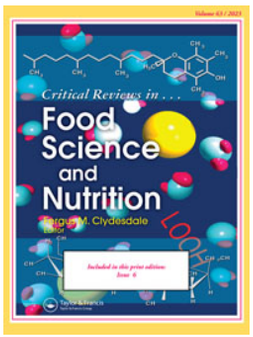

Critical Reviews in Food Science and Nutrition

ISSN: (Print) (Online) Journal homepage: https://www.tandfonline.com/loi/bfsn20

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To cite this article: Lili Song, Sisi Wen, Qin Ye, Heqiang Lou, Yadi Gao, Vivek K. Bajpai, María Carpena, Miguel-Angel Prieto, Jesus Simal-Gandara, Jianbo Xiao, Xianghe Meng & Jiasheng Wu (2023) Advances on delta 5-unsaturated-polymethylene-interrupted fatty acids: Resources, biosynthesis, and benefits, Critical Reviews in Food Science and Nutrition, 63:6, 767-789, DOI: 10.1080/10408398.2021.1953960

To link to this article: https://doi.org/10.1080/10408398.2021.1953960

Published online: 16 Aug 2021.

CRITICAL REVIEWS IN FOOD SCIENCE AND NUTRITION
2023, VOL. 63, NO. 6, 767–789
https://doi.org/10.1080/10408398.2021.1953960

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REVIEW

Advances on delta 5-unsaturated-polymethylene-interrupted fatty acids: Resources, biosynthesis, and benefits

Lili Song[a]*, Sisi Wen[b]*, Qin Ye[c], Heqiang Lou[a], Yadi Gao[a], Vivek K. Bajpai[d], María Carpena[e], Miguel-Angel Prieto[e], Jesus Simal-Gandara[e], Jianbo Xiao[e,f], Xianghe Meng[b], and Jiasheng Wu[a]

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ABSTRACT

Though the knowledge on delta 5-unsaturated-polymethylene-interrupted fatty acids (Δ5-UPIFAs) is being updated, the issue of their integration still exists within the field. Thus, this review systematically summarizes the sources, biosynthesis and metabolism, analytical methods, preparation, and health-promoting roles of Δ5-UPIFAs. In plants, the content of Δ5-UPIFAs is higher, which is an ideal source. In animals, although the content of Δ5-UPIFAs is not high, there are many species, which is the possible source of some special Δ5-UPIFAs. At present, although the extraction of Δ5-UPIFAs is mainly from plants, the fermentation by organisms, especially for genetically modified microorganisms engineering maybe be a substitue of pepration of Δ5-UPIFAs. Δ5-UPIFAs have been proved to possess multi-beneficial effects, such as lipid lowering, anti-inflammation and so on, so it has a certain potential application value. However, related knowledge of the underlying molecular mechanisms regarding Δ5-UPIFAs limited, and how Δ5-UPIFAs work is not clear. Further clinical and human studies about Δ5-UPIFAs are also needed. Studies on tapping new resources, developing structured lipide rich in Δ5-UPIFA and enhancing delivery were quite deficient. This review emphasizes the further directions on Δ5-UPIFAs with scientific suggestions to pay more attention to the applications of Δ5-UPIFAs in food, pharmaceutical and cosmetic industries.

KEYWORDS

Delta 5-unsaturated-polymethylene-interrupted fatty acids; sources; preparation; determination; healthy benefits

1. Introduction

Delta 5-unsaturated-polymethylene-interrupted fatty acids (Δ5-UPIFAs) are unusual fatty acids, where the first- and second- double bonds are separated by multiple methylene groups $(-CH2-)_{n\geq 2}$, and the first double bond appears in the fifth carbon atom near the carboxyl end of fatty acids (Wang et al. 2020; Wolff and Christie 2002). Although there are several hosts of Δ5-UPIFAs, in this review, we have mainly focused on major Δ5-UPIFAs such as taxoleic acid (TA), pinolenic acid (PA), coniferonic acid (CA), sciadonic acid (SA), and juniperonic acid (JA) due to their natural abundance relatively in large amounts. The chemical structures of the five Δ5-UPIFAs are shown in Figure 1.

In general, Δ5-UPIFAs occur in organisms with a wide range of contents. Δ5-UPIFAs are found relatively in larger amounts in various gymnosperms (Sayanova et al. 2007; Wolff 1998; Wolff et al. 2001) than other organisms, such as algae (Kim and Itabashi 2012), marine invertebrates (Go et al. 2002; Litchfield, Tyszkiewicz, and Dato 1980), fungi (Jamieson and Reid 1972a, 1972b; Jareonkitmongkol, Shimizu, and Yamada 1993), and molds (Saito and Ochiai 1996). The total contents of Δ5-UPIFAs in the conifer seed oil and *Pinus pumila* kernel oil were about 20%–32% and 20% of total fatty acids, respectively (Chen et al. 2016). Moreover, the pine nut oil contains 14%–19% PA of total fatty acids (Xie, Miles, and Calder 2016). In contrast, only small amounts were found to present in algae (<2.5%) (Kim and Itabashi 2012). In plants, the content of Δ5-UPIFAs is higher, which is an ideal source. In animals, although the content of Δ5-UPIFAs is not high, there are many species, which is the possible source of some special Δ5-UPIFAs. At present, the extraction of Δ5-UPIFAs is mainly from plants, so the special Δ5-UPIFAs in animals needs to be better extracted and utilized. And the content of Δ5-UPIFAs in nature is not very large, so we can consider some new technologies to prepare, such as microbial fermentation and so on.

Δ5-UPIFAs are bioactive and have been proved to possess multi-beneficial effects, e.g., serum lipid reducing ability (Ells et al. 2012; Huang et al. 2014; Tsai et al. 2018), anti-inflammatory effect, increasing insulin sensitivity (Calder, et al. 2015; Christiansen et al. 2015), and anti-tumor activity (Chen et al. 2011; Das 2011; Park et al. 2018), suggesting that Δ5-UPIFAs are worthy of being studied in terms of

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*Lili Song and Sisi Wen contributed equally to this work.

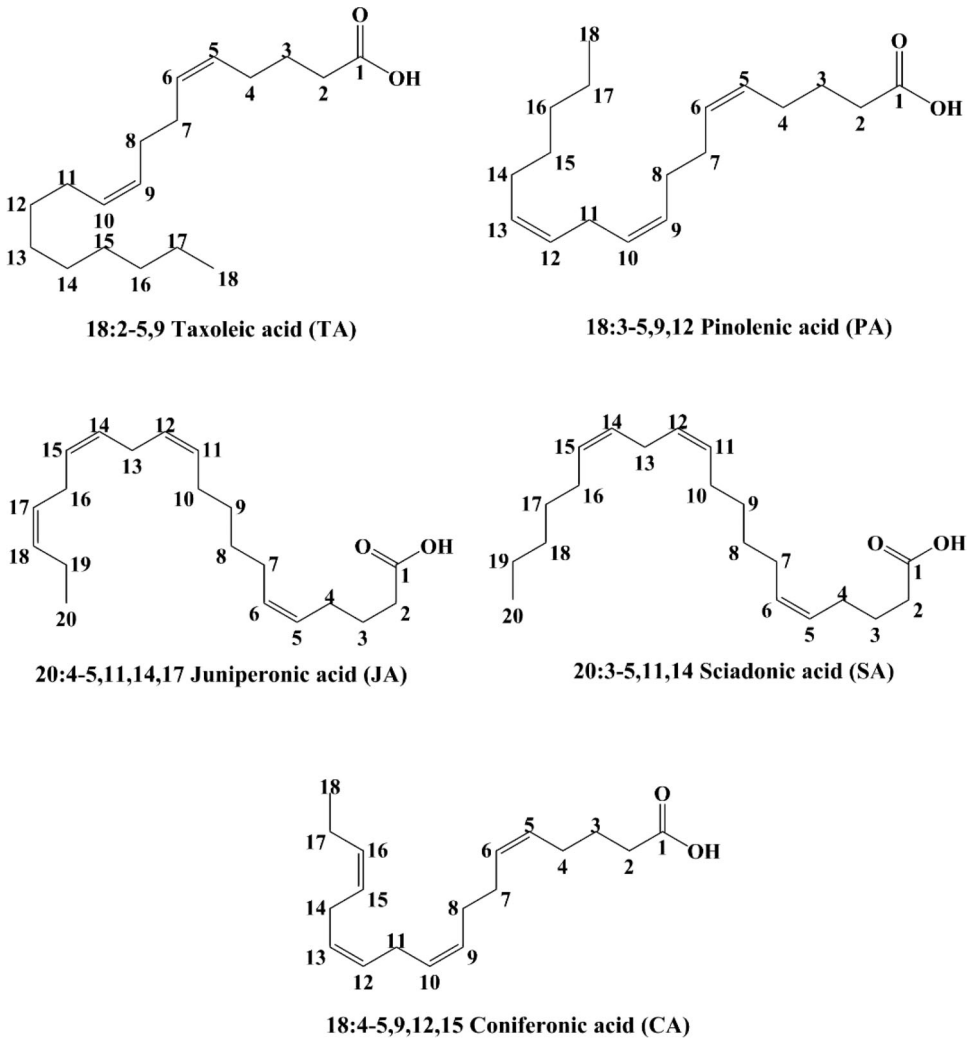

Figure 1. Chemical structures of major Δ5-UPIFAs.

improving the quality of life and health. However, further clinical and human studies are needed.

The potential health benefits of Δ5-UPIFAs from marine invertebrates and pine nut oil were discussed previously (Barnathan 2009; Xie, Miles, and Calder 2016; Wolff et al. 2002). Xie, Miles, and Calder (2016) described the potential health benefits of pine nut oil and PA, while Δ5-UPIFAs contain other fatty acids in addition to PA. Wolff et al. (2002) only discussed some plant sources and the content is incomplete. There's another review briefly discussed the metabolism, activity and distribution of Δ5-UPIFAs in marine organisms. There are a series of Δ5-UPIFAs with increasing amount of interests, suggesting that it is necessary to summarize the updating knowledge about Δ5-UPIFAs with increasing development of research on them. However, there is no comprehensive and systematic review of Δ5-UPIFAs, such as animal and plant sources, activities, preparation methods and so on. In this review, a detailed summary of the sources, biosynthesis and metabolism, analytical methods, preparation, and health-promoting roles of Δ5-UPIFAs has been presented comprehensively to provide the innovative information for better understanding and the future applications of Δ5-UPIFAs in food, pharmaceutical, and cosmetic industries. To the best of our knowledge, this is the first comprehensive review of the current scientific knowledge on Δ5-UPIFAs. The framework of the review is presented in Figure 2.

2. Sources

Δ5-UPIFAs occur naturally in various organisms as a minor component compared with common fatty acids (Saito and Ochiai 1996; Sayanova et al. 2007; Ventrella et al. 2008). In this review, the sources of Δ5-UPIFAs have been mainly categorized in 3 groups that include plant sources, animal sources, and microbial sources. Unless otherwise stated, the percentage of Δ5-UPIFAs herein refers to the ratio of the described acids to total fatty acids.

