分散液液微萃取技术在乳制品分析中的应用

杨新月[1#], 华震宇[1#], 赵艳坤[1,2], 郑 楠[2], 刘慧敏[2], 范盈盈[1],
武亚婷[1], 安 静[1], 郭万慧[1], 陈 贺[1*]

[1. 新疆农业科学院农业质量标准与检测技术研究所, 农业农村部农产品质量安全风险评估实验室(乌鲁木齐),
新疆农产品质量安全实验室, 乌鲁木齐 830091; 2. 中国农业科学院北京畜牧兽医研究所,
农业农村部奶及奶制品质量安全控制重点实验室, 北京 100193]

摘 要: 乳制品是人体重要的营养来源, 其品质和安全关乎公众健康。由于乳制品基质效应明显, 使相关指标的检测分析更具挑战性, 亟需发展简便、高效、绿色的前处理方法改善基质干扰。分散液液微萃取技术(dispersive liquid-liquid microextraction, DLLME)是一种新型样品前处理方法, 具有操作简单、溶剂消耗低和萃取效率高的特点, 已广泛用于复杂基质样品中痕量无机/有机分析物的前处理。本文阐述了不同类型 DLLME 的原理特点, 就 DLLME 技术在乳制品检测分析中的应用研究展开文献调研, 展望了该技术在乳制品提取中的优化方向和应用前景, 为乳制品分析提供新的技术选择。

关键词: 分散液液微萃取; 乳制品; 样品前处理

Application of dispersive liquid-liquid microextraction in dairy product analysis

YANG Xin-Yue[1#], HUA Zhen-Yu[1#], ZHAO Yan-Kun[1,2], ZHENG Nan[2], LIU Hui-Min[2],
FAN Ying-Ying[1], WU Ya-Ting[1], AN Jing[1], GUO Wan-Hui[1], CHEN He[1*]

(1. *Institute of Quality Standards & Testing Technology for Agro-products*, *Xinjiang Academy of Agricultural Sciences*, *Ministry of Agriculture and Rural Affairs-laboratory of Quality and Safety Risk Assessment for Agro-products*, *Key Laboratory of Agro-products Quality and Safety of Xinjiang*, *Urumqi* 830091, *China*; 2. *Institute of Animal Science*, *Chinese Academy of Agricultural Sciences*, *Key Laboratory of Quality & Safety Control for Milk and Dairy Products of Ministry of Agriculture and Rural Affairs*, *Beijing* 100193, *China*)

ABSTRACT: Dairy product is an important source of nutrition for human body, its quality and safety has direct relationship with public health. Because of the obvious matrix effect of dairy product, the detection and analysis of related indexes are more challenging, and it is urgent to develop simple, efficient and green pretreatment methods to improve matrix interference. A new pretreatment method, dispersive liquid-liquid microextraction (DLLME), has widely applied in complex samples, such as the analysis of trace organic or inorganic samples. The DLLME has many

基金项目: 国家自然科学基金项目(32060797)、国家农产品质量安全风险评估项目(GJFP20220304)、新疆天山创新团队项目(2022D14016)、新疆重大科技专项(2022A02006-1)、新疆科技援疆计划项目(2022E02012)

Fund: Supported by the National Natural Science Foundation of China (32060797), the National Agricultural Product Quality & Safety Risk Assessment Project (GJFP20220304), the Tianshan Innovation Team Project of Xinjiang (2022D14016), the Major Science and Technology Projects of Xinjiang Uygur Autonomous Region (2022A02006-1), and the Science and Technology Support Project of Xinjiang (2022E02012)

#杨新月、华震宇为共同第一作者

#YANG Xin-Yue and HUA Zhen-Yu are Co-first Authors

***通信作者:** 陈贺, 硕士, 研究员, 主要研究方向为奶及奶制品质量安全风险评估与营养品质评价。E-mail: 1441536011@qq.com

***Corresponding author:** CHEN He, Master, Professor, Institute of Quality Standards & Testing Technology for Agro-products, Xinjiang Academy of Agricultural Sciences, No.403 Nanchang Road, Urumqi 830091, China. E-mail: 1441536011@qq.com

advantages, such as simple operation, low solvent consumption and high extraction efficiency, *ect*.. This review compared the different types of DLLME, clarified the principles, and also investigated the application of DLLME technology in dairy product detection and analysis, prospected the optimization direction and application prospect of this technology in dairy product extraction, which provides a new technical choice for dairy product analysis.

KEY WORDS: dispersive liquid-liquid microextraction; dairy product; sample pretreatment

0 引 言

乳制品是人体重要的食物来源，主要包括液体乳、乳粉和其他乳制品，由于工艺、形态和质构的差异，其品质也各有参差，不同乳制品主要检测的品质指标各有差异[1]。乳制品基质复杂且干扰物影响大，无法直接用仪器检测相关指标，往往需要通过分离、富集和清除等前处理步骤来消除部分干扰物的不良影响[2−3]。因此，乳制品的相关分析对于样品前处理方法提出了更高的要求。

分散液液微萃取技术(dispersive liquid-liquid microextraction, DLLME)是一种微型化液相萃取的前处理方法。REZAEE 等[4]于 2006 年首次提出 DLLME 技术，用于检测水样中有机化合物的组分含量。通过萃取剂在分散剂中强大的分散能力，在样品溶液中分散成的细小液滴，形成萃取剂-分散剂-样品溶液三相乳浊液体系，增大了萃取剂和分析物的接触面积，使分析物在样品溶液和萃取剂之间快速达到分配平衡而完成萃取，通过离心即可分离出含有分析物的萃取剂[5−8]。与传统的溶剂萃取法相比，DLLME 具有操作简单、萃取快、有机溶剂消耗少和富集倍数高的特点。

