction conditions	Reactor	TAGs analysis	Reference
Lipozyme RM IM Lipozyme TL IM	PPP and hazelnut oil FA, and GLA	PA 70% in *sn*-2 position, ~10% GLA, and ~44% OA	55 °C, *n*-hexane	Test tubes[a]	—	2005 [86]
Lipozyme RM IM	PPP and hazelnut oil FA, and SA	42.5% OA, 7% SA, and 70% PA in the *sn*-2 position	65 °C, *n*-hexane	Test tubes[a]	—	2005 [87]
Novozym 435, Lipase IM 60	PPP and OA	74% OPO, PA 90.7% in *sn*-2 position	50 °C, no solvent	10 mL glass vial	Silver-ion HPLC	2004 [88]
Lipozyme RM IM	FAs and lard	71% PA in the *sn*-2 position	61 °C, no solvent	Conical flask	—	2003 [89]
Immobilized *Fusarium* and *Rhizopus* lipase	PPP and OA	OPO 36 mol%	50 °C, no solvent	Screw-capped vessel	2 × NARP LC	2001 [90]
Immobilized RDL	AA and PPP	56.9/3.2 mol% of AA in *sn*-1,3/2 position, APA 75.9%	40 °C, no solvent	Screw-capped vessel	NARP LC	2000 [91]
Lipozyme IM, immobilized RDL, RML	PPP, 2-monopalmitin (2-MP), and OA	OPO in up to 78% yield containing 96% PA in *sn*-2 position	Vacuum/*n*-hexane	Closed vessel	GC	1999 [92]
Lipozyme IM and *papaya latex*	FAs and PPP	PA > 90% in *sn*-2 position	60 °C, no solvent	Screw-capped tube	GC	1998 [93]
Lipozyme IM, immobilized RDL, RJL, RNL	PPP, MAG, and OA	72% OPO, PA 94% in *sn*-2 position, 84% yield at >95% purity	Methyl-*t*-butyl ether	Closed vessel	GC	1998 [94]
Immobilized M10 ™ from *Mucor jauanicus*	OA and butterfat	Modified butterfat	40 °C, no solvent	Hollow-fiber reactor	NARP LC	1997 [95]

RBF: round-bottom flask; CA: capric acid; OA: oleic acid; PPP: tripalmitoylglycerol; PUFA: polyunsaturated fatty acids; PBR: Pebble-bed reactor ; HPLC: High-performance liquid chromatography; ALA: *a*-Linoleic acid ; APCI-MS: Atmospheric pressure chemical ionization mass spectrometers; FA: fatty acid; AA: arachidonic acid; SA: stearic acid; MA: myristic acid; GLA: gamma-linolenic acid; SDA: stearidonic acid; RDL: *Rhizopu delemar* lipase; RJL: *Rhizopus javanicus* lipase; RNL: *Rhizopus niveus* lipase; APA: 1, 3-arachiarachidonoy1-2-palmitoyl-glycerol; C52: TAG with a carbon number of 52.

[a] Test tubes or flasks with screw-caps.

研究。Wei等[59]在正己烷（*n*-hexane）体系和无溶剂系统中，利用高纯度的PPP和由山茶籽油制备的油酸，通过两种*sn*-1,3位选择性脂肪酶（Lipozyme RM IM和Lipozyme TL IM）催化合成了高纯度*sn*-OPO。研究发现，Lipozyme RM IM适用于*n*-hexane体系，而Lipozyme TL IM适用于无溶剂系统。*Sn*-2位的PA相对含量分别达到了92.92%和86.62%，而*sn*-OPO的含量分别为32.34%和40.23%。Zou等[48]通过由Lipozyme RM IM催化的酸解反应从巴沙鲶鱼油和芝麻油脂肪酸中制备出*sn*-2棕榈酸甘油酯。在最佳条件下，酶促产物中*sn*-2 PA含量占总PA的67.7%。这种方法的特点是步骤简单、副产物少。酸解反应的主要副产物是FFA，该副产物可通过分子蒸馏法有效去除。然而，由于酰基转移的存在，*sn*-2位脂肪酸迁移到了*sn*-1,3位，从而影响了目标物结构TAG的产量。因此，通过酸解反应获得的高纯度结构TAG的产率相对低于通过醇解反应获得的TAG产率。