There are limited sources of Δ5-UPIFAs species in microorganisms. In animals, distribution of Δ5-UPIFAs is abundant but with limited amounts. In marine animals, homologous non-methylene diene fatty acids such as 20:2-5,11 and 20:2-5,9 limit the application of Δ5-UPIFAs (Zhukova 1986). In contrast, the content of Δ5-UPIFAs in gymnosperms is relatively high with less amount of isomeric existence. In comparison, gymnosperms are ideal raw materials for the preparation of Δ5-UPIFAs. In gymnosperms,

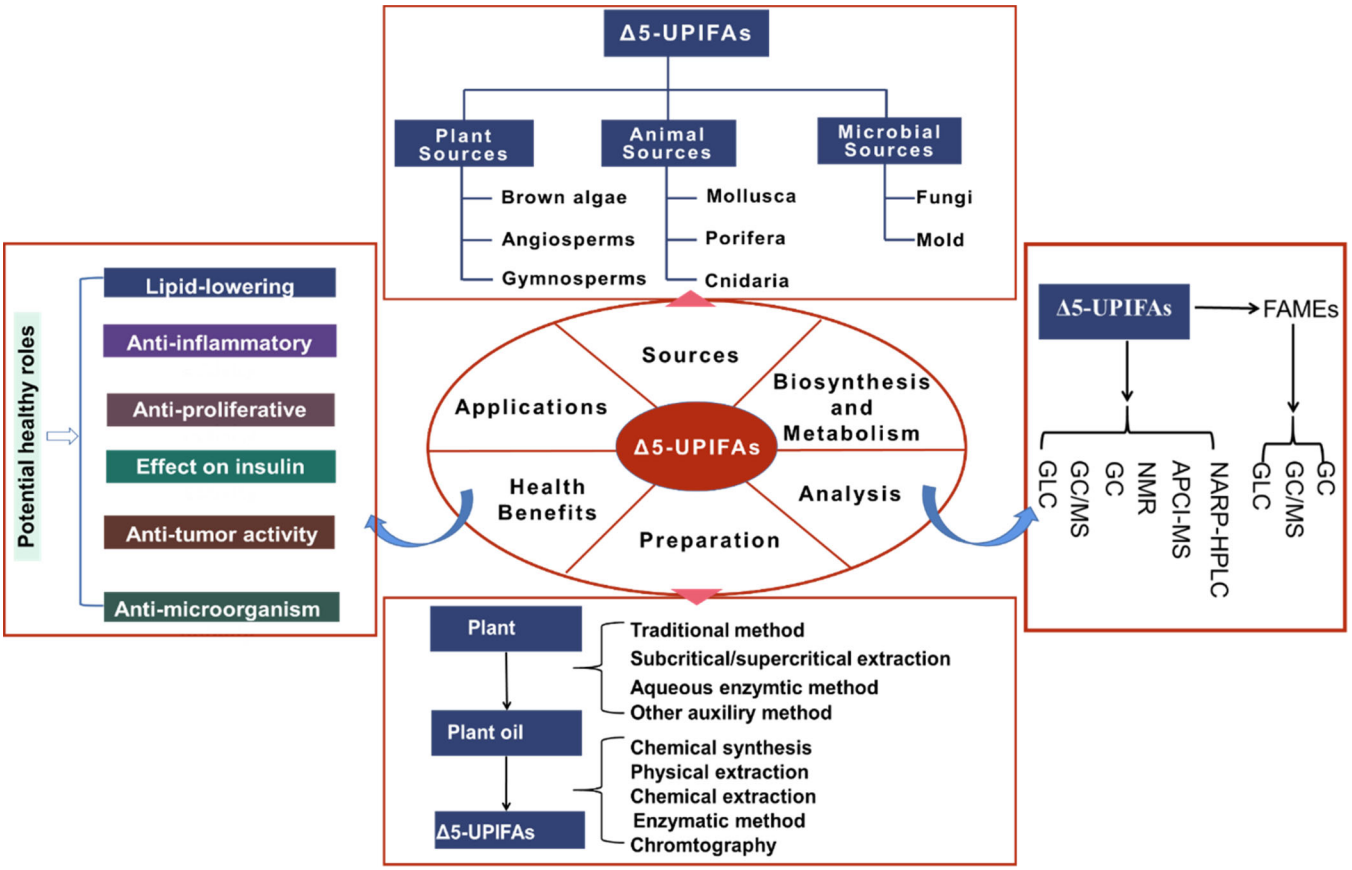

Figure 2. Framework of the review.

Ephedraceae campylopoda is an ideal source of JA with about 22% of content. *Larix deciduas* is an ideal source of PA, in which PA content is about 28.45%. The distribution of Δ5-UPIFAs content in each plant, animal and microbial species is shown in Tables 1–3, respectively. Interaction diagram among animal, plant and microbial sources is shown as Figure 3.

2.1. Plant sources of Δ5-UPIFAs

The total amounts of Δ5-UPIFAs in plant vary from 0.59% to 31.92%. The range of Δ5-UPIFAs in algae, gymnosperms and angiosperms belongs to 0.59%–2.34%, 1%–8.4% and 0.28%–31.92%, respectively. Among algae, gymnosperms and angiosperms, gymnosperms represent the highest content of Δ5-UPIFAs, reaching 31.92%. Plants are the most concentrated sources of Δ5-UPIFAs, among which gymnosperms contain the most abundant of Δ5-UPIFAs (Sayanova et al. 2007; Wolff 1998; Wolff et al. 2001). In addition, small amounts are found in ferns (Nekrasov et al. 2019), algae (Kim and Itabashi 2012) and angiosperms (Sayanova et al. 2007). In gymnosperms, Pmaceae, Taxodlaceae, Cupressaceae, and Pinaceae members have been found to contain large amounts of Δ5-UPIFAs (Jamieson and Reid 1972). In gymnosperms, each species contains different kinds of Δ5-UPIFAs, and their abundance is different. The major fatty acid PA, which is mainly distributed in *Tsuga* sp., *Larix* sp. and *Picea* sp., found with content range of

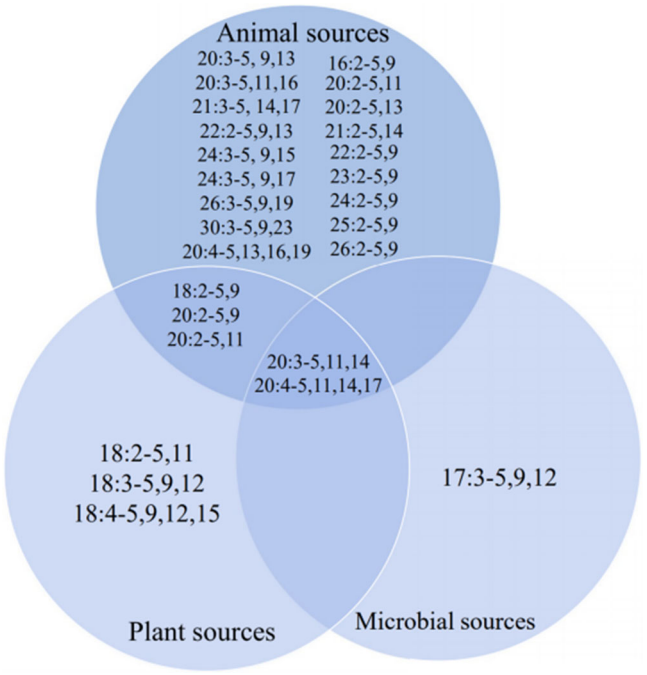

Figure 3. Interaction diagram among animal, plant and microbial sources.

11.8% to 28.45%. The second abundant fatty acid SA, which is mainly distributed in *Podocarpus andinus*, *Torreya grandis* and *Podocarpus nagi*, found within the content range of 9.29% to 23.88%. Another fatty acid JA, which is mainly distributed in *E. gerardiana*, *E. campylopoda* and *E. viridis*,

Table 1. Plant sources of Δ5-UPIFAs.