目前，已有综述围绕乳制品中某一特定指标的检测技术(例如：药物残留、塑化剂等)进行归纳汇总，缺乏对于前处理方法在乳制品检测中应用研究进展的详细描述。本文阐述了 DLLME 的类型，系统地概述 DLLME 前处理方法及该技术在乳制品中的应用进展，以期为乳制品分析提供理论支撑。

1 DLLME 的不同类型

常规的 DLLME 通常使用 30~300 μL 的四氯化碳、氯仿、辛醇、低共熔溶剂和离子液体等作为萃取剂，分散剂以 0.5~3.0 mL 乙醇、乙腈、丙酮等为主，萃取 3~10 min(图 1)。近年来，为了增加 DLLME 的模式和应用场景，提高萃取效率，不同研究人员通过改变萃取辅助方式和萃取介质来优化 DLLME。由此，衍生出 DLLME 的多种类型。

1.1 按萃取辅助方式分类

1.1.1 超声辅助分散液液微萃取

超声辅助分散液液微萃取(ultrasound assisted-DLLME, UA-DLLME)是利用超声波加快萃取剂与分散剂在样品溶液中的溶解，提高萃取剂的分散程度[9−10]。QIAO 等[11]运用该方法快速提取瓶装水、茶饮料和牛奶中内分泌干扰物，并取得良好的效果。UA-DLLME 加快了萃取剂与分析物之间的传质速率，但持续超声所产生的热量会使分析物重新溶解回溶剂中，导致萃取量下降，延长超声时间也可能使萃取剂挥发。

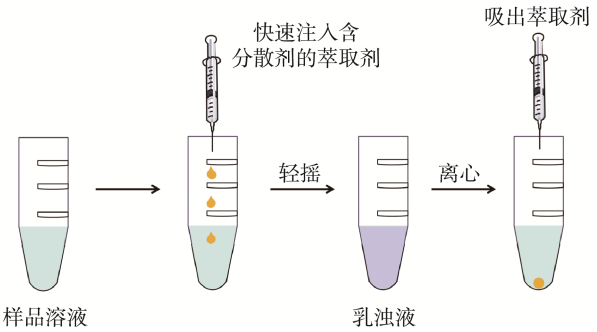

图 1 DLLME 示意图

Fig.1 Schematic of DLLME method

1.1.2 涡流辅助分散液液微萃取

涡流辅助分散液液微萃取(vortex assisted-DLLME, VA-DLLME)是利用涡旋加快萃取剂与样品溶液的混合，增强两相之间的均匀性和传质，有助于萃取剂形成细小液滴[12−13]。VA-DLLME 具有快速简便、环境污染小等优点，在塑化剂、抗生素等分析中得到了应用[14−15]。

1.1.3 空气辅助分散液液微萃取

空气辅助分散液液微萃取(air-assisted-DLLME, AA-DLLME)是将萃取剂和样品溶液反复吸入、排出注射器，直至形成混浊体系，达到平衡态，整个过程未使用分散剂[16−17](图 2)。值得注意的是，AA-DLLME 具有操作简单、低成本和普遍适用性，相比于超声和涡流辅助，该方法大大提高了萃取效率和富集倍数，已成功检测环境水中内分泌干扰物的含量，富集倍数可达 134 倍[18]。

1.2 按萃取介质分类

1.2.1 低密度溶剂-DLLME

低密度溶剂(low density solvent, LDS)是将密度比水小的溶剂如甲苯、辛醇等作为萃取剂，离心后，萃取剂漂浮在样品溶液上层，可直接吸取进样[19−20]。LAOSUWAN 等[21]用该方法快速测定水产品中 4 种金属离子，方法灵敏度提高了 64~230 倍。LDS-DLLME 操作简单，特别是，通过离心使 DLLME 体系更加分明，降低杂质干扰。

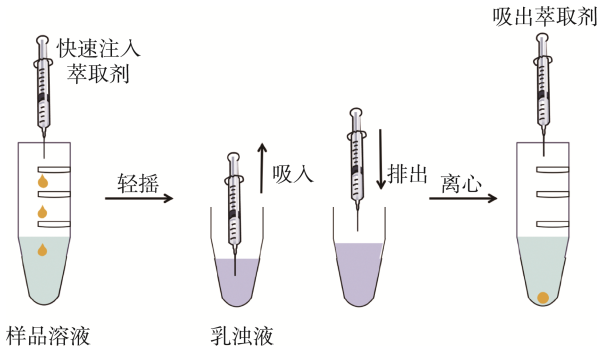

图 2　AA-DLLME 示意图

Fig.2　Schematic of AA-DLLME method

1.2.2　离子液体-DLLME

离子液体(ionic liquid, IL)是一类由有机阳离子与有机/无机阴离子构成的非分子型溶剂。在 DLLME 展现出良好的特性，包括热/化学稳定性、低挥发性、可设计性，以及与高效液相色谱(high performance liquid chromatography, HPLC)的兼容性[22−23]。与传统的单滴液液微萃取相比，AA-IL/IL-DLLME 使分析物的接触面积更大，更容易转移到萃取相中，缩短萃取时间。同时，该方法具有溶剂消耗少和绿色环保的优势，在萃取黄曲霉毒素、合成色素等指标中得到了有效应用[24−25]。

1.2.3　低共熔溶剂-DLLME

低共熔溶剂(deep eutectic solvents, DES)是一类由氢键受体和氢键供体组成的多组分低共熔混合物，也被称为“类离子液体”[26−28]。与 IL-DLLME 相比，DES-DLLME 具有低毒且操作成本低的优点，萃取剂的制备更简便，已应用于合成色素的测定[29−30]，但该方法可选择的氢键受体和供体有限。