4.2. 酯交换反应

酯交换反应是采用*sn*-1,3位选择性脂肪酶催化*sn*-2位富含PA的TAG与FFA酯或油脂的反应。图3显示了*sn*-1,3位选择性脂肪酶催化的酯交换反应方案。FFA酯主要包括乙酯和甲酯，而富含OA/PUFA的天然油脂通常用作酰基供体。此反应过程中所用到的材料通常价格便宜且分布广泛，这使得酯交换反应在结构TAG的工业生产中非常受欢迎。然而，反应的最终产物是具有相似物理性质的不同的TAG混合物，这使得我们很难通过纯化得到纯度较高的结构TAG。因此，选择种类和比例合适的的油脂至关重要。

一些研究人员选择猪油作为底物[47,70]。Zou等[99]在填充床反应器中通过采用Lipozyme RM IM催化猪油与植物油混合物的酯交换反应制备了*sn*-2棕榈酸甘油酯。植物油是高油酸油（如葵花籽油和低芥酸菜籽油）、微生物类油（如藻油和微生物油）、棕榈仁油和棕榈油的混合物。最终产物在*sn*-2位的PA含量为39.2%，并且

其脂肪酸分布与HMF高度相似。Srivastava等[85]使用LIP1或Lipozyme RM IM作为生物催化剂，通过PPP与OA或OA甲酯的酯交换反应合成了结构TAG。结果表明，在两种脂肪酶中，酯交换反应中OA甲酯的OA酯化比例均高于酸解反应中OA的酯化比例，且Lipozyme RM IM比LIP1更适合用于制备*sn*-2棕榈酸甘油酯。

4.3. 醇解和酯化反应

为了克服上述反应方案的缺点以及获得更多的结构TAG，我们研究利用醇解反应来合成*sn*-2棕榈酸甘油酯。*Sn*-1,3位选择性脂肪酶催化的醇解反应方案如图4所示。该方法是一个两步反应，每步都需要*sn*-1,3位选择性脂肪酶。首先，我们选择天然油脂与醇进行反应，从而形成了富含PA的2-MAG；然后，将纯化的2-MAG用FFA酯化以获得更高产率的目标物结构TAG。Schmid等[94]通过PPP的醇解反应制备了*sn*-OPO；在第一步反应中，研究人员研究了不同脂肪酶（Lipozyme RM IM、*Rhizopus delemar*和*Rhizopus javanicus*）对富含棕榈酸的2-MAG产量和纯度的影响。结果表明，固定在硅藻土上的*Rhizopus delemar*可使富含棕榈酸的2-MAG产量达到最高，进而使结晶后的2-MAG在甲基叔丁基醚中的纯度达到95%。

在第二步反应中，将纯化后的2-TAG与OA在*n*-hexane中采用固定在硅藻土上的Lipozyme RM IM或*Rhizopus delemar*进行酯化。最终产物在*sn*-2位的PA含量为92%~94%的、在*sn*-1,3位的OA含量为83%~89%的，而*sn*-OPO的产量达到了70%~72%。如表2所示，醇解反应仅被用于少数研究，其中*sn*-OPO含量都超过了70%，并且*sn*-2位的PA含量通常大于90%。该反应过程避免了酰基转移问题并获得了较纯的结构TAG。然而，反应过程的复杂性导致了成本增加。因此，这种方法在工业生产中不是常用的方法。

5. 市售 *sn*-2 棕榈酸甘油酯产品

结构TAG是一种具有一定营养、口感质地或者物理化学性质的脂类物质，可被广泛应用于食品加工和特殊医学领域。许多研究专注于结构TAG的商业化。如今，我们已经生产出商业化的*sn*-2棕榈酸甘油酯产品，该产品的组成与分布通常与HMF的相同。这些产品已作为营养强化剂被添加到婴儿配方奶粉中。

Loders Croklaan公司建立了*sn*-2棕榈酸甘油酯的首条生产线[100]。1995年，该公司申请注册Betapol®作为其商标，获批后，公司开始将*sn*-2棕榈酸甘油酯产品作为婴儿配方奶粉配料在欧洲进行生产。Betapol®是通过由固定化的*sn*-1,3位选择性脂肪酶（*Rhizomucor miehei*）对从高油酸葵花籽油获得的富含油酸的FFA与富含PPP脂肪的棕榈硬脂进行的酸解反应合成的。生产过程是在装有酶的填充床反应器中进行，该生产过程采用了两步法反应以提高脂肪酸的转化率[101]。Betapol®的TAG分子量分布组成如下：ECN:DB为52:2（33.4 mol%）和酰基碳数（CAN）:DB为52:3（10.5 mol%），其中主要的TAG位置异构体分别是*sn*-OPO（82.2 mol%）和*sn*-OPL/LPO（82.0 mol%）[102]。