Species		Contents of Δ5NMIPUFAs	Reference
Brown algae	*S. fulvelum*	20:2-5,9 (0.59%), 20:3-5,11,14 (0.68%), 20:4-5,11,14,17 (0.57%)	(Kim and Itabashi 2012)
	S. horneri	20:2-5,9 (1.03%), 20:4-5,11,14,17 (0.12%)	
	S. Boreale	20:2-5,9 (2.34%)	
	S. thunbergi	20:2-5,9 (0.93%), 20:3-5,11,14 (0.07%), 20:4-5,11,14,17 (0.15%)	
	S. yezoense	20:2-5,9 (0.38%), 20:3-5,11,14 (0.08%), 20:4-5,11,14,17 (0.13%)	
Angiosperms	*Anemone leveillei*	20:3-5,11,14 (8.4%)	(Sayanova et al. 2007)
	Phymatosoruspustulatus	20:3-5,11,14 (0.6%), 20:4-5,11,14,17 (0.4%)	(Nekrasov et al. 2019)
	A. yokoscense	20:3-5,11,14 (1.9%), 20:4-5,11,14,17 (0.1%)	
Gymnosperms	*Agathisrobusta*	20:2-5,11 (0.05%), 20:3-5,11,14 (0.06%), 20:4-5,11,14,17 (0.17%)	(Wolff, Christie, Pédrono, and Marpeau 1999)
	A. araucana	18:2-5, 9 (0.2%), 18:3-5, 9, 12 (1.32%), 20:2-5, 11 (0.10%), 20:3-5, 11, 14 (8.9%), 20:4-5,11,14,17 (0.65%) (in the leaves)	(Wolff, Christie, et al. 2000)
	A. bidwillii	18:2-5,9 (0.08%), 18:3-5,9,12 (0.34%), 20:2-5,11 (0.20%), 20:3-5,11,14 (1.31%), 20:4-5,11,14,17(0.13%) (in the seeds)	
	A. cunninghamii	18:2-5,9 (0.05%), 18:3-5,9,12 (1.38%), 20:2-5,11 (0.15%), 20:3-5,11,14 (1.42%), 20:4-5,11,14,17(0.06%) (in the seeds)	
	A. dammara	18:2-5,9 (0.03%), 18:3-5,9,12 (0.09%), 20:2-5,11 (0.05%), 20:3-5,11,14 (0.30%), 20:4-5,11,14,17(0.14%) (in the leaves)	
	Abies spp.	18:2-5,9 (0.61%), 18:3-5,9,12 (1.23%), 20:2-5,11 (0.03%), 20:3-5,11,14 (0.19%)	
	Cedrus spp.	18:2-5,9 (6.2%), 18:3-5,9,12 (11.8%), 20:2-5,11 (0.1%), 20:3-5,11,14 (0.8%)	(Wolff et al. 2002)
	E. campylopoda	18:2-5,11 (2.0%), 20:2-5,11 (1.2%), 20:3-5,11,14 (5.4%), 20:4-5,11,14,17 (21.9%)	
	E. gerardiana	18:2-5,9 (0.4%), 18:2-5,11 (1.7%), 18:4-5,9,12,15 (0.5%), 20:2-5,11 (1.5%), 20:3-5,11,14 (7.5%), 20:4-5,11,14,17 (19.2%)	(Wolff, Christie, Pédrono, Marpeau, et al., 1999)
	E. przewalskii	18:2-5,9 (0.5%), 18:2-5,11 (2.0%), 18:4-5,9,12,15 (0.5%), 20:2-5,11 (1.1%), 20:3-5,11,14 (4.4%), 20:4-5,11,14,17 (8.8%)	
	E. viridis	18:2-5,9 (1.0%), 18:2-5,11 (1.9%), 18:3-5,9,12 (0.2%), 18:4-5,9,12,15 (0.2%), 20:2-5,11(1.5%), 20:3-5,11,14 (6.6%), 20:4-5,11,14,17 (11.7%)	
	Ephedraceaenevadensis	18:2-5,9 (1.4%), 18:2-5,11 (4.0%), 18:3-5,9,12 (0.1%), 18:4-5,9,12,15 (0.2%), 20:2-5,11(1.6%), 20:3-5,11,14 (5.6%), 20:4-5,11,14,17 (8.9%)	
	H.mertensiana	18:2-5,9 (2.2%), 18:3-5,9,12 (19.4%), 20:2-5,11 (0.1%), 20:3-5,11,14 (1.3%)	
	*Keteleeria*spp.	18:2-5,9 (3.5%), 18:3-5,9,12 (1.5%), 20:2-5,11 (1.7%), 20:3-5,11,14 (2.9%)	(Wolff et al. 2002)
	Larixdeciduas	18:2-5,9 (2.73%), 18:3-5, 9,12 (28.45%), 20:2-5,11 (0.16%), 20:3-5,11,14 (0.58%)	
	Larixleptolepis	18:2-5,9 (2.2%), 18:3-5,9,12 (25.8%), 20:2-5,11 (0.1%), 20:3-5,11,14 (0.5%)	(Wolff, Deluc, and Marpeau 1996)
	Larix spp.	18:2-5,9 (2.37%), 18:3-5,9,12 (28.05%), 20:2-5,11 (0.13%), 20:3-5,11,14 (0.55%)	
	P. amabilis	18:2-5,9 (7.8%), 18:3-5,9,12 (7.3%), 20:2-5,11 (1.2%), 20:3-5,11,14 (3.6%)	(Wolff et al. 2001)
	P. strobus	18:2-5,9 (1.74%), 18:3-5,9,12 (25.29%), 20:2-5,11 (0.2%), 20:3-5,11,14 (1.93%)	(Wolff et al. 2002)
	P. thunbergi	18:2-5,9 (2.8%), 18:3-5,9,12 (18.1%), 20:2-5,11 (0.5%), 20:3-5,11,14 (5.8%)	(Wolff, Pédrono, et al. 2000)
	Piceaabies	18:2-5,9 (3.42%), 18:3-5,9,12 (22.81%), 20:2-5,11 (0.08%), 20:3-5,11,14 (0.82%)	
	*Picea*spp.	18:2-5,9 (3.32%), 18:3-5,9,12 (23.76%), 20:2-5,11 (0.06%), 20:3-5,11,14 (0.92%)	(Lisa et al. 2007)
	Pinuspinea	18:2-5,9 (1.8 mg/g TFA), 18:3-5,9,12 (3.0 mg/g TFA), 20:2-5,11 (1.3 mg/g TFA), 20:3-5,11,14 (18.8 mg/g TFA) (in Tunisian Pinuspinea)	(Wolff, Deluc, and Marpeau 1996)
	Podocarpusandinus	18:2-5,9 (2.0 mg/g TFA), 18:3-5,9,12 (2.6 mg/g TFA), 20:2-5,11 (3.8 mg/g TFA), 20:3-5,11,14 (14.4 mg/g TFA) (in Mediterranean Pinuspinea) 20:3-5,11,14 (16.7%)	(Nasri et al. 2005)
	PodocarpusNagi	20:2-5,11 (0.88%), 20:3-5,11,14 (23.88%)	(Wolff 1998)
	T. baccata	18:2-5,9 (9.6%), 18:3-5,9,12 (0.4%), 20:2-5,11 (0.2%), 20:3-5,11,14 (1.5%), 20:4-5,11,14,17 (0.2%)	(Takagi and Itabashi 1982)
	T. chinensis	18:2-5,9 (16.4%)	(Wolff, Deluc, and Marpeau 1996)
	grandisFort.Ex. Lindl	20:2-5,11 (0.82%), 20: 3-5,11,14 (12.72%)	(Destaillats, Wolff, and Angers 2001)
	T. grandis Fort. Var. Merrillii	20:2-5,11 (0.82%), 20:3-5,11,14 (9.19%)	(Shi et al. 2018)
	Thuyaplicata	18:2-5,9 (0.67%), 18:3-5,9,12 (0.06%), 20:2-5,11 (0.67%), 20:3-5,11,14 (4.27%), 20:4-5,11,14,17 (11.5%)	(Wolff et al. 1997)
	Tsuga spp.	18:2-5,9 (2.0%), 18:3-5,9,12 (20.4%), 20:2-5,11 (0.2%), 20:3-5,11,14 (2.6%)	(Wolff et al. 2002)

Table 2. Animal sources of Δ5-UPIFAs.

Species		Content of Δ5-UPIFAs	Reference
Mollusca	*Crassostrea virginica*	20:2-5,13 + 20:2-5,11 (1.71%)	(Paradis and Ackman 1977)
	Calytogenaphaseoliformis	20:3-5,13,16 (4.8%), 20:4-5,13,16,19 (8.3%), 21:3-5,14,17 (5.2%), 21:2-5,14 (6.1%)	(Saito 2007)
	Mytilusgalloprovincialis	20:2-5,11 (1.9%–5.5%), 20:2-5,13 (0.8%–2.5%)	(Ventrella et al. 2008)
	Nassaserata	20:3-5,11,14 (0.05%), 20:4-5,11,14,17 (0.25%)	(Go et al. 2002)
	Nassariusalbescens	20:3-5,11,14 (0.02%), 20:4-5,11,14,17 (0.26%)	
	Nodilittorinasubnodosa	20:3-5,11,14 (0.07%), 20:4-5,11,14,17 (0.26%)	
	Littorinascarba	20:3-5,11,14 (0.06%), 20:4-5,11,14,17 (0.30%)	
	Planaxissulcata	20:3-5,11,14 (0.05%), 20:4-5,11,14,17 (0.26%)	
	Monodontaturbinata	20:3-5,11,14 (0.02%), 20:4-5,11,14,17 (0.09%)	
	Gibula cineraria	20:3-5,11,14 (0.06%), 20:4-5,11,14,17 (0.11%)	
	Littorinaneritoide	20:3-5,11,14 (0.02%), 20:4-5,11,14,17 (0.29%)	
	Melanoidestuberculata	20:3-5,11,14 (0.41%), 20:4-5,11,14,17 (0.02%)	
	Thedoxusneritoides (endemic)	20:3-5,11,14 (0.25%), 20:4-5,11,14,17 (0.05%)	
	Pyrguliabarroisi were	20:3-5,11,14 (0.39%), 20:4-5,11,14,17 (0.04%)	
	Melanopsispraemorsum	20:3-5,11,14 (0.22%), 20:4-5,11,14,17 (0.01%)	
	Melanopsispraemorsum	20:3-5,11,14 (0.22%), 20:4-5,11,14,17 (0.01%)	
	Cellana grata	20:3-5,11,14 (0.2%), 20:2-5,9 (<0.1%), 24:2-5,9 (<0.1%), 24:3-5,9,15 (0.1%),24:3-5,9,17 (<0.1%)	(Kawashima and Ohnishi 2006; Kawashima et al. 2008)
	*Chromodoris*spp.	20:2-5,11 (1.3%), 20:2-5,13 (2.5%), 24:2-5,9 (1.0%), 25:2-5,9 (4.0%), 26:2-5,9 (6.0%)	(Zhukova 2007)
	Phyllidiacoelestis	20:2-5,11 (0.8%), 20:2-5,13 (2.0%), 24:2-5,9 (1.8%), 25:2-5,9 (1.7%), 26:2-5,9 (0.9%)	
Porifera	*Chondrillanucula*	30:3-5,9,23 (34%)	(Litchfield, Tyszkiewicz, and Dato 1980)
	microcionaprolifera	26:2-5,9 (14%), 26:3-5,9,19 (31%)	(Jefferts, Morales, and Litchfield 1974)
Cnidaria	*PalythoaCaribaorum*	18:2-5,9 (1%), 20:2-5,9 (2%), 22:2-5,9 (0.9%), 24:2-5,9 (0.7%) (in the phospholipids)	(Carballeira and Reye 1995)
	CondylactisGigantea	18:2-5,9 (0.8%), 20:2-5,9 (3%), 21:2-5,9 (0.1%) 22:2-5,9 (0.8%), 24:2-5,9 (0.1%) (in the phospholipids)	
	Stoichactis Helianthus	16:2-5,9 (2.1%), 18:2-5,9 (4.1%), 20:3-5,9,13 (2.3%), 20:2-5,9 (2.3%), 21:2-5,9 (0.6%), 22:3-5,9,13 (0.3%), 22:2-5,9 (2.1%), 23:2-5,9 (0.2%) (in the phospholipids)	(Carballeira and Medina 1994)

Table 3. Microbial sources of Δ5-UPIFAs.

Species		Content of Δ5-UPIFAs	Reference
Fungi	*Mortierella alpine* IS-4	Under the optimum growth temperature, the yield of 20:4-5,11,14,17 was 6.4 mg20: 4-Δ5/g dry mycelium. The yield of 20:3-5,11,14 was 27 mg/gdry mycelia.	(Jareonkitmongkol, Shimizu, and Yamada 1993)
	Ascophyllum nodosum	20:3-5,11,14 (3.6%)	(Jamieson and Reid 1972)
Mold	*Polysphondyliumpallidum*	17:3-5,9,12 (1.5–2.0%)	(Saito and Ochiai 1996)

found within the content range of 11.5% to 21.9%. *T. chinensis*, *P. amabilis* and *Cedrus* sp. were found to be abundant in TA within the content range of 6.2% to 16.4%. The detailed information on the content of Δ5-UPIFAs in each plant species is shown in Table 1.

2.2. Animal sources of Δ5 NMIPUFAs

Compared with plants, the contents of Δ5-UPIFAs in animal sources are less with about 0.11% to 45%, and most of the Δ5-UPIFAs are concentrated in marine animals (Table 2). Mollusca was reported to contain the most Δ5-UPIFAs producing species, where the Δ5-UPIFAs contents reach up to 20% (Barnathan 2009). In Mollusca, large amounts of Δ5-UPIFAs were reported in oysters (Paradis and Ackman 1977), clams (Saito 2007), and mussels of the Lamellibranchia (Go et al. 2002). In addition, small amounts of Δ5-UPIFAs were found in the sponges of the poriferain (Jefferts, Morales, and Litchfield 1974; Litchfield, Tyszkiewicz, and Dato 1980) and anemones of the Cnidaria phylum (Carballeira and Medina 1994; Carballeira and Reye 1995).

