

Liquid–liquid chromatography as a promising technology in the separation of food compounds

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1 | INTRODUCTION

Liquid–liquid chromatography (LLC) is a preparative separation technique in which both the stationary and mobile phases are liquid. In LLC, the immiscible phases of a pre-equilibrated biphasic solvent system are used as the mobile and stationary phases. Centrifugal fields are used to keep one of the phase stationary, while the other one is pumped through the “column.” The different partitioning of the compounds present in an injected mixture between the two phases results in elution at different time along the column, according to their partition coefficients. (Berthod, 2002). LLC operation units generally consist of “hydrostatic” centrifugal partition chromatography or “hydrodynamic” counter-current chromatography units (Pauli et al., 2008). LLC has many advantages over classic liquid–solid chromatographic methods, that is, lack of irreversible adsorption, lower solvent utilization, saving of time and laboratory effort, possibility to use crude samples, and practically full recovery of sample and solvents. The developed separation conditions can be easily scaled-up for industrial applications (Bouju et al., 2015). Limitations of the LLC are associated with the retention and stability of the liquid stationary phase during the process. Problems with stationary phase may result from sample injection, properties of the biphasic solvent system and conditions of the process (e.g., temperature, rotations). These issues need to be addressed during the optimization of the isolation. Despite the low recognition of LLC, the features mentioned above may open the way to broader applications in the future. For instance, LLC is a

powerful tool for separating complex chemical mixtures, such as plant extracts. LLC has been employed in the isolation of a wide variety of natural compounds, for example, alkaloids (Leitão et al., 2021), polyphenols (Li et al., 2022), terpenoids (Song et al., 2016), saponins (Wang et al., 2022), coumarins (Kozioł et al., 2020; Widelski et al., 2021), and many other (Friesen et al., 2015; Luca et al., 2019; Skalicka-Woźniak & Garrard, 2014) (Figure 1).

In this commentary, we shortly reviewed research works in which LLC has been applied in food research. Extensive possibilities of LLC applications include separating compounds with potentially beneficial properties, exploring the composition of food supplements and pigments, or assessing the contamination of foods with mycotoxins, pesticides, or pyrrolizidine alkaloids (PAs).

2 | COLORANTS AND PIGMENTS

The chemical composition of various food colorants and pigments was explored using LLC. The technique has been successfully used to separate complex pigments and their impurities (Kabasawa et al., 1991, 1992; Ogura et al., 1994; Oka et al., 1998), as well as to isolate natural colorants (e.g., betanins, anthocyanins, β -carotene). Those studies were also vital in developing advanced operating LLC methods (Oka et al., 2002). The use of LLC in the purification of color compounds from natural products includes, among others, the isolation of betanins (magenta dye) from red beetroots (Spórna-Kucab et al., 2013), isolation of anhydrosafflor yellow B

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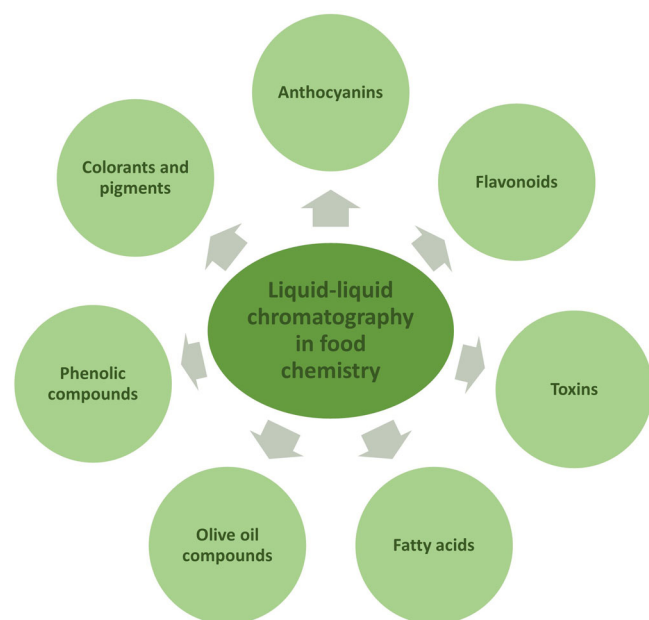