1.2.4　悬浮固化分散液液微萃取

悬浮固化分散液液微萃取(DLLME based on solidification of floating organic droplet, DLLME-SFO)是将熔点低于 10~25℃的溶剂作为萃取剂，离心后经低温处理，凝固的萃取剂漂浮在样品溶液上层，易分离(图 3)[31]。值得关注的是，DLLME-SFO 将萃取剂在冰浴中固化，可促进其从样品溶液中分离，有利于萃取剂的收集，在药物残留等分析中得到了应用[32-33]；但由于萃取剂的低熔点性，可选择的萃取剂有限。

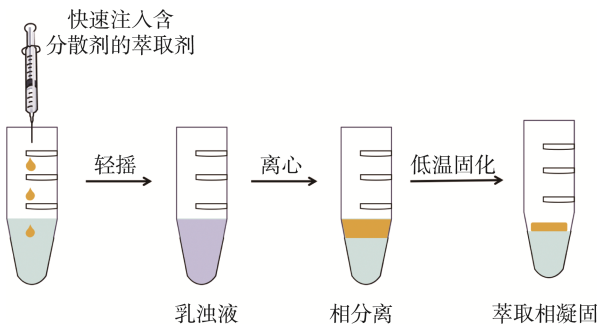

图 3　DLLME-SFO 示意图

Fig.3　Schematic of DLLME-SFO method

2　分散液液微萃取技术在乳制品中的应用

乳制品富含蛋白质、脂肪和维生素等营养物质，有益于人体健康。但乳制品从生产、运输、加工到上市需要经过多重环节，期间会受到生产、运输、储藏环境的影响，造成药物残留、塑化剂、抗生素等不同程度的污染。为了保证乳制品品质和安全，需要对这些指标进行监控，但由于乳制品基质效应明显，前处理技术会影响分析结果的准确性。

2.1　液体乳

HOMAIE 等[34]分别以四氯化碳和对氯苯酚 DES 为萃取剂，乙腈为分散剂，结合 HPLC-紫外检测器(ultraviolet absorption detector, UV)检测，比较 2 种萃取剂对牛奶中 4 种植物甾醇(豆甾醇、β-谷甾醇、菜油甾醇和芸苔甾醇)的萃取效果，基于四氯化碳的 DLLME 和 DES-DLLME 的检出限分别为 0.3~0.9 ng/mL 和 0.09~0.32 ng/mL，结果表明，DES-DLLME 具有更低的检出限，另外，DES 可以直接注入 HPLC 系统，缩短萃取时间，获得更高的萃取效率和良好的可重复性。QUIGLEY 等[35]采用氯甲酸乙酯衍生化反应，以 100 μL 氯仿为萃取剂，0.89 mL 乙腈为分散剂，建立了 DLLME-气相色谱-质谱法(gas chromatography-mass spectrometry, GC-MS)测定牛奶中 3 种游离氨基酸的分析方法，具有更快的衍生时间(<1 min)和更低的检出限(0.31~0.84 μg/mL)。营养类成分的检测多以 HPLC 为主，分离效果好，可避免衍生化、皂化等步骤。

在分析液体乳的营养指标的同时，科研工作者对其污染物分析也有一定的研究(表 1)。SZARK 等[45]将 QuEChERS (quick、easy、cheap、effective、rugged、safe)与 DLLME 相结合，通过 GC-MS 技术应用于酸奶中药物残留的快速检测，与单独使用 QuEChERS 法相比，该方法更经济、更省时，大大提高了分析物的富集。PETRARCA 等[46]以氯仿为萃取剂，借助超声辅助，通过液相色谱-串联质谱法(liquid chromatography-tandem mass spectrometry, LC-MS/MS)测定牛奶中除虫菊酯的含量。GC-MS 可以分离、识别和量化复杂样品中的微量成分，具有挥发性高、极性特征小和热稳定性好的优点，但在进样前需要进行溶剂置换；LC-MS/MS 方法较灵敏，具有高通量、高分辨率的优点。

在液体乳的生产、包装和加工过程中，接触的管材和包装材料中含有一定量的塑化剂(plasticizers, PAEs)，这可能会迁移到乳制品中，PAEs 污染在一定程度上会影响消费者的健康。YAN 等[47]以 40 μL CCl_4 为萃取剂、0.8 mL 甲醇为分散剂，通过 GC-火焰离子化检测器(flame ionization detection, FID)同时测定瓶装牛奶中 6 种邻苯二甲酸酯类物

质, 发现 PAEs 的含量均不超过 6.4 ng/g。与 GC 相比, HPLC 在分析 PAEs 异构体时具有较高的灵敏度和选择性, 中国出入境食品检验检疫行业标准 SN/T 3147—2017《出口食品中邻苯二甲酸酯的测定》除 GC-MS 外, 还列入了 HPLC-MS/MS。

相比于其他前处理方法, 已经报道的 DLLME 在液体乳中主要通过在前处理过程中加入乙腈、盐等试剂沉淀蛋白质、去除脂肪, 并结合萃取剂对目标物的特异性选择以减少基质干扰, 所建立的方法可大大节省前处理时间。

2.2 乳 粉

VINAS 等[48]基于四氯乙烷萃取剂, 结合液相色谱(liquid chromatography, LC)-FLD 检测, 开发了一种用于检测配方奶粉中维生素 B_1 的分析方法。SADRYKIA 等[49]首次将 DLLME 用于婴儿配方食品中维生素 E 的测定, 以氯仿和乙腈为萃取剂和分散剂对目标物快速分离、净化, 整个过程避免了烦琐的皂化步骤, 20 min 内即可完成前处理, 缩短了反应时间。