Among these marine animals, *Chondrilla nucula*, a member of Porifera phylum was reported to be rich in 30:3-5,9,23 Δ5-UPIFA (34%), whereas *Microciona prolifera* contained 26:2-5,9 and 26:3-5,9,19 Δ5-UPIFAs with 14% and 31% of content, respectively. The data indicate that these two species are potential sources of ultra-long chain Δ5-UPIFAs. Other members of Mollusca and Cnidaria, although cover a large range of Δ5-UPIFAs, the content in animal sources was higher than that of plants, except for five species (*Phyllidia coelestis*, *Chromodoris* sp., *Calytogena phaseoliformis*, *Microciona prolifera* and *Chondrilla nucula*), where the total content of Δ5-UPIFAs was less than 5%. *Microciona prolifera* and *Chondrill anucula* were found to be the major sources of ultra-long chain Δ5-UPIFAs and contained especially 26:3-5,9,19 (31%) and 30:3-5,9,23 (34%).

2.3. Microbial sources of Δ5-UPIFAs

There are a fewer kind of microorganisms which contain high amount of Δ5-UPIFAs as compared to animals and

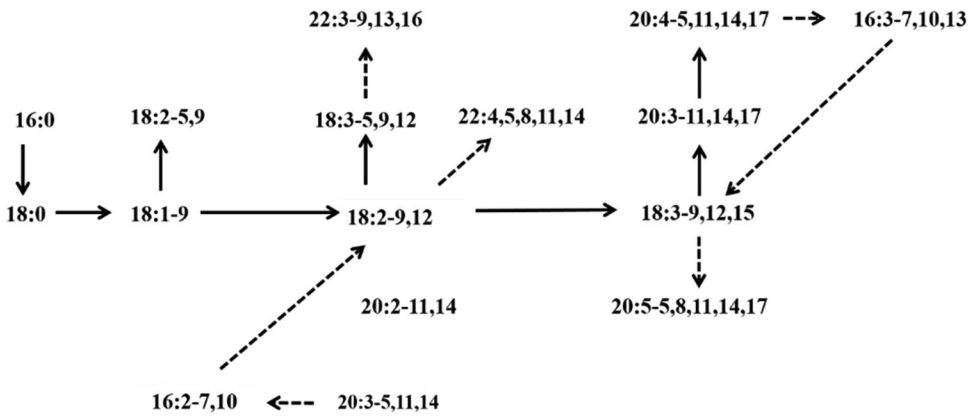

Figure 4. Possible pathway of Δ5-UPIFAs synthesis and metabolism.

plants, and small amounts of Δ5-UPIFAs exist in some fungi and molds (Table 3). 5-UPIFAs were reported to present in two kinds of fungi, *Mortierella alpine* IS-4 and *Ascophyllum nodosum*. A filamentous fungus, *Mortierella alpine* IS-4, was found to contain SA and JA Δ5-UPIFAs (Jareonkitmongkol, Shimizu, and Yamada 1993). The optimal temperature for the accumulation of 20:4-5,11,14,17 in fungal mycelia was 12 °C, with a yield of 6.4 mg 20:4-Δ5/g dry weight mycelium. At 20 °C, the fungal mycelia had high content of 20:3-5,11,14 with a yield of 27 mg/g dry weight mycelium, accounting for about 7% of the total mycelial fatty acids. More than 75 mol% of the two fatty acids was found in the triacylglycerol fraction. *Ascophyllum nodosum* (Jamieson and Reid 1972) contained a considerable amount of 20:3-5,11,14, which reached to a maximum concentration of 3.6%. In addition to fungi, Saito and Ochiai (1996) found that all-*cis*-17:3-5,9,12 (about 1.5%–2.0%) was identified from the cellular slime mold *Polysphondylium pallidum* below 25 °C.

Generally, different sources from animal, plant and microorganisms have their own characteristics. Gymnosperms are the most abundant source of Δ5-UPIFAs, making this group suitable for extraction and preparation of Δ5-UPIFAs. Animal sources, although not rich in content, are the main sources of uncommon ultra-long chain fatty acids, which is a very unique source of ultra-long chain fatty acids for humans. Although the contents of Δ5-UPIFAs are low in microorganisms (ranging from 2%–7%), this group of microorganisms has unique advantages over animals and plants due to fast reproduction process and short fermentation cycle.

3. Biosynthesis and metabolism

Although the specific biosynthesis and metabolic pathways of Δ5-UPIFAs have not been established completely, some valuable knowledge on this aspect has been put forward. The possible pathways of Δ5-UPIFAs synthesis and metabolism presented in this article are shown in Figure 4. The solid line represents the biosynthesis pathway and the dotted line belongs to the metabolic pathway.

3.1. Biosynthesis

According to the position of ethylene bond and the number of carbon atoms in the Δ5-UPIFAs chain, it is presumed that Δ5-UPIFAs are formed by Δ5-desaturation of oleic acid, linoleic acid, and α-linolenic acid or by Δ5 desaturation of other extension products of UPIFAs (Cook et al. 1991; Monroig et al. 2016; Wolff, Christie, Pédrono, and Marpeau 1999). The synthesis pathway of the Δ5-UPIFAs was first demonstrated in 1993 (Jareonkitmongkol, Shimizu, and Yamada 1993) by comparing the differences in monetization among different strains. The results showed that Δ5-desaturase is involved in the formation of these fatty acids. Liu et al. (2014) used cloning technology to study the role of desaturase in mollusks. The results demonstrated that desaturase possibly participates in the biosynthesis of JA and SA (Δ5-UPIFAs) by introducing double bond on C20:3 (n-3) and C20:2 (n-6) at Δ5 carbon.

Irene, Johnathan, and Olga (2016) reconstituted the pathway for the biosynthesis of SA and established a robust transformation system in the transgenic seeds of *Camelina sativa* via iterative metabolic engineering. Further, Wu et al. (2018) used high-throughput Illumina sequencing technology to generate the tree transcriptome, providing the first comprehensive genomic analysis of SA biosynthesis, as well as revealed pivotal information regarding the genes associated with SA biosynthesis. Findings indicated that there is an active fatty acid elongation and desaturation system in the organism, allowing UPIFAs to be de novo synthesized. Based on the above information it was speculated that the active FA elongation and desaturation system (Δ5-desaturase) in vivo allows the de novo synthesis of Δ5-UPIFAs (Ding et al. 2020). Moreover, at first, it is likely to perform synthesis extension step followed by desaturation.

More specific synthesis examples of Δ5-UPIFAs in organisms have also been reported in the literature (Cook et al. 1991; Sayanova et al. 2007; Ullman and Sprecher 1971; Wolff, Christie, Pédrono, and Marpeau 1999). Ullman and Sprecher (1971) reported that incubation of radioactive eicosa-11,14-dienoic acid or eicosa-11-enoic acid with rat liver microsomes resulted in the formation of desaturated SA and eicosa-5,11-dienoic acid. Wolff, Christie, Pédrono, Marpeau, et al. (1999) found that in the seeds of gymnosperms rich in SA, part of the linoleic acid (LA, 18:2-9,12)

was prolonged to eicosadienoic acid (EDA, 20:2-11,14), which was then desaturated by conifer specific Δ5-desaturase to form SA. Similar results were found when EDA was incubated with RAW264.7cells (Huang et al. 2011). Monroig et al. (2016) further used cloning technology to study the role of desaturation and chain elongation in mollusks. It was shown that UPIFAs could be synthesized by introducing double bonds on C20:3 (n-3) and C20:2 (n-6) at Δ5 carbon after extending C_{18} to C_{20}.

3.2. Metabolism

Metabolism of Δ5-UPIFAs is an important and complex biochemical reaction in the body. It refers to the process of digestion, absorption, synthesis and decomposition in the body with the help of various related enzymes, which is of great significance to life activities. Previously, Tanaka et al. (1998) performed experiments on various fatty acids (FAs) to study the positions of fatty acid double bonds recognized by the rat liver fatty acid chain extension system (FACES). It was found that the condensing enzyme of FACES could recognize the methylene-interrupted *cis* double bond structure vicinal to the carboxyl group in the fatty acid molecule.

Later, Tanaka et al. (2007) conducted a detailed study on the metabolism of SA and JA in Swiss 3T3 cells. It was found that JA was partially degraded to α-linoleic acid and then elongated into EPA in the cell lipid. After two *β*-oxidations in the peroxisome, SA was partially degraded to 16:2-7,10 and then elongated into linoleic acid in the microsomes (Chen et al. 2012). In another study, it was found that fatty acid desaturase in rat liver cannot desaturate SA to arachidonic acid (Tanaka et al. 2001). Tanaka et al. (2014) further studied the metabolism of SA at cellular level and observed similar results. The results indicated that only C_{20} UPIFA, not C_{18} UPIFA, was effectively converted into essential fatty acids by fatty acid remodeling systems in rodent and human cells. Chuang et al. (2009) studied the metabolism of PA in RAW264.7 cells, and it was found that PA could be metabolized as 22:3-9,13,16. Similar findings were observed in a research conducted on human liver cancer HepG2 cells (Tanaka et al. 1999). These findings conclude that Δ5-UPIFAs may be metabolized by the metabolic desaturase-stretching enzyme pathway. For example, the possible metabolic pathway of PA is that PA is elongated to 20:3-7,11,14, where a small portion of 20:3-7,11,14 is further extended to 22:3-9,13,16.

4. Analysis

In order to clarify the functions of Δ5-UPIFAs, identification and quantification of Δ5-UPIFAs are equally important. Therefore, great efforts have been furnished to develop quali-quantitative techniques for determining the composition of Δ5-UPIFAs over the past decades. Δ5-UPIFAs fractionation was mainly accomplished by using chromatography techniques. Since many classes of lipids are nonvolatile, it is necessary to derivatize them before GC analysis. Though the derivatization increases the complexity of the analysis, requires additional sample preparation and use of internal standards, GC is still a commonly used technique in fatty acid detection. With the development of separation techniques, e.g. ion mobility spectrometry (IMS), the composition of Δ5-UPIFAs could be improved. Some common methods used for the detection of fatty acids are shown in Table 4.

4.1. Basic chromatography

After the derivatization of fatty acids into methyl esters, the GC-flame ionization detector (FID) system was used for routine determination of pine species (Mikkelsen, Jessen, and Ballin 2014). GC-Mass spectrometer (MS) system was further used to identify FAs (Madhumita, Guha, and Nag 2019). On the basis of applied GC-MS conditions, methodology of the derivatization can be modified. The conversion of fatty acids into the diethylamide derivative is being measured to determine the position of double bonds in polyunsaturated fatty acids. The derivative is easy to prepare in one step, and the method is suitable for the determination of long-chain fatty acid mixture samples.