FIGURE 1 Applications of liquid–liquid chromatography (LLC) in food chemistry.

pigment (yellow dye) from safflower (Huang et al., 2021) or purification of β -carotene (orange dye) (Tao et al., 2021). The application of LLC in the isolation of pigments of natural origin generally provided higher yields and prevented the molecules' degradation catalyzed by the stationary phase in high-performance liquid chromatography (HPLC). Furthermore, using food-grade solvent systems to isolate food-grade compounds may open up a new way to isolate compounds with potential health benefits (Spórna-Kucab et al., 2015).

LLC is a developing technique for the downstream isolation of anthocyanins from complex mixtures. For instance, the first LLC purification methods of anthocyanins were developed by Renault et al. These methods were less time- and solvent-consuming than previously described procedures, such as paper chromatography, ion exchange chromatography, or HPLC (Renault et al., 1995, 1997). The separations were carried out in oxygen-free acidic media, preserving thus the anthocyanins' bioactivity. In addition, anthocyanins from grape pomace were purified with LLC with high efficiency in preparative scale (Lima et al., 2021). All these studies indicate the possibility of using LLC as a faster and more efficient alternative to conventional separation technologies to isolate anthocyanins. For instance, this technology can provide higher sample recovery, higher availability of the stationary phase for interactions with the solute, higher stability in acidic or basic conditions, higher range of solvent systems polarities, and lower solvent consumption and maintenance costs than in HPLC systems (Nunes et al., 2022).

As a group of lipid-soluble natural pigments, the unstable structures of carotenoids require a more careful

selection of the separation method, thus limiting their large-scale preparation. Preserving the structure of carotenoids is a critical issue due to the numerous biological activities of these compounds, that is, reduction of oxidative damage, immunomodulatory activity, and prevention of cardiovascular, eye, and cancer diseases. Thus, applying solid phase-free and easily scale-up techniques like LLC seems to be the perfect solution to this challenge. For instance, LLC was applied to isolate carotenoids from *Lycium barbarum* L. (Solanaceae) fruits (goji berries). The method was efficient for the large-scale preparation of carotenoids based on its improvements, that is, high sample injection and low solvent consumption (Gong et al., 2021).

3 | PHENOLIC COMPOUNDS

Phenolic compounds include over 8000 known structures. They are present in all plant organs, including fruits and vegetables. Therefore, phenolics are vital ingredients in the human diet (Alara et al., 2021). Developing efficient extraction and isolation methods for the structure determination and health impact studies of these compounds is crucial. LLC has been applied in isolating a wide range of polyphenolic compounds, such as anthocyanins and flavonoids. A broad variety of anthocyanins have been isolated with LLC from red wine, elderberry and blood orange juice, blackberries, purple corn, black rice, grape seeds, pine bark, cinnamon bark, cocoa seeds, and eggplant (Degenhardt et al., 2001; Fan, Li, et al., 2020; Hillebrand et al., 2004; Jeon et al., 2015; Phansalkar et al., 2018; Schwarz et al., 2003). The polyphenolic profile (together with other classes of compounds) in tea or coffee extracts has also been explored with the use of LLC (Fan et al., 2022; Si et al., 2006; Stodt et al., 2015; Yanagida et al., 2006; Yuan et al., 2004). LLC has also been applied in separating polyphenols from red wine and rum aged in oak barrels (Fan et al., 2015; Regalado et al., 2011). Furthermore, LLC has been employed to examine the polyphenols composition of numerous fruits, that is, blackcurrants (He et al., 2009; Mbeunkui et al., 2012), citrus fruits (Rodríguez-Rivera et al., 2014; Zhu et al., 2013), apples (Castillo-Fraire et al., 2019; Lu et al., 2019), or passion fruit (Pan et al., 2020). Recent studies concerned the application of LLC in isolating gallic acid from *Cornus officinalis* (Cornaceae). Purified gallic acid was added to starch-based products to improve the food quality. LLC improved the separation efficiency (loading capacity and purity) compared with conventional chromatography on the macroporous resin (Tan et al., 2022). In another study, dihydromyricetin was isolated from the Asian tea herb *Ampelopsis grossedentata* (Vitaceae) with LLC. The purified polyphenol, obtained with an enhanced purity and in a shorter isolation time compared to the previously used