部分学者也关注了乳粉中可能的有害物质(表 2)。GAO 等[50]为了减少挥发性有机溶剂的消耗, 以疏水性[C_6MIM][PF_6]和亲水性[C_4MIM][BF_4]分别用作萃取剂和分散剂, 结合 UA-IL-DLLME 和 RP-HPLC-光电二极管阵列(photo-diode array, PDA)检测, 成功用于婴幼儿配方乳粉中磺胺类药物的提取、分离和测定, 方法的检出限和回收率分别为 2.94~16.7 μg/kg 和 90.4%~114.8%, 该方法具有色谱分离快、高灵敏度和高通量的优点。FARAJI 等[51]开发了一种新型、灵敏的多苯磺酰氯柱前衍生化法, 以低密度溶剂 1-辛醇和乙腈分别为萃取剂和分散剂, 结合 HPLC-UV/可见光谱(visible spectroscopy, VIS), 分析测定了原料奶和婴儿配方奶粉中的三聚氰胺。该方法简化了浓缩步骤, 减少了有机试剂的使用, 有效增强了灵敏度(检出限为 0.1 μg/L)和极性化合物三聚氰胺的疏水性, 表现出良好的准确性和重现性。

对比发现, 乳粉和液体乳的样品形态不同, 与常规液体乳处理相比, 通常需要提前用温水将乳粉溶解, 而后使用提取液提取。在此过程中, 既要注意提取液的选择, 尽可能使目标物完全提取, 也要根据相似相溶原理选择与目标物有相互作用的萃取剂, 所建立的 DLLME 体系具有一定的创新性。

表 1 DLLME 在液体乳中的应用
Table 1 Application of DLLME in milk

DLLME 类型	萃取剂/分散剂	分析物	分析方法	回收率/%	检出限	文献
DLLME	四氯化碳(35 μL)/乙醇(0.8 mL)	胆固醇	HPLC-UV	93.3~105	0.01 μg/L	[33]
DES-DLLME	对氯苯酚(39 μL)/乙腈(1.25 mL)	豆甾醇、*β*-谷甾醇、菜油甾醇、芸苔甾醇	HPLC-UV	82~91	0.09~0.32 ng/mL	[34]
DLLME	氯仿(100 μL)/乙腈(0.89 mL)	游离氨基酸	GC-MS	68.82~108	0.31~0.84 μg/mL	[35]
DLLME	氯仿(1 mL)/乙腈(1.9 mL)	磺胺类药物	反向色谱(reversed phase, RP)-HPLC-FLD	90.8~104.7	0.6~1.03 μg/L	[36]
UA-DLLME	四氯化碳(100 μL)/异丙醇(0.8 mL)	邻苯二甲酸酯	GC-MS	70~130	0.1~0.9 ng/g	[37]
DES-DLLME	DES(250 μL)/乙腈(100 μL)	三嗪和苯脲类除草剂	HPLC-二极管阵列检测器(diode array detector, DAD)	80.21~118.62	0.41~0.59 μg/L	[38]
DLLME	四氯化碳(80 μL)/乙醇(2 mL)	维生素 D_3	HPLC-UV	86.6~113.3	0.9 ng/mL	[39]
DLLME	氯仿(250 μL)/丙酮(1.2 mL)	苯甲酸、山梨酸	HPLC-UV	101~105	苯甲酸: 0.1 μg/mL, 山梨酸: 0.08 μg/mL	[40]
DES-DLLME	DES (200 μL)/硫酸铵(5 mL)	氟喹诺酮类药物	HPLC-UV	87.8~114.1	0.01 μg/mL	[41]
IL-DLLME	[C_4MIM-TEMPO]Cl/甲醇	磺胺类药物	HPLC-UV	97.2~101.6	0.534~0.891 μg/L	[42]
IL-DLLME	b-MIM-Cl (140 μL)/NH_4PF_6 水溶液(980 μL)	四环素类药物	HPLC-DAD	75.8~109.7	0.12~0.45 μg/L	[43]
UA-IL-DLLME	[C_6MIM][Tf_2N] (100 μL)	硒	石墨炉原子吸收光谱法	/	12 ng/L	[44]

注: /表示无, 下同。

表 2　DLLME 在乳粉中的应用
Table 2　Application of DLLME in milk powder

DLLME 类型	萃取剂/分散剂	分析物	分析方法	回收率/%	检出限	文献
DLLME	四氯乙烷(90 μL)/乙腈(0.5 mL)	维生素 B_1	LC-FLD	98.7	0.09 ng/mL	[48]
DLLME	氯仿(200 μL)/乙腈(2 mL)	维生素 E	HPLC-UV	89.12~109.98	5 mg/L	[49]
UA-IL-DLLME	$[C_6MIM][PF_6]$ (70 μL)/$[C_4MIM][BF_4]$ (100 μL)	磺胺类药物	RP-HPLC-PDA	90.4~114.8	2.94~16.7 μg/kg	[50]
LDS-DLLME	1-辛醇(60 μL)/乙腈(1.0 mL)	三聚氰胺	HPLC-UV/VIS	90~104.2	0.1 μg/L	[51]
DLLME	氯仿(200 μL)/乙腈(2 mL)	大环内酯类抗生素	LC-MS/MS	89.5~105	HPLC-DAD: 0.3~1.4 ng/g; LC-MS/MS: 0.03~0.72 ng/g	[52]
DLLME	二氯甲烷(300 μL)/乙醇(1 mL)	丙森锌	GC-MS	98.1~102.3	0.15 mg/kg	[53]
DLLME	四氯乙烯(30 μL)/CAN (440 μL)	双酚 A 双酚 B	GC-MS	68~114	双酚 A: 60 ng/L; 双酚 B: 30 ng/L	[54]