4.2. Hyphenated chromatography

To improve the resolution of complex mixtures of lipids, HPLC and GC are coupled with different kinds of detectors, such as MS detectors. A non-aqueous reversed-phase high-performance liquid chromatography (NARP-HPLC) with atmospheric pressure chemical ionization (APCI)-MS detection (Lisa et al. 2007) has been developed successfully to determine the total contents of Δ5-UPIFAs in seed oils of *Larix decidua*, *Picea abies* and *Abies alba* with about 32%, 27%, and 20%, respectively. The method was further applied to determine Δ5-UPIFAs in the oils extracted by supercritical carbon dioxide technique (Wang et al. 2007). A new simple method to identify triacylglycerols in oils and fats was performed using online coupling of non-aqueous reversed phase chromatography-electrospray ionization-mass spectrometry (NARP-LC-ESI-MS^2) with silver nitrate ($AgNO_3$) as a post-column additive (Acheampong et al. 2011). This methodology was applied to *Pinus koraiensis* seed oil and it was possible to detect all TAGs even those at a low level. Silver cationization improved the sensitivity.

The combination of chromatographic methods and other chemical means can broaden the application of the method. The composition of lipids and FAs in conifer trees (*Pinus sylvestris* L. and *Picea obovata* Ledeb.) were studied by gas-liquid chromatography (GLC)/MS (Nokhsorov, Dudareva, and Petrov 2019). For both species, the FA lipid composition of needles included a high content of species-specific Δ5-UPIFAs (11.6–18.6%). The positional distribution of various Δ5-acids in the seed triacylglycerols from several conifer species was established after partial chemical degradation with Grignard reagent by GLC (Blaise et al. 1997). The results showed that Δ5-acids are mainly distributed in the 1,3-position. This method made the detection range of

Table 4. Analysis methods of Δ5-UPIFAs.

Research topic	Method	Pretreatments	References
Identification of structure	GC-FID	Methyl esterification and filtration	(Mikkelsen, Jessen, and Ballin 2014)
	GC-MS	Methyl esterification and filtration	(Li et al. 2015; Ponphaiboon et al. 2018)
	NARP-HPLC	Filtration	(Lisa et al. 2007; Wang et al. 2007; Acheampong et al. 2011)
	HPLC	Filtration	(Li et al. 2015; Ponphaiboon et al. 2018)
	Solvent-Mediated Covalent Adduct Chemical Ionization Triple Quadrupole Tandem Mass Spectrometry	Methylation and filtration	(Wang et al. 2020).
	Nuclear Magnetic Resonance Spectroscopy	Drying and filtering	(Gao et al. 2020; Madhumita, Guha, and Nag 2019; Rakhmatullin et al. 2017; Vlahov 2006)
Determination of double bond positions in PUFAs	GC-MS	Methyl esterification and filtration	(Nilsson and Liljenberg 1991)
Determination of distribution of Δ5-acids	Partial Chemical Cleavage + GLC	Methyl esterification and filtration	(Blaise et al. 1997)
	^{13}C Nuclear Magnetic Resonance Spectroscopy	Drying and filtering	(Gunstone, Seth, and Wolff 1995; Gunstone and Wolff 1996)

the chromatographic methods beyond the limit of substance structure confirmation.

4.3. Nuclear magnetic resonance spectroscopy

^{13}C nuclear magnetic resonance (NMR) is an essential technique for the characterization of various compounds. By using this technique, several components have been examined (Vlahov 2006) and the distribution of Δ5-acids between α and β chains has been profiled (Gunstone, Seth, and Wolff 1995; Gunstone and Wolff 1996; Rakhmatullin et al. 2017). The disadvantages of ^{13}C NMR are mostly caused by the complication of the spectra and long relaxation time of the insensitive ^{13}C nuclei, leading to crowded and non-quantitative spectra (Gao et al. 2020).

4.4. Advanced chromatography

Recently, a newly CH_3CN CACI-triple quadrupole system was constructed for identification of UPIFAs prominent in pine and ginkgo nuts and for Δ5 monoenes (Wang et al. 2020). The uncommon fragmentation and H•transfer rules of Δ5 fatty acids extended the parameters previously developed for normal methylene interrupted fatty acids, enabling positive structural identification without purified chemical standards.

5. Preparation

5.1. Extraction of seed oils

Generally, the preparation method of Δ5-UPIFAs is to extract the plant seed oil first, followed by concentration and purification. The preparation of Δ5-UPIFAs requires seed oil as a raw material at a proper state, which can improve the preparation process. Therefore, the first operation in the preparation involves transferring the target analytes to a liquid phase. Extraction methods such as supercritical fluid extraction, pulsed electric field assisted extraction, enzymatic hydrolysis, and mechanic expression are commonly used for oil extraction from seeds (Incegul et al. 2020).

5.1.1. Traditional methods

The most commonly used conventional methods for obtaining oils are organic solvent extraction (Dhibi et al. 2014) and mechanical press (He et al. 2016; Zadernowski, Naczk, and Czaplicki 2009). Pressing of nuts yielded only 49.2% of the total lipids present in *P. sibirica* nuts (Saito and Ochiai 1996), while 86.4% oil was extracted from *Calophyllum inophyllum* by hexane extraction (Bhuiya et al. 2020). Both methods have advantages and disadvantages. The oil produced by the pressing method had good quality and pure flavor (Bendini et al. 2011). The disadvantage of the method was considered in terms of its low extraction rate with several impurities in the final product. Conventional Soxhlet extraction is a very simple methodology that requires little training, and it can extract more sample mass than supercritical fluid extraction. Moreover, no filtration is required after leaching and it is facilitated by the low cost of the basic equipment. The most serious drawbacks of Soxhlet extraction as compared to other techniques are the long time requirement for extraction and a large amount of extracting wasted, which are not only expensive to address, but also the source of additional environmental problems (Castro and Priego-Capote 2010).

5.1.2. Subcritical/supercritical extraction

Supercritical fluid extraction is another useful method to extract oils. Under 60 °C and 40 MPa, the yield of oil extracted from *Geoffroea decorticans* was 40% (Salinas et al. 2020). Supercritical fluid extraction has the advantages of fabricating products without toxic residues, no degradation of active principles, and with high purity (Pereira and Meireles 2010; Zheng et al. 2020). Additionally, it has shortcomings such as low processing capacity, high equipment investment, and production costs (Ibáñez, Mendiola, and Castro-Puyana 2016; Rai, Mohanty, and Bhargava 2015).

Table 5. Preparation of seed oils.

Material	Method	Oil	Reference
Pinushalepensis Mill	Hexane solvent extraction	Pine seed oil	(Bhuiya et al. 2020)
Pinussibirica	Mechanical press	Pine seed oil	(Zadernowski, Naczk, and Czaplicki 2009)
Geoffroeadecorticans	Supercritical fluid extraction	Almond oil	(Salinas et al. 2020)
Pine kernel	Aqueous enzymatic extraction	Pine nut oil	(Li et al. 2011)
Xanthocerassorbifolia	Subcritical fluid extraction	Yellow horn seed oil	(Gu et al. 2019)
CeibaPentandraGaertn	Ultrasonic-assisted extraction	Kapok seed oil	(Senrayan and Venkatachalam 2020)
Roasted sunflower seed cake	PEF-assisted extraction	Sunflower oil	(Shorstkii, Khudyakov, and Mirshekarloo 2020)

A method similar to supercritical fluid extraction is subcritical fluid extraction. For each extraction, 1,000 mL of subcritical n-butane was added, and the ratio of plant sample to n-butane was 1:10 (g/mL). The extraction was performed at 35 °C for 30 min over 3 cycles (Gu et al. 2019). The extraction results of *Xanthoceras sorbifolia* Bunge (yellow horn) seed oil using subcritical n-butane and supercritical CO_2 indicated that subcritical n-butane extraction had higher yield (58.79%) than supercritical CO_2 extraction method (56.47%). Supercritical extraction is much safer due to its relatively low pressure, lower equipment investment and lower operating cost than supercritical CO_2 (Sun et al. 2018). In addition, sub-critical extraction preserves oil quality adequately (Hou, Li, and Qiu 2018).

5.1.3. Aqueous enzymatic method

Alcalase endo-protease enzyme was selected to extract oil from pine kernel. Under enzyme additive amount (1.97%), hydrolysis time (3 h), hydrolysis temperature (51 °C), materials to water rate (1:5), and pH (8.4), the total oil extraction rate was 89.12% (Li et al. 2011). The aqueous enzyme method is an environmentally friendly and efficient method. When auxiliary processing such as microwave ultrasound is applied in the AEP process, its process efficiency is further enhanced (Chen et al. 2016).

5.1.4. Other auxiliary methods

Ultrasound is one of the emerging and novel techniques in the oil extraction process. The oil was extracted from kapok seed using ultrasonic acoustic cavitation (UCAE) (Senrayan and Venkatachalam 2020). UCAE produced 92.29% of oil recovery with a shorter extraction time of 10 min. In addition, the energy consumption of subcritical fluid extraction was 80 folds lower than Soxhlet and 50 folds lesser than SE. As a novel industry scale technology, pulsed electric field (PEF) has already been demonstrated as an innovative solution for electroporation of oil cells during the extraction process (Kotnik et al. 2015). Sunflower seed oil was extracted with n-hexane assisted by PEF with electric field strength of 7 kV/cm and energy consumption of 6.1 kJ/kg (Shorstkii, Khudyakov, and Mirshekarloo 2020). PEF-assisted sunflower oil extraction could recover 55.3% of the oil that normally remains in the sunflower oil seeds. Furthermore, the use of PEF treatment had minor effects on the main chemical characteristics (acid and peroxide value, etc.) and color parameters of the sunflower oil. PEF can help to keep native flavor and nutrients intact (Zhang et al. 2017). The preparation methods with transferring the target analytes to a liquid phase involved are shown in Table 5.

5.2. Δ5-UPIFAS preparation

The contents of Δ5-UPIFAs occurring in seeds are not ideal, and appropriate preparation methods can expand their application. Although there are many kinds of Δ5-UPIFAs, attention has been mainly focused on SA, JA, and PA due to their relatively high content in nature. Several preparation methods of Δ5-UPIFAs are shown in the following subsequent sections:

5.2.1. Chemical synthesis

Chemical synthesis is a common method in the production of various compounds. Some researchers have synthesized Δ5-UPIFAs from simple fatty acids. Carballeira et al. (2002) synthesized all-*cis*-5,9-16:2 from 1,5-hexadiyne, however, no double bond isomerization was observed. The total yield of the six-step synthesis using 1,5-hexanediyne as the starting material was 8%. Vik et al. (2010) used eicosapentaenoic acid (EPA) as a starting material to synthesize JA, and as a result, JA was obtained with a total yield of 19% following 8 steps methodology (Figure 5).

Although Δ5-UPIFAs can be obtained by chemical synthesis, the low yield and several reaction steps are not the best choices for industrial applications. Since the acceptance of chemically synthesized substances is not reckoned as high as that of natural products, extraction of Δ5-UPIFAs from natural products could be another good option.