TABLE 1 Examples of the applications of LLC in the isolation of food components.

Source	Isolated compounds	Purity of the isolate	Yield	Time of isolation	References
Safflower (<i>Carthamus tinctorius</i>)	Anhydrosafflor yellow B	98%	6.52 ± 1.01 mg/100 mg of extract	320 min	Huang et al. (2021)
<i>Lycium barbarum</i> fruits	Carotenoids: zeaxanthin (A), zeaxanthin monopalmitate (B), zeaxanthin dipalmitate (C)	87.9% (A), 88.9% (B), 91.2% (C)	28.5 mg of A, 45.8 mg of B, and 161.5 mg of C/720 mg of extract	651 min	Gong et al. (2021)
Black Currant (<i>Ribes nigrum</i>)	Anthocyanins delphinidin-3-rutinoside (A), cyanidin 3-rutinoside (B), delphinidin 3-glucoside (C), cyanidin 3-glucoside (D)	-	16 mg of A, 11 mg of B, 5 mg of C, 3 mg of D/430 mg of extract	-	Degenhardt et al. (2001)
Red wine	Malvidin 3-glucoside (E)	-	43 mg of E/1.1 g of extract	-	
Red Cabbage (<i>Brassica oleracea</i>)	Derivatives of cyanidin-3-sophorosido-5-glucoside (1–5)	-	30 mg (1/2), 32 mg (3), 5 mg (4), 4 mg (5) of compounds 1, 2, 3, 4, 5/30 mg of extract	-	
Peel of eggplant (<i>Solanum melongena</i>)	Delphinidin-3-rutinoside	99.1%	-	45 min	Fan, Li, et al. (2020)
Black currant (<i>R. nigrum</i>) fruit	Phenolic acids: protocatechuic acid (A), caffeic acid (B), 4-hydroxybenzoic acid (C), myricetin (D)	89% (A), 94% (B), 95% (C), 96% (D)	0.8 mg of A, 1.0 mg of B, 0.5 mg of C, and 2.5 mg of D/500 mg of extract	480 min	He et al. (2009)
Black currant (<i>R. nigrum</i>) fruit	Flavonols: myricetin-3-O-rutinoside (A), myricetin-3-O-hexoside (B), myricetin-3-O-(6"-malonyl)-glucoside (C), kaempferol-3-O-glucoside (D), quercetin-3-O-rutinoside (E), quercetin-3-O-hexoside (F), and myricetin (G)	-	145.5 mg of A, 79.7 mg of B, 17.4 mg of C, 20.5 mg of D, 55.1 mg of E, 25.8 mg of F, 129.1 mg of G/10.5 g of extract	I step 180 min, II steps up to 290 min	Mbeunkui et al. (2012)
Ougan (<i>Citrus reticulata</i>) albedo	Poncirin	96.56%	2.1 mg/60 mg of refined extract	260 min	Zhu et al. (2013)
Apple (<i>Malus pumila</i>) leaves	Polyphenols: unknown quercetin-glycoside of flavonoid (A), quercetin 3-O-rhamnoside (B), quercetin 3-O-xyloside (C)	96.45% (A), 90.18% (B), 92.23% (C)	2.52 mg of A, 3.20 mg of B, 2.84 mg of C/300 mg of extract	420 min	Lu et al. (2019)
Passion fruit (<i>Passiflora edulis</i>) seeds	Polyphenols (stilbens): scirpusin B (A) and piceatannol (B)	90.2% (A), 94.8% (B)	15.4 mg of A and 28.9 mg of B/300 mg of extract	300 min	Pan et al. (2020)
<i>Cornus officinalis</i>	Gallic acid	98.72%	106 mg/500 mg of extract	460 min	Tan et al. (2022)
<i>Ampelopsis grossedentata</i>	Dihydromyricetin	97.13%	96 mg/300 mg of extract	120 min	Xue et al. (2022)
Fish oil	Eicosapentaenoic acid ethyl ester (A), docosahexaenoic acid ethyl ester (B)	96.92% (A), 95.12% (B)	62.5 mg of A and 355.4 mg of B/480 mg of fish oil	170 min	Fa, Li, et al. (2020) and Fan, Wen, et al. (2020)
Fish oil	Eicosapentaenoic acid (A), docosahexaenoic acid (B)	95.5% (A), 96.9% (B)	79.6 mg of A and 328.3 mg of B/500 mg of refined fish oil	300 min	Li et al. (2019)