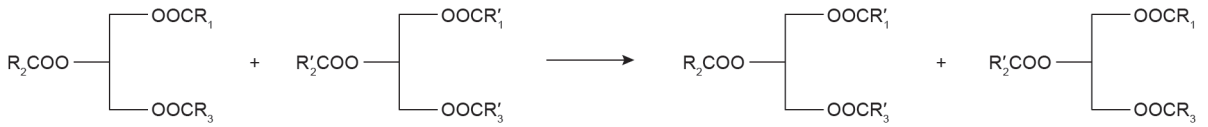

图3. *Sn*-1,3位选择性脂肪酶催化酯交换反应方案。

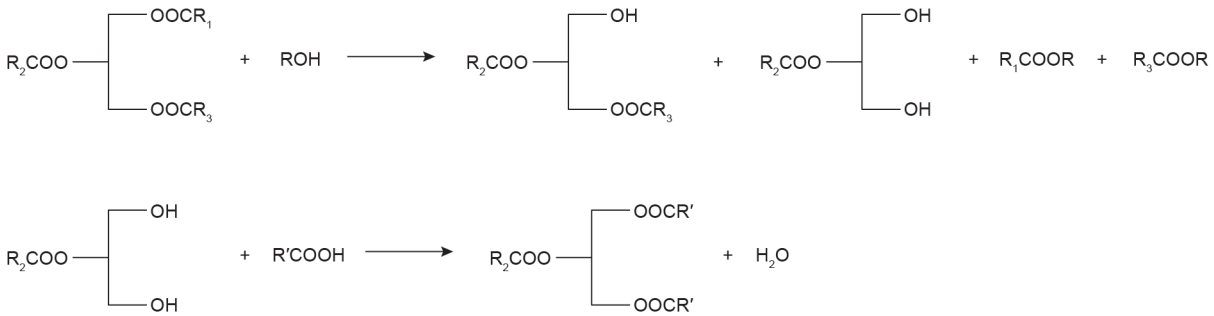

图4. *Sn*-1,3位选择性脂肪酶催化酸解反应方案。

目前，市场上有几种商业化的*sn*-2棕榈酸甘油酯产品，如由瑞典卡尔斯港市的Advanced Lipids公司生产的INFAT®。INFAT®在甘油碳骨架*sn*-2位的PA含量为70%~75%。在中国市场，*sn*-2棕榈酸甘油酯产品由新加坡丰益国际集团（Wilmar International）和浙江贝家生物科技有限公司生产。其他商业化的*sn*-2棕榈酸甘油酯产品包括惠氏公司（Bonamil）、雀巢集团（Alsty）和纽迪希亚公司（Cow & Gate Premium）。如获取更多信息，请读者参阅Ferreira等[101]和Happe等[103]的书籍。

6. 结论

在过去的20年，研究人员利用脂肪酶催化技术开发了几种结构TAG，如CBE、*sn*-2棕榈酸甘油酯和*sn*-1,3-山嵛酸-2-油酸TAG等[1]。其中，*sn*-2棕榈酸甘油酯是一种成功的结构TAG，许多制造商已将其添加到婴儿配方奶粉中作为营养补充剂。

除PA外，我们还应重视对婴儿配方奶粉中微量脂肪酸的研究，以使其达到与HMF相似的TAG组成。据报道，某些LCPUFA，如花生四烯酸（AA）和EPA主要附着在甘油碳骨架的*sn*-1,3位，而一些微量脂肪酸，如支链脂肪酸在*sn*-2位的含量较高（达60%）[104]。因此，深入研究HMF的TAG结构和组成将为研究新型结构TAG提供更详细的信息。

脂肪酶在结构TAG合成中有着广阔的应用前景。尽管脂肪酶与化学催化剂相比有明显的优势，但是其工业应用相对较慢，这主要是由于脂肪酶的成本较高。筛选更加便宜的具有*sn*-1,3位选择性的脂肪酶，并研究提高其稳定性的方法，将是我们未来的研究重点。

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Conflict of interest statement

Wei Wei, Cong Sun, Xiaosan Wang, Qingzhe Jin, Xuebing Xu, Casimir C. Akoh, and Xingguo Wang declare that they have no conflict of interest or financial conflicts to disclose.

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