5.2.2. Physical extraction

Following organic solvent extraction, purification with an appropriate solvent is the most common method for extraction and preparation of Δ5-UPIFAs. Chen et al. (2012) obtained SA from *Podocarpus nagi* seeds with a purity of more than 98% by column chromatography followed the organic dissolution and extraction. However, this purification method is complex and time-consuming, which could be more suitable for small-scale purposes at the laboratory scale but not suitable for industrial applications. Urea complexation is a technique to eliminate saturated fatty acids and monounsaturated fatty acids (Vázquez et al. 2017). Wanasundara and Shahidi (1999) successfully used urea complexation to produce omega-3 fatty acid concentrates from seal oil (SBO). Urea complexation was also applied to prepare PA from *Pinus koraiensi* soil (Lee et al. 2004), after which PA concentration increased from 14.1% to 45.1%

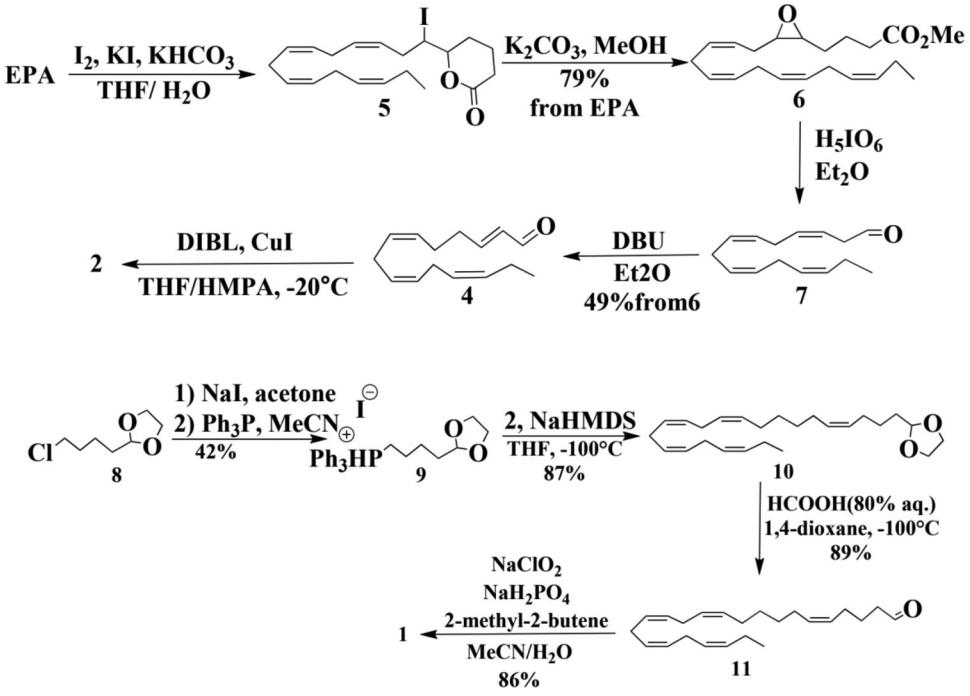

Figure 5. The reaction process of synthesis of JA from EPA (Reproduced from Vik et al. 2010).

with the corresponding yield of 1.88%. It was further used to enrich PA content to 43.8% from the initial content 16.8% in pine nut oil (Kim and Hill 2006). Though the urea complexation method increased the concentration of PA, the final concentration was not ideal, suggesting that this method was not a suitable for preparation option. Moreover, urea complexation is simply an auxiliary method for concentration.

5.2.3. Chemical extraction

Similar to physical extraction, the target components can be collected by chemical extraction (Hase et al. 1992). As a difference, chemical extraction involves some chemical reactions. PA was isolated from Scandinavian distilled tall oil fatty acid (TOFA) by iodinated esterification (Hase et al. 1992), and it was found that TOFA contained more than 10% PA. The above-mentioned iodide lactone process was repeated by using 57% pinolenic acid methyl ester prepared from TOFA by urea fractionation. It was found that the purity of PA prepared from TOFA by the iodolactone method was 80%, and the purity of PA prepared from the concentrate was 96%.

The results showed that when preparing PA from TOFA, iodolactone could be a good method. Combining of iodolactone with some pre-purification methods, such as urea fractionation, the purity of the preparation can be improved. However, the elution of column chromatography is still involved in the preparation process, which makes the method difficult to carry out large-scale production in the industry.

5.2.4. Enzymatic methods

5.2.4.1. Distribution of Δ5-UPIFAs. Fatty acids can occupy any of three positions on the glycerol backbone, namely *sn-1*, *sn-2*, and *sn-3* ("*sn*" stands for "stereospecific numbering") (Hunter 2001). Different fatty acids have different preferences at different positions. Δ5-acids only exist at *sn-1* in triacylglycerols using ^{13}C-NMR spectroscopy, and they are independent based on their chain length (C_{18} or C_{20}) and unsaturation (2–4 double bond) (Gunstone, Seth, and Wolff 1995; Gunstone and Wolff 1996). Takagi and Itabashi (1982) found that *cis*-5-olefin fatty acids were mainly distributed at the 1,3-position of triacylglycerols of 20 gymnosperms seed lipids, as also reported previously (Wolff, Dareville, and Martin 1997). In animals, researchers have found that 18:2-5,9 has no preference for *sn-1* or *sn-2* positions in sponge phosphatidylethanolamines (Carballeira, Emiliano, and Morales 1994). Blaise et al. (1997) studied the position distribution of Δ5-acid in triacylglycerol of conifer seeds by partial chemical cleavage method. They found that at least for pine species, Δ5-acids usually esterify only on one external position of TAG at a time, and rarely esterify two positions simultaneously. Gresti et al. (1996) also demonstrated that there was only one molecule of Δ5-UPIFAs in the individual TAG species. These results may provide a new simple method to get high purity Δ5-UPIFAs. In view of these characteristics, enzymatic preparation of Δ5-UPIFAs seems to be a feasible alternative.

5.2.4.2. Enzymatic hydrolysis. In order to increase the contents of Δ5-UPIFAs, lipase RM-IM in *Rhizomocor miehei* was used as a biocatalyst to catalyze the esterification of ethanol and fatty acids (Kim et al. 2018). The free fatty acids were obtained from saponified and acidified pine nut oil. Δ5-OAs were concentrated in the unesterified fatty acid part. The optimal conditions for the enzymatic reaction included the molar ratio of fatty acid to ethanol, temperature and enzyme loading as 1:7, 25 °C, respectively, with 5%

of the total weight of the substrate. After 3 times concentration, in which the last unesterified part was used as a raw material, the content of Δ5-OAs was 96 mol% with the total yield of 6 mol%. Though the purity of Δ5-OA was greatly improved, the product was a mixture of Δ5-UPIFAs. Similarly, Jie and Rahmatullah (1995) used an enzymatic method with cylindrical lipase to enrich 20:3-5,11,14 and 20:4-5,11,14,17 (Δ5-UPIFAs) from *Biota orientalis* seed oil. The enrichment process was repeated twice after conducting urea fractionation to remove saturated fatty acids. The yield of fatty acid was 20% and up to 41% acylglycerols rich in *cis*-5, 20:3 and 20:4 were obtained.

No et al. (2015) prepared PA by esterifying pine nut oil with lauryl alcohol using *Candida* lipase. As a result, PA with a purity greater than 95 mol% was obtained when the ratio of urea to fatty acid was greater than 3:1 (wt/wt), whereas 100% pure PA was produced when the ratio of urea to fatty acid was 5:1, with a yield of 8.7 mol%.

Different from the above-mentioned enzyme specificity of fatty acids, Meng et al. (2020) utilized the specificity of the enzyme for *sn-1,3* to prepare SA. Lipozyme RM IM was used as a *sn-1,3* specific biocatalyst for enzymatic acid hydrolysis reaction (Sahin, Akoh, and Karaali 2005). *T. grandis* seed oil was subjected to a two-step enrichment process involving specific lipase-catalyzed ethanolysis and urea complexing. The content of SA in the fatty acid ethyl ester was increased to 80.14% from an initial value of 9.95%.

5.2.4.3. Immobilized enzyme hydrolysis. To maintain the activity of enzyme and improve its stability, immobilized enzyme hydrolysis was applied (Lee et al. 2011). Novozym 435 not only showed significant activity on long-chain PUFA, but also displayed *sn-1,3*-domain specificity for the methanolysis of TAG (Irimescu, Iwasaki, and Hou 2002; Sun, Zhu, and Bi 2014). An immobilized novozym 435 was used to catalyze the alcoholysis of pine nut oil in a batch reactor to produce PA concentrate. The optimal reaction conditions included addition of immobilized lipase novozym 435 (5%), temperature (25 °C), the molar ratio of PA: ethanol (1:80), substrate molar ratio (1:80), and reaction time (60 min) that resulted PA yield in the form of FAEE as 60.7 mol%.

Although the yield was high by using a batch reactor, it could not be produced on a large scale. The circulating packed bed reactor (RPBR) can significantly improve the stability of the enzyme and reduce enzyme loss, easily applicable for large-scale production (Zhao et al. 2012). An RPBR was used to produce PA concentrate from pine nut using lipase novozym 435 as a biocatalyst. The optimal temperature, the molar ratio (molar ratio of pine nut oil to ethanol), and residence time of lipase-catalyzed ethanol cracking were: 45 °C, 1:50, and 3 min, respectively. Under these conditions, the PA content in the concentrate within the first 10 minutes of the reaction was the highest as 36.1 mol%.

5.2.5. Chromatography

Chromatography is a common micro separation method at the laboratory scale. In recent years, some researchers have separated Δ5-UPIFAs by chromatographic method. Hammann et al. (2015) used counter current chromatography (CCC) to separate two Δ5-UPIFAs (SA and JA) from *Podocarpus falcatus* seed oil. The component enriched with the target compounds was subjected to subsequent CCCs. The solvent system of CCC-4 was n-hexane/methanol/water 400:364:36 (v/v/v). After three CCC treatments, the purity of both JA and SA obtained reached 99%.

Although the fatty acids produced by CCC are of high purity, the operation is complex and not conducive to industrial production. In order to simplify the operation process, semi-preparative supercritical chromatography was used to obtain Δ5-UPIFAs from *Biota orientalis* seed oil (Montañés, Tallon, and Catchpole 2017). The optimal reaction conditions were as follows: outlet pressure, 170 bar, temperature, 333 K, wavelength, 200 nm, flow rate changes, and 7.5–12.5 mL CO_2/min, without any co-solvent. Column aminophenyl was used as a stationary phase for SA enrichment in the form of FAEE gamma amino phenyl, and the purity of SA was 75.6%. Column aminophenyl was used as a stationary phase for JA enrichment in the form of FAEE gamma silica, and the purity of JA collected was more than 95%.

The preparation methods demonstrated in this article are shown in Table 6. During chemical synthesis, although the reactants are somehow easy to get as starting materials, the steps are complex. Compared with synthetic substances, people are more likely to accept substances that exist naturally. Although in the preparation of chromatography, some advanced instruments can be used to prepare substances with high purity, the treatment capacity of the chromatographic column is limited, thus making difficult to be applied to large-scale industrial production. Also, the purity of the product obtained when the urea complex is used alone is not ideal; however, it can get a better preparation effect by combining it with other preparation methods. Target products with high purity can be prepared by selecting appropriate enzymes, and the appearance of immobilized enzymes makes the enzymatic method possible to be applied for industrial production.

6. Health benefits of Δ5-UPIFAs

Δ5-UPIFAs have been proved to possess several beneficial physiological functions, including lipid-lowering ability, anti-proliferation effect, anti-inflammatory activity, anti-tumor activity, anti-microbial activity, and enhancing insulin sensitivity etc.