(Continues)

TABLE 1 (Continued)

Source	Isolated compounds	Purity of the isolate	Yield	Time of isolation	References
Diatom (<i>Nanofrustulum shiloi</i>)	Eicosapentaenoic acid	99%	698.97 mg/1200 mg of algal oil	I step 51 min, II step 200 min	Bárcenas-Pérez et al. (2022)
Rye grains (<i>Secale cereale</i>)	Alkylresorcinols (1–5)	>99% in either case	51.8 mg of 1, 77.4 mg of 2, 57.2 mg of 3, 28.8 mg of 4, and 11.5 mg of 5/1 g hydrogenated and transesterified rye grain extract	305 min	Hammerschick et al. (2021)
Olive oil deodorizer distillate (<i>Olea europaea</i>)	Squalene	95.5%	935 mg/5 g of olive oil deodorizer distillate	240 min	Xynos et al. (2016)
Olive oil processing wastewater (<i>O. europaea</i>)	Hydroxytyrosol	>95%	430 mg/6 g of refined extract	180 min	Xynos et al. (2015)
Olive oil (<i>O. europaea</i>)	Hydroxytyrosol (A), tyrosol (B), and (E)/(Z)-oleocanthal (C)	96.1% (A), 94.5% (B), 89.2% (C)	34.8 mg of A/400 mg of phenolic fraction 25.1 mg of B and 19.2 mg of C/800 mg of phenolic fraction	60 min	Adhami et al. (2015)

Abbreviation: LLC, liquid–liquid chromatography.

column chromatography processes on polyamide and macroporous resins, was proven to improve corn starch quality (Xue et al., 2022). Those studies indicate the promising future role of LLC in food and dietary supplement enrichment.

4 | FATTY ACIDS AND PLANT OILS

Omega-3 fatty acids, such as eicosapentaenoic acid and docosahexaenoic acid, are essential macronutrients in the human diet. Abundant sources of these compounds are commonly used as dietary supplements, for example, fish oils (Cleland et al., 2006). However, the separation of such lipid compounds by chromatographic methods is challenging. Interestingly, as a very adaptable method, LLC seems to be one of the few efficient techniques capable of isolating nonpolar lipid compounds.