2.3　奶　酪

添加抗菌性的物质能有效抑制或延缓奶酪表面微生物的生长, 但需要进行严格的质量控制(表 3)。纳他霉素是国际上唯一获准的抗真菌生物防腐剂, GB 2760—2014《食品安全国家标准 食品添加剂使用标准》规定纳他霉素在干酪和再制干酪及其类似品中最大使用量为 0.3 g/kg。SOROURADDIN 等[55]开发了 DLLME 与火焰原子吸收光谱法相结合的新型分析方法, 以三氯乙烷和乙醇为萃取剂和分散剂, 成功用于奶酪中纳他霉素的提取、富集和测定, 在此过程中, 分散剂和萃取剂混合后快速注入样品中, 加入 Zn^{2+}, 纳他霉素-锌复合物快速转移至萃取剂中, 达到平衡态, 方法回收率和检出限分别为 86%~98%和 1.8 ng/mL, 具有良好的精密度(<3.9%)。ABEDI 等[56]以 1-辛醇和丙酮为萃取剂和分散剂, 结合 GC-FID 检测, 快速萃取奶酪中山梨酸和苯甲酸, 避免了衍生化步骤, 克服耗时长的问题, 方法具有较高的回收率(97%~103%), 较好的重现性(相对标准偏差小于 6.1%)、较快的萃取时间(10 min)和无基质干扰等优点。

奶酪等固体乳制品与上述的液体乳和乳粉又是一种不同的存在形态(固态), 在前处理中需要提前将样品切成小块(约 5 mm 厚), 放置于滤纸约 1 h, 以去除渗透的水, 而后用电动混合器将样品均质, 在制样环节则需要消耗约 1.5 h。针对这类样品, DLLME 萃取快的优势则突显出来, 从样品制备结束到萃取完成大约需要 15 min 即可上机分析, 但固体乳制品处理耗时较长的问题仍有待优化。

2.4　乳脂(黄油)

乳脂(黄油)是将牛奶中的稀奶油和脱脂乳分离后, 使稀奶油成熟并经搅拌而成的奶制品。ROOSTA 等[60]首次采用超声辅助反胶束分散液-液微萃取(ultrasound-assisted reverse micelles dispersive liquid-liquid microextraction, USA-RM-DLLME)开发了用于黄油中乙偶姻的测定方法, 以水和表面活性剂为萃取介质避免了有机溶剂的使用, 方法的回收率和检出限分别为 93.9%~107.8%和 0.2 mg/L, 具有灵敏度高、线性范围宽(0.6~200 mg/L)、萃取时间短(12.5 min)和分离时间短(3 min)的优点。EBADNEZHAD 等[61]以乙基(甲基)氯化铵/新戊酸为萃取剂, 乙醇为分散剂, 分析黄油中 4 种植物甾醇的含量, 该方法未稀释样品即可提高灵敏度, 且检出限低(0.51~1.3 ng/g)、富集因子高(340~425)。

表 3　DLLME 在奶酪中的应用
Table 3　Application of DLLME in cheese

DLLME 类型	萃取剂/分散剂	分析物	分析方法	回收率/%	检出限	文献
DLLME	三氯乙烷(116 μL)/乙醇(1.5 mL)	纳他霉素	火焰原子吸收法	86~98	1.8 ng/mL	[55]
LDS-DLLME	1-辛醇(60 μL)/丙酮(475 μL)	山梨酸、苯甲酸	GC-FID	88~103.7	山梨酸: 150 ng/g 苯甲酸: 140 ng/g	[56]
UA-DLLME	氯仿(500 μL)/乙腈(3 mL)	黄曲霉毒素 B_1、黄曲霉毒素 M_1	RP-HPLC-FLD	/	黄曲霉毒素 B_1: 0.1 μg/kg 黄曲霉毒素 M_1: 0.01 μg/kg	[57]
LDS-DLLME	1-辛醇(60 μL)/丙酮(600 μL)	生物胺	GC-MS	97~103	5.9~14.0 ng/g	[58]
DES-DLLME	DEAC-香芹酚(64 μL)/乙腈(1.3 mL)	黄曲霉毒素 M_1	HPLC-FLD	94	0.74 ng/kg	[59]

相比于奶酪等固体乳制品，黄油样品在 40℃下融化 5 min，即可进行 DLLME 操作，大大缩短了样品处理的时间。

3 结束语

DLLME 与多种检测手段相结合，为乳制品的成分及污染物分析提供了一种可行的检测方法。与 GC、HPLC、MS 等检测技术有效结合，可用于分析蛋白质、甾醇、脂肪等营养成分和抗生素、塑化剂等有害物质，通过提高富集倍数、萃取回收率与灵敏度，表现出较好的分析效果。

DLLME 作为一种新型的前处理方法，较其他萃取技术相比，具有操作简单、萃取快和绿色污染小的特点。基于相似相溶原理，已发展出一些新的辅助方式和萃取介质，AA-DLLME 借助空气有效改善了分析物和萃取相的传质，减少分散剂的使用；DES 和 IL 是新型萃取剂，制备简单，绿色友好。将 DES/IL 与 AA-DLLME 结合，进一步提高了分析物的萃取效率。

但是，DLLME 在乳制品分析中还处于初探阶段，仍有待进一步拓展，包括：(1)扩宽分析物种类。乳制品中 β-乳球蛋白、乳铁蛋白等生物活性成分分析较为复杂，可以尝试使用新型的前处理方法简化检测过程，DLLME 就提供了新的可能。(2)开发具有更优异性能的萃取剂。结合分析物的化学结构，通过多种相互作用力(如 π-π 作用、离子交换、静电作用、氢键、疏水和亲水作用等)，设计与分析物有作用力的萃取剂，提高目标物的选择性。因此，探索和发现更多新型萃取剂，结合适宜的检测技术，获得更好的富集效果和检测数据，是 DLLME 技术在乳制品分析应用中重要的发展方向之一。

参考文献

[1] WITARD OC, BATH SC, DINEVA M, *et al*. Dairy as a source of iodine and protein in the UK: Implications for human health across the life course, and future policy and research [J]. Front Nutr, 2022, 9: 800559.