6.1. Lipid-lowering activity

Obesity is a chronic energy imbalance that occurs when energy intake in the body is greater than energy expenditure. The fatty acid composition of the edible oil can significantly affect the levels of various lipids and lipid profile in the human body. The lipid lowering effect of Δ5-UPIFAs is shown in Figure 6.

Table 6. Preparation methods of Δ5-UPIFAs.

Methods		Materials	Reaction processes	Products	Purities	Yields	References
The procedure is the same as the preparation of SA.	JA	>95%	_				
Chemical synthesis	Six step synthesis	1,5-hexadiyne	1,5-Hexadiyne is coupled with 1-bromohexane and n-butyllithium. The 1,5-dodecyne is then alkylated with (4-bromobutoxy)-tert-butyldimethylsilane and n-BuLi to obtain 1-(tert-butyldimethylsilyloxy)-5,9-hexadecadiyne. The silicon group is deprotected with TBAF, and the alcohol is oxidized with PCC. The aldehyde is then oxidized with $NaClO_2$ to obtain 5,9-hexadecane acid. Finally, hydrogenation is performed on the catalyst to obtain 5,9-hexadecadienoic acid.	16:2-5,9	_	8%	(Carballeira et al. 2002)
	Eight step synthesis	EPA	The EPA treated with potassium carbonate in methanol. After epoxide was obtained by further reaction, it was oxidized with periodate. Then the unsaturated aldehydes were obtained by DBU treatment. The aldehyde was then selectively reduced and reacted with triphenylphosphine iodide. After the reaction product was cracked with formic acid aqueous solution, JA was prepared with 2-methyl-2-butene as scavenger.	JA	_	19%	(Vik et al. 2010)
Physical extraction	Extraction with n-Hexane and purification with column chromatography	*P. nagi* seeds	The saponified seed oil was acidified with HCl. After extraction with n-hexane, SA was prepared by silver column chromatography.	SA	≥98%	0.22g SA methyl ester was obtained from 2 g seed powder.	(Chen et al. 2012)
	Urea complexation	*Pinuskoraiensis*oil	Pine nut oil was hydrolyzed in 95% ethanol and acidified with HCl. LPAFAE was recovered with n-hexane. Dissolve LPAFAE in ethanol, add urea and react for 30 min. HPAFAE was recovered by urea complexation.	PA	45.1%	1.80%	(Lee et al. 2004)
Chemical extraction	Iodinated esterification	TOFA (10%PA) 57% pinolenic acid methyl ester	TOFA is dissolved in an aqueous solution of potassium bicarbonate containing THF and potassium iodide. Iodine is added to obtain iodonolactone. The lactones were separated by column chromatography. Then the collected components are regenerated with fatty acids.	PA	80% 96%	_ _	(Hase et al. 1992)
Enzymatic method	Repeated lipase-catalyzed esterification with lipase RMIM in Rhizomocormiehei	Pine nut oil	The optimal conditions: the molar ratio of fatty acid to ethanol, temperature, and enzyme loading were set to 1:7, 25 °C, and 5% of the total weight of the substrate, respectively. Enzyme	Δ5-olefin acids (18:2-Δ5,9,18:3-Δ5,9,12 and 20:3-Δ5,11,14)	96mol%	6mol%	(Kim et al. 2018)

			catalyzed esterification repeated three times.				
	Enzymatic enrichment with cylindrical lipase	*B.orientalis* seed oil	Firstly, conduct urea fractionation to remove saturated fatty acids, and then repeat esterification twice with Cylindrical lipase at 25 °C for 6 h.	C_{20}*cis*-5		polyunsaturated fatty acids (SA and JA)	41%
	20%	(Jie and Rahmatullah 1995)					
	A combination of enzymatic esterification by using Candida lipase and urea complexation	Pine nut oil	In the first step, the fatty acids present in pine nut oil were selectively esterified with lauryl alcohol using Candida lipase. In the second step, the urea complexation is performed on the PA-enriched part from the first step.	PA	100%	8.7 mol%	(No et al. 2015)
	lipase-catalyzed ethanolysis with an immobilized Candida antarctica lipase	Pine nut oil	Lipase catalyzed ethanol hydrolysis of pine nut oil, PA: ethanol= 1:80 molar ratio. The mixture reacted with immobilized lipase Novozym 435 of Candida antarctica (5% of the total substrate weight) at 300 rpm and 25 °C for 60 min.	PA	30mol%	60.7 mol%	(Lee et al. 2011)
Circulating packed bed reactor (RPBR) with lipase Novozym435 as catalyst	Pine nut oil	In RPBR, lipase Novozym435 catalyzes the production of PA by ethanol cracking. The optimum temperature, molar ratio (the molar ratio of pine nut oil to ethanol) and residence time were 45 °C, 1:50 and 3 min, respectively.	PA	36.1 mol%	9mol%	(Zhao et al. 2012)	
Chromatography	Countercurrent chromatography	Seed oil of *Podocarpusfalcatus*	After the fatty acid methyl ester was prepared with 1% methanol sulfuric acid, FAME was injection PTC-1000 CCC instrument. Collect the fraction rich in the target component and repeat the CCC operation.	SA JA	99% 99%	_ _	(Hammann et al. 2015)
	Semi-preparative supercritical chromatography	*Biota orientalis* seed oil	Ethyl ester was prepared in anhydrous ethanol with KOH as catalyst. FAEE was injected into SFC device.	SA	75.60%	_	(Montañés, Tallon, and Catchpole 2017)

Note: "–" meant not mentioned.

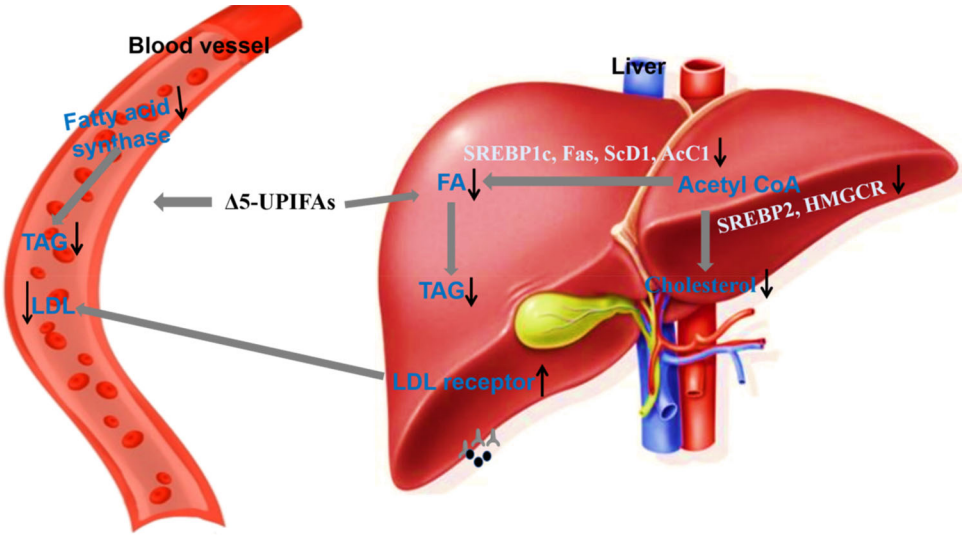

Figure 6. Proposed mechanism of action of Δ5-UPIFAs to lower lipid. Δ5-UPIFAs can reduce the mRNA levels of genes involved in fatty acid and cholesterol synthesis, thereby down-regulating the lipid anabolic pathway of cells, lowering VLDL in serum, lowering liver and serum TAG and cholesterol. They enhance liver uptake of LDL, thus reducing LDL.

6.1.1. In *vivo* experiments

Asset et al. (1999) reported the effect of seed oils on lipoprotein metabolism in male Wistar rats. These oils contained two special fatty acids of Δ5-UPIFAs, namely PA and SA. In rats treated with *Pinus pinaster* seed oil, very low-density lipoproteins (VLDL) triglycerides reduced by 40% and VLDL cholesterol reduced by 33%. The levels of serum total triglycerides reduced by 16% and VLDL triglycerides reduced by 21% in rats treated with *P. koraiensis* seed oil. The results showed that Δ5-UPIFAs have VLDL triglycerides reducing effect, whereas SA has a greater potential to reduce VLDL cholesterol than PA.

It was also reported that fatty acid substances of sea pine oil could reduce the plasma cholesterol, triglyceride and low-density lipoprotein (LDL) cholesterol in hApoB mice, and showed good effect on the blood lipid index of apolipoprotein B in 3–4 months aged female mice when compared to groups administrated with coconut oil and sunflower oil (Asset et al. 2001), suggesting that the effect on lipid-lowering may be caused by Δ5-UPIFAs.

Ikeda et al. (1992) observed lower plasma levels of cholesterol and liver triacylglycerol in male Sprague Dawley mice when administrated with *Biota orientalis* seed oil (BSO) which contains SA and JA. The results suggested that BSO specifically inhibited the synthesis and/or promoted the catabolism of triglycerides. The role of BSO can be attributed to SA and JA. Similar results that SA could modify the lipid metabolism in rats particularly by reducing the triacylglycerol concentration were also reported (Pedrono et al. 2018). Although SA from Torrey seed oil was proved to reduce the activity of some liver enzymes involved in fatty acid synthesis, the expression of genes related to lipid metabolism in rat liver had no correlation with dietary fat content (Endo et al. 2007). Further research confirmed that the inhibition of fatty acid synthesis was dose-dependent (Endo, Tsunokake, and Ikeda 2009).

Ferramosca et al. (2008) studied the effect of PA on male ICR mice and found that PA could significantly reduce the weight gain, liver weight, the plasma triglyceride, and total cholesterol. PA also exhibited positive effect on liver lipids, and the activities of mitochondrial and cytoplasmic enzymes related to fatty acid synthesis in the liver were significantly reduced in the animals fed with pine oil and corn oil.

6.1.2. In *vitro* experiments

Lee et al. (2004) conducted research on HepG2 cells to study the effects of PA on cholesterol levels. The results indicated that the high-pinolenic acid-containing FA extract showed LDL-reducing properties by enhancing liver uptake of LDL.

Similar to other polyunsaturated fatty acids, PA, present in Korean pine oil, acts as a ligand for PPARα and PPARδ nuclear receptors, due to being a re-gioisomer of γ-linolenic acid. These nuclear receptors are involved in lipid oxidative metabolism (Wang 2010). Lee and Han (2016) studied the mechanism of function of PA present in Korean pine oil on down-regulating lipid synthesis and metabolism pathways in HepG2 cells. To do so, HepG2 cells were cultured with six kinds of fatty acids (palmitic acid, oleic acid, γ-LA, PA, eicosapentaenoic acid, and α-LA). The results showed that PA participates in down-regulating the ACSL3 and ACSL4 adipogenesis pathway by reducing the mRNA level of fatty acid synthesis related genes (SREBP1c, FAS and SCD1), cholesterol synthesis related genes (HMGCR), and the protein uptake-related gene (LDLr), thereby down-regulating the lipid anabolic pathway of HepG2 cells. PA was also reported to prevent lipid accumulation in HepG2 cells via up-regulating the AMPK/SIRT1 signaling pathway (Zhang et al. 2019).