LLC allowed the separation of fatty acid methyl esters from shark liver oil (Bordier et al., 1994; Du et al., 1996). The latest studies indicate the effectiveness of LLC in the purification of eicosapentaenoic and docosahexaenoic acids. These two acids were isolated using innovative water-free biphasic solvent systems consisting of guanidinium ionic liquids, which provided high yield and loading capacity. Applying ionic liquids in LLC hastens the possibility of more economical and environmentally friendly isolation processes (Fan, Wen, et al., 2020; Li et al., 2019). In another study, eicosapentaenoic acid was isolated by LLC from the biomass of *Nanofrustulum shiloi*, a species of marine diatoms. The proposed approach led to the high efficiency of the isolation process and high purity of the obtained fatty acid (Bárcenas-Pérez et al., 2022). At this point, it is important to mention the groundbreaking research of the W. Vetter group, which led to the development of efficient methods for isolating lipid compounds by LLC. The research concerned the selection of biphasic solvent systems for specific lipid classes. All advantages and limitations of the chosen solvent systems were addressed (Schröder & Vetter, 2011; Vetter et al., 2017). Thus, the purification of phytosterols from plant oils (Schröder & Vetter, 2012), fatty acids from microalgae strains (Hammann et al., 2013), alkylresorcinols from rye grains (Hammerschick et al., 2021), dicarboxylic fatty acids from the head tissue of Nile tilapia (*Oreochromis niloticus*) (Lehnert et al., 2021), and fatty acids from fungi species (Müller et al., 2022) was successively achieved.

Olive oil obtained from olives (the fruits of *Olea europaea*, Oleaceae) is rich in fatty acids, along with squalene, sterols, and phenolic compounds (e.g., esters of tyrosol, hydroxytyrosol or oleuropein) (Boskou, 2006). LLC was repeatedly used to separate olive oil compounds and industrial by-products. LLC has proven to be highly efficient in the isolation of compounds such as squalene

(Xynos et al., 2016), secoiridoids (Vougogiannopoulou et al., 2015), hydroxytyrosol (Xynos et al., 2015), oleuropein (Boka et al., 2015), and many others (Angelis et al., 2021). Once again, LLC proved numerous benefits over traditional chromatography, that is, prevention of isomers decomposition due to stationary phase interactions, lower solvent consumption, and higher speed of isolation (Adhami et al., 2015).

Thus, LLC can be regarded as an innovative method for separating health-beneficial fatty acids and other lipid compounds from various sources, such as fish oils, microalgae, or plant oils. Furthermore, the efficiency of the process implies the possibility of using LLC on a large scale in the food industry.

5 | TOXINS

LLC can play a significant role in isolating and fractionating toxins from food products such as pesticides, mycotoxins, or PAs. For instance, LLC was used to explore the presence of pesticides (namely methomyl, fenobucarb, and carbaryl pesticides) in vegetable and fruit samples (Ito et al., 2008). LLC was also successfully applied in the isolation of mycotoxins from foods, for example, *Alternaria* mycotoxins from wine and juice (Fan et al., 2016), *Fusaria* B-type fumonisins (Hu et al., 2020) and trichothecene mycotoxins from *Fusarium* (Liu et al., 2018). In these studies, LLC has shown to be more advantageous over hitherto used techniques, such as column chromatography on macroporous resins, anion-exchange resins, or octadecylsilyl silica. The same applies to food contamination with hepatotoxic and cancerogenic PAs. LLC can be used to explore the presence of PAs in natural products or foods. For instance, one recent study presented the purification of PAs from honey by LLC. Products like honey can be contaminated with PAs due to bees foraging on the PAs-producing plants (Letsyo, 2022).

6 | CONCLUSION

This short commentary was intended to present the potential use of LLC in isolating food ingredients by reviewing the studies related to this aspect. Notwithstanding, LLC could find a suitable and well-deserved place in food production and quality control of foods. Numerous studies have proven its usefulness in separating ingredients necessary in food processing (e.g., dyes and compounds improving the quality of food products). A summary of various applications of the LLC for separation of food compounds is presented in Table 1. LLC has an unquestionable position as an efficient tool in isolating compounds that can be used as dietary supplements or in determining toxins contamination in foods. In the future, the usefulness of the LLC may be increased through its miniaturization and high throughput of the instruments.

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