[2] SERESHTI H, ZAREI-HOUSSEINABADI M, SOLTANI S, *et al*. Green vortex-assisted emulsification microextraction using a ternary deep eutectic solvent for extraction of tetracyclines in infant formulas [J]. Food Chem, 2022, 396: 133743.

[3] YU X, ZHONG T, ZHANG YJ, *et al*. Design, preparation, and application of magnetic nanoparticles for food safety analysis: A review of recent advances [J]. J Agric Food Chem, 2022, 70(1): 46–62.

[4] REZAEE M, ASSADI Y, MILANII H, *et al*. Determination of organic compounds in water using dispersive liquid-liquid microextraction [J]. J Chromatogr A, 2006, 1116(1–2): 1–9.

[5] ALCANTARA GKS, CALIXTO LA, ROCHA BA, *et al*. A fast DLLME-LC-MS/MS method for risperidone and its metabolite 9-hydroxyrisperidone determination in plasma samples for therapeutic drug monitoring of patients [J]. Microchem J, 2020, 156: 104894.

[6] 易冰清, 何纯点, 涂小珂, 等. 分散液液微萃取结合气相色谱-质谱法快速测定葡萄酒中 20 种农药残留[J]. 食品安全质量检测学报, 2021, 12(19): 7651–7659.
YI BQ, HE CD, TU XK, *et al*. Rapid determination of 20 kinds of pesticide residues in wine by dispersive liquid-liquid microextraction combined with gas chromatography-mass spectrometry [J]. J Food Saf Qual, 2021, 12(19): 7651–7659.

[7] GALUCH MB, MAGON T, SILVEIRA R, *et al*. Determination of acrylamide in brewed coffee by dispersive liquid-iquid microextraction (DLLME) and ultra-performance liquid chromatography tandem mass spectrometry (UPLC-MS/MS) [J]. Food Chem, 2019, 282: 120–126.

[8] GRAU J, AZORIN C, BENEDE JL, *et al*. Use of green alternative solvents in dispersive liquid-liquid microextraction: A review [J]. J Sep Sci, 2022, 45(1): 210–222.

[9] MALAEI R, RAMEZANI AM, ABSALAN G. Analysis of malondialdehyde in human plasma samples through derivatization with 2,4-dinitrophenylhydrazine by ultrasound-assisted dispersive liquid-liquid microextraction-GC-FID approach [J]. J Chromatogr B, 2018, 1089: 60–69.

[10] GAO X, SI XX, YUAN YX, *et al*. Ultra-trace extraction of two bactericides via ultrasound-assisted dispersive liquid-liquid microextraction [J]. J Chromatogr Sci, 2021, 59(2): 182–190.

[11] QIAO LZ, SUN RT, TAO Y, *et al*. New low viscous hydrophobic deep eutectic solvents for the ultrasound-assisted dispersive liquid-liquid microextraction of endocrine-disrupting phenols in water, milk and beverage [J]. J Chromatogr A, 2021, 1662: 462728.

[12] WANG YX, ZHAO SJ, YANG LY, *et al*. Determination of 12 quinolones in honey by vortex-assisted dispersive liquid liquid microextraction performed in syringe based on deep eutectic solvent combine with ultra performance liquid chromatography-mass spectrometry [J]. Eur Food Res Technol, 2022, 248(1): 263–272.

[13] SANTANA-MAYOR A, HERRERA-HERRERA AV, RODRIGUEZ-RAMOS R, *et al*. Development of a green alternative vortex-assisted dispersive liquid-liquid microextraction based on natural hydrophobic deep eutectic solvents for the analysis of phthalate esters in soft drinks [J]. ACS Sustain Chem Eng, 2021, 9(5): 2161–2170.

[14] SANTANA-MAYOR A, HERRERA-HERRERA AV, RODRIGUEZ-RAMOS R, *et al*. Development of a green alternative vortex-assisted dispersive liquid-liquid microextraction based on natural hydrophobic deep eutectic solvents for the analysis of phthalate esters in soft drinks [J]. ACS Sustain Chem Eng, 2021, 9(5): 2161–2170.

[15] SELAHLE SK, NOMNGONGO PN. Determination of fluoroquinolones in the environmental samples using vortex assisted dispersive liquid-liquid microextraction coupled with high performance liquid chromatography [J]. Int J Environ Anal Chem, 2019, 100(3): 282–294.

[16] AZOOZ EA, AL-WANI HSA, GBURI MS, *et al*. Recent modified air-assisted liquid-liquid microextraction applications for medicines and organic compounds in various samples: A review [J]. Open Chem, 2022, 20(1): 525–540.

[17] EL-DEEN AK, SHIMIZU K. A green air assisted-dispersive liquid-liquid microextraction based on solidification of a novel low viscous ternary deep eutectic solvent for the enrichment of endocrine disrupting compounds from water [J]. J Chromatogr A, 2020, 1629: 461498.

[18] EL-DEEN AK, SHIMIZU K. A green air assisted-dispersive liquid-liquid microextraction based on solidification of a novel low viscous ternary

deep eutectic solvent for the enrichment of endocrine disrupting compounds from water [J]. J Chromatogr A, 2020, 1629: 461498.

[19] ZHU BL, MENG L, CAO J, *et al*. Simultaneous determination of 10 new psychoactive piperazine derivatives in urine using ultrasound-assisted low-density solvent dispersive liquid-liquid microextraction combined with gas chromatography-tandem mass spectrometry [J]. J Forensic Sci, 2021, 66(2): 748–757.

[20] LAOSUWAN M, MUKDASAI S, SRIJARANAI S. A simple in syringe low density solvent-dispersive liquid liquid microextraction for enrichment of some metal ions prior to their determination by high performance liquid chromatography in food samples [J]. Molecules, 2020, 25(3): 552.

[21] LAOSUWAN M, MUKDASAI S, SRIJARANAI S. A simple in syringe low density solvent-dispersive liquid liquid microextraction for enrichment of some metal ions prior to their determination by high performance liquid chromatography in food samples [J]. Molecules, 2020, 25(3): 552.

[22] WANG YL, LI B, SARMAN S, *et al*. Microstructural and dynamical heterogeneities in ionic liquids [J]. Chem Rev, 2020, 120: 5798–5877.

[23] FENG JJ, LOUSSALA HM, HAN S, *et al*. Recent advances of ionic liquids in sample preparation [J]. Trac-trend Anal Chem, 2020, 125: 115833.

[24] ZHAO RY, AN J, SUN YM, *et al*. A simple and low-cost sample preparation for the effective extraction, purification and enrichment of aflatoxins in wheat by combining with ionic liquid-based dispersive liquid-liquid microextraction [J]. Microchem J, 2021, 164: 106036.

[25] ZHANG YQ, SUN YM, YANG Z, *et al*. A simple one-step transferred sample preparation for effective purification and extraction of auramine O in bean product by combining air-assisted ionic liquid-based dispersive liquid-liquid microextraction [J]. Microchem J, 2020, 159: 105571.

[26] ACHKAR T, GREIGE-GERGES H, FOURMENTIN S. Basics and properties of deep eutectic solvents: A review [J]. Environ Chem Lett, 2021, 19(4): 3397–3408.

[27] YILMAZ E, SOYLAK M. A novel and simple deep eutectic solvent based liquid phase microextraction method for rhodamine B in cosmetic products and water samples prior to its spectrophotometric determination [J]. Spectrochim Acta A, 2018, 202: 81–86.

[28] ZHU SQ, ZHOU J, JIA HF, *et al*. Liquid-liquid microextraction of synthetic pigments in beverages using a hydrophobic deep eutectic solvent [J]. Food Chem, 2018, 243: 351–356.

[29] YILMAZ E, SOYLAK M. A novel and simple deep eutectic solvent based liquid phase microextraction method for rhodamine B in cosmetic products and water samples prior to its spectrophotometric determination [J]. Spectrochim Acta A, 2018, 202: 81–86.

[30] ZHU SQ, ZHOU J, JIA HF, *et al*. Liquid-liquid microextraction of synthetic pigments in beverages using a hydrophobic deep eutectic solvent [J]. Food Chem, 2018, 243: 351–356.

[31] XU LJ, MIAO XH, YANG ZG, *et al*. Solid-phase extraction combined with dispersive liquid-liquid microextraction based on solidification of floating organic droplet for simultaneous determination of organochlorine pesticides and polychlorinated biphenyls in fish [J]. Food Anal Method, 2019, 12(8): 1871–1885.

[32] MAO XJ, WAN YQ, LI ZM, *et al*. Analysis of organophosphorus and pyrethroid pesticides in organic and conventional vegetables using QuEChERS combined with dispersive liquid-liquid microextraction based on the solidification of floating organic droplet [J]. Food Chem, 2020, 309: 12575.

[33] DANESHFAR A, KHEZELI T, LOTFI HJ. Determination of cholesterol in food samples using dispersive liquid-liquid microextraction followed by HPLC-UV [J]. J Chromatogr B, 2009, 877(4): 456–460.

[34] HOMAIE O, MOGADDAM MRA, TAMIZI E, *et al*. Comparison of organic and deep eutectic solvents based dispersive liquid-liquid microextraction for the analysis of phytosterols in cow milk combined with high-performance liquid chromatography-ultraviolet detector [J]. J Sep Sci, 2021, 44(22): 4167–4180.

[35] QUIGLEY A, CONNOLLY D, CUMMINS W. Determination of selected amino acids in milk using dispersive liquid-liquid microextraction and GC-MS [J]. Anal Methods, 2019, 11(28): 3538–3545.

[36] DANESHFAR A, KHEZELI T, LOTFI HJ. Determination of cholesterol in food samples using dispersive liquid-liquid microextraction followed by HPLC-UV [J]. J Chromatogr B, 2009, 877(4): 456–460.

[37] TUNCEL SG, SENLIK D. Determination of phthalates in milk by ultrasound-assisted dispersive liqui-liquid microextraction and gas chromatography-mass spectrometry [J]. Anal Lett, 2016, 49(9): 1334–1343.

[38] FENG J, ZHAO Z, LI JL, *et al*. Vortex-assisted dispersive liquid-liquid microextraction based on the solidification of sedimentary deep eutectic solvents for the determination of triazine and phenylurea herbicides in milk samples [J]. Anal Methods, 2022, 14(4): 460–468.

[39] GHALEBI M, TAMIZI E, AHMADI S, *et al*. A dispersive liquid-liquid micro-extraction technique for the pre-concentration and quantification of vitamin D_3 in milk and yogurt samples using a non-aqueous HPLC method [J]. Iran J Pharm Res, 2019, 18(2): 677–685.

[40] JAVANMARDI F, NEMATI M, ANSARIN M, *et al*. Benzoic and sorbic acid in soft drink, milk, ketchup sauce and bread by dispersive liquid-liquid microextraction coupled with HPLC [J]. Food Addit Contam B, 2015, 8(1): 32–39.

[41] YU KL, YUE ME, XU J, *et al*. Determination of fluoroquinolones in milk, honey and water samples by salting out-assisted dispersive liquid-liquid microextraction based on deep eutectic solvent combined with MECC [J]. Food Chem, 2020, 332: 127371.

[42] YAO T, DU KF. Simultaneous determination of sulfonamides in milk: In-situ magnetic ionic liquid dispersive liquid-liquid microextraction coupled with HPLC [J]. Food Chem, 2020, 331: 127342.

[43] KAYNAKER M, ANTEP M, MERDIVAN M. Determination of tetracyclines in milk, eggs and honey using in-situ ionic liquid based dispersive liquid-liquid microextraction [J]. J Anal Chem, 2018, 73(1): 23–29.

[44] TUZEN M, PEKINER OZ. Ultrasound-assisted ionic liquid dispersive liquid-liquid microextraction combined with graphite furnace atomic absorption spectrometric for selenium speciation in foods and beverages [J]. Food Chem, 2015, 188: 619–624.

[45] SZARK A, BUCIKOVA K, KOSTIC I, *et al*. Development of a multiresidue QuEChERS-DLLME-fast GC-MS method for determination of selected pesticides in yogurt samples [J]. Food Anal Method, 2020, 13(10): 1829–1841.

[46] PETRARCA MH, CCANCCAPA-CARTAGENA A, MASIA A, *et al*. Comparison of green sample preparation techniques in the analysis of pyrethrins and pyrethroids in baby food by liquid chromatography-tandem mass spectrometry [J]. J Chromatogr A, 2017, 1497: 28–37.

[47] YAN HY, CHENG XL, LIU BM. Simultaneous determination of six phthalate esters in bottled milks using ultrasound-assisted dispersive liquid-liquid microextraction coupled with gas chromatography [J]. J Chromatogr B, 2011, 879(25): 2507–2512.

[48] VINAS P, LOPEZ-GARCIA I, BRAVO-BRAVO M, *et al*. Dispersive liquid-liquid microextraction coupled to liquid chromatography for thiamine determination in foods [J]. Anal Bioanal Chem, 2012, 403(4): 1059–1066.

[49] SADRYKIA F, SHAYANFAR A, VALIZADEH H, *et al*. A fast and simple method for determination of vitamin E in infant formula by dispersive liquid-liquid microextraction combined with HPLC-UV [J]. Food Anal Method, 2019, 12(1): 23–31.

[50] GAO SQ, YANG X, YU W, *et al*. Ultrasound-assisted ionic liquid/ionic liquid-dispersive liquid-liquid microextraction for the determination of sulfonamides in infant formula milk powder using high-performance liquid chromatography [J]. Talanta, 2012, 99: 875–882.

[51] FARAJI M, ADELI M. Sensitive determination of melamine in milk and powdered infant formula samples by high-performance liquid chromatography using dabsyl chloride derivatization followed by dispersive liquid-liquid microextraction [J]. Food Chem, 2017, 221: 139–146.

[52] CAMPILLO N, VINAS P, FEREZ-MELGAREJO G, *et al*. Dispersive liquid-liquid microextraction for the determination of macrocyclic lactones in milk by liquid chromatography with diode array detection and atmospheric pressure chemical ionization ion-trap tandem mass spectrometry [J]. J Chromatogr A, 2013, 1282: 20–26.

[53] BODUR S, ERARPAT S, GUNKARA OT. A new derivatization method for the determination of propineb in black tea and infant formula samples using dispersive liquid-liquid microextraction followed by gas chromatography-mass spectrometry [J]. Talanta, 2020, 213: 120846.

[54] CUNHA SC, ALMEIDA C, MENDES E, *et al*. Simultaneous determination of bisphenol A and bisphenol B in beverages and powdered infant formula by dispersive liquid-liquid micro-extraction and heart-cutting multidimensional gas chromatography-mass spectrometry [J]. Food Addit Contama A, 2011, 28(4): 513–526.

[55] SOROURADDIN SM, FARAJZADEH MA, HASSANYANI A. Determination of natamycin in dairy products using dispersive liquid-liquid microextraction and indirect flame atomic absorption spectrometry [J]. Food Anal Method, 2017, 10(7): 2529–2538.

[56] ABEDI AS, MOHAMMADI A, AZADNIYA E, *et al*. Simultaneous determination of sorbic and benzoic acids in milk products using an optimised microextraction technique followed by gas chromatography [J]. Food Addit Contam A, 2014, 31(1): 21–28.

[57] KARASEVA NM, AMELIN VG, TRET'YAKOV AV. QuEChERS coupled to dispersive liquid-liquid microextraction for the determination of aflatoxins B_1 and M_1 in dairy foods by HPLC [J]. J Anal Chem, 2014, 69(5): 461–466.

[58] MOHAMMADI M, KAMANKESH M, HADIAN Z, *et al*. Determination of biogenic amines in cheese using simultaneous derivatization and microextraction method followed by gas chromatography-mass spectrometry [J]. Chromatographia, 2017, 80(1): 119–126.

[59] MASROURI M, MOGADDAM MRA, FARAJZADEH MA, *et al*. Combination of solvent extraction with deep eutectic solvent based dispersive liquid-liquid microextraction for the analysis of aflatoxin M_1 in cheese samples using response surface methodology optimization [J]. J Sep Sci, 2021, 44(7): 1501–1509.

[60] ROOSTA M, GHAEDI M, DANESHFAR A. Optimisation of ultrasound-assisted reverse micelles dispersive liquid-liquid micro-extraction by Box-Behnken design for determination of acetoin in butter followed by high performance liquid chromatography [J]. Food Chem, 2014, 161: 120–126.

[61] EBADNEZHAD H, MOGADDAM MRA, FARAJZADEH MA, *et al*. Development of an ultrasonic and heat-assisted liquid-liquid extraction method combined with deep eutectic solvent-based dispersive liquid-liquid microextraction for the extraction of some phytosterols from cow milk butter samples [J]. J Iran Chem Soc, 2021, 18(9): 2483–2491.

(责任编辑：张晓寒 于梦娇)

作者简介

杨新月，硕士，助理研究员，主要研究方向为农产品质量与安全。
E-mail: 897855095@qq.com

华震宇，硕士，高级实验师，主要研究方向为农产品质量与安全。
E-mail: 780016831@qq.com

陈　贺，硕士，研究员，主要研究方向为奶及奶制品质量安全风险评估与营养品质评价。
E-mail: 1441536011@qq.com