Based on the above findings it is summarized that the effect of Δ5-UPIFAs on lipids is mainly mediated via reducing the VLDL in serum, triglycerides (TAG) in liver and serum, and cholesterol. The mechanism adopted by Δ5-UPIFAs could be lowering the mRNA levels of genes related to fatty acid and cholesterol synthesis and genes related to lipoprotein uptake, that were involved in down-regulating

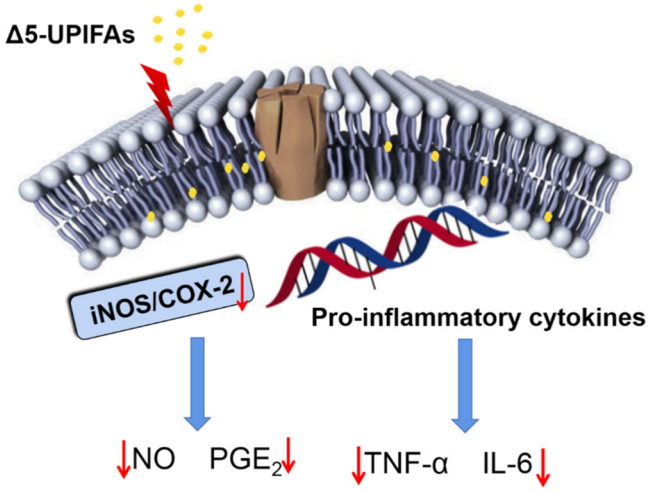

Figure 7. Proposed mechanism of action of Δ5-UPIFAs on anti-inflammatory activity. SA is first taken up by cells and then incorporated into phospholipids. SA incorporates membrane phospholipids to cause changes in membrane structure and fluidity, thereby affecting body-mediated reactions, carrier processes and signal transduction. SA regulates the signal transduction pathway of cells, inhibits the production of pro-inflammatory mediators and gene expression. Inhibition of pro-inflammatory mediators is partly due to reduced expression of iNOS and COX-2.

the fat production pathway, thereby down-regulating the lipid anabolic pathway of HepG2 cells, thus increasing LDL uptake by the liver for its reduction.

6.2. Anti-inflammatory activity

Inflammation is a direct defensive reaction that occurs quickly when any body tissue is damaged physically or chemically and attacked by the inflammatory factors such as immunological and biological receptors (Gorman and Park 2004). Although inflammation has beneficial role in eliminating the causative agents and repairing damaged tissues, excessive inflammation is often accompanied by the occurrence of other diseases, which easily causes cardiovascular disease and high cancer risk (Gorman and Park 2004). Therefore, it is necessary to develop more anti-inflammatory substances of natural origin. Some studies have found that Δ5-UPIFAs have anti-inflammatory effects by reducing inflammatory factors, such as AA and prostaglandin E2 (PGE2) etc. The anti-inflammatory effect of Δ5-UPIFAs has shown in Figure 7.

Huang et al. (2014) incubated murine RAW264.7 and rat peritoneal macrophages with PA at a dose of 25 or 50 μM and further stimulated the cells with LPS, as a result, PA significantly reduced PGE2 production in a dose-dependent fashion. On the other hand, incorporation of SA into the host epithelial phospholipids resulted in a reduction in PGE2 production during the initial infection with *Candida albicans* and *Candida dublin* (Ells et al. 2012). For this, macrophages were cultured in DMEM medium with different concentrations of SA (Chen et al. 2012), and it was found that SA significantly reduced the production of NO, factor-α (TNF-α), interleukin-6 (IL-6), type 2 cyclooxygenase (COX-2), and inducible nitric oxide synthase (iNOS). The down-regulation of PGE2 may be due to reduced COX-2 expression, or due to competition between the extended products of PA, Δ7Eta and AA. Also, SA incorporation inhibited the expression of total mitogen activated protein kinase (MAPK) and phosphorylated MAPK as well as translocation of NF-JB p65. These inhibitory effects can partly attribute to the inactivation of MAPK signal.

Tanaka et al. (1999) used the HepG2 cell system to study the effect of several polyunsaturated fatty acids (PUFAs) on the AA concentration in the phosphatidylinositol (PI). The polyunsaturated fatty acids of PI in control cells were mainly incorporated mead acid 20:3-5,8,11 (MA) and AA. The addition of EPA reduced the proportion of MA from the control value of 12.0 to 5.0%, but the AA concentration remained unchanged. SA reduced both AA and MA concentrations by about 50%. The PA reduced the levels of MA and AA from the control values of 12.0% and 15.9% to 6.3% and 7.0%, respectively. In contrast, colonial acid with a trans double bond at position Δ5 could not decrease the AA and MA concentrations of the PI fraction. Therefore, it can be concluded that Δ5-UPIFAs showed a modification effect on PI and can reduce the AA content in PI. Similar results on PA and SA were reported previously when LPS-stimulated murine microglial BV-2 cells were treated with 50 μM PA or SA (Chen et al. 2015). These findings were further supported with similar results obtained in murine macrophage cells when treated with JA (Tsai et al. 2018), where JA suppressed the production of NO, IL-6, TNF-α, and the expression of iNOS. Furthermore, JA suppressed the expression of phosphorylated MAPK. Above findings suggest that the anti-inflammatory properties of JA could be due to the incorporation of JA into cellular phospholipids with subsequent modulation of membrane-mediated MAPK signaling.

6.3. Anti-proliferative activity

Morishige et al. (2008) obtained JA with a purity of 98% from *Biota orientals* seeds and observed the inhibitory effect of JA on the proliferation of Swiss 3T3 cells. The results indicate that the proliferation of JA-loaded cells was almost half of that of LA-loaded cells. The anti-proliferative capacity of JA is comparable to that of EPA. In contrast, SA, the omega-6 analog of JA, did not show anti-proliferative activity. Therefore, the omega-3 double bond rather than the non-methylene-interrupted structure in JA is necessary for its anti-proliferative activity.

6.4. Enhancing insulin sensitivity

FFA1, FFA2, FFA3, and FFA4 are free fatty acid receptors involved in insulin-responsive tissues. PA is highly potent and efficient as a ligand for FFA1 and FFA4. Christiansen et al. (2015) used the acute glucose tolerance test to study the effect of PA on C57BL/6 mice metabolism. The male mice aged 6–7 weeks were fed with standard laboratory feed. After 5 hours of fasting, the mice were given oral glucose load (3 g/kg), 30 minutes before receiving glucose, PA (100 mg/kg) or PNO (1 g/kg). For control group, corn oil (1 g/kg) was injected into the abdominal cavity of the mice.

The co-activation of FFA1 and FFA4 by PA was seemed to enhance the secretion of glucose-dependent insulin and promoted effective glucose metabolism. In summary, the mechanism of increasing insulin sensitivity conferred by PA could be due to its dual agonist efficacy that can synergistically activate FFA1 and FFA4, thereby enhancing glucose-dependent insulin secretion and insulin sensitivity (Xie, Miles, and Calder 2016).

6.5. Anti-tumor activity

A pilot study first demonstrated the presence of SA in hormone-positive BC tissue in vivo (Park et al. 2018). Chen et al. (2011) studied the effect of PA on MDA-MB-231 human breast cancer cells. The invasiveness of cells with DHA and PA decreased by 29.6% and 25.4%, respectively, suggesting that PA can inhibit cell metastasis by inhibiting cell invasion and movement and may have the ability to inhibit tumor metastasis. The anti-tumor mechanism of Δ5-UPIFAs could be attributed to their ability to reduce cell viabilities of tumor cells, thereby inhibiting tumor metastasis, and promoting the formation of lipid peroxides to promote tumor cell apoptosis (Das 2011).

6.6. Anti-bacterial activity

Tasdemir et al. (2007) studied six kinds of pure components and complex fatty acid mixtures (FAMA to FAMG) obtained from MeOH extract of Turkish sponge *Agelas oroides*. Inhibition of e*noyl-[acyl-carrier-protein] reductase* (FabI) of *Plasmodium falciparum* (PfFabI), *Mycobacterium tuberculosis* (MtFabI), and *Escherichia coli* (EcFabI) measures the enzymatic activity by tracking the oxidation of NADH. FAMA consisted of a mixture of 23:2-5,9 and 24:2-5,9 (1:2) and was active against both MtFabI and EcFabI. FAMB was composed of a mixture of 24:2-5,9, 25:2-5,9 and 26:2-5,9 at 3:3:2, which inhibited PfFabI (IC_{50}, 0.35 μg/mL), but showed not activity against MtFabI and EcFabI. These findings indicated that (5Z,9Z)-23:2-5,9 is an active ingredient in FAMA that inhibited EcFabI and MtFabI with IC_{50}=9.4 μg/mL for both, and IC_{50}=0.5 μg/mL for EcFabI.

Carballeira et al. (2002) used 16:2-5,9 for determining its antibacterial activity against *Pseudomonas aeruginosa* (ATCC 27853), *Escherichia coli* (ATCC 25922), *Staphylococcus aureus* (ATCC 25923), and *Streptococcus faecalis* group D (ATCC 29212) by using assay protocol of National Committee of Clinical Laboratory Standards. It was found that 5,9-hexadecadienoic acid showed moderate antibacterial activity against Gram-positive bacteria *Staphylococcus aureus* (MIC-80 μM) and *Streptococcus faecalis* (MIC-200 μM), as such kind of fatty acids have not shown antimicrobial activity Gram-negative bacteria *Pseudomonas aeruginosa* and *E. coli*. Suzuki et al. (2000) studied the inhibitory effect of several unsaturated fatty acids on topoisomerase I, and reported similar findings. I. It was observed from the results that the presence of double bonds in fatty acid molecules is necessary to inhibit topoisomerase I, and the length of the carbon chain affects its inhibitory effect.

There are several possibilities of using fatty acid synthases (FASs) as antibacterial principles. Fatty acid synthases (FASs) are synthesized by repeating the four sub-sequent reactions of condensation, reduction, dehydration, and reduction. In mammals and other higher eukaryotes, all these reactions are catalyzed by type I fatty acid synthase (FAS I) (Smith 1994). On the other hand, bacteria, plants, and algae contain the type II fatty acid synthase system (FAS II) (Schweizer and Hofmann 2004). Type II fatty acid system has also been found to present in some parasites, including malaria parasites and *Plasmodium falciparum* (Waller et al. 1998). FabI is a key enzyme in all FAS-II systems because it catalyzes the last step of NADH-dependent reduction in each elongation cycle. Therefore, Δ5-UPIFAs may play a bacteriostatic and insect-resistant role by inhibiting FabI. Additionally, the antibacterial activity of Δ5-UPIFAs may also be due to the inhibitory effect of the fatty acid on topoisomerase. Physiological functions of Δ5 NMIPUFAs are shown in Table 7.

7. Applications

Nowadays, the materials rich in Δ5-UPIFAs are widely favored by the food industry. Δ5-UPIFAs can not only be used alone to exert their biological activity, but also can be used in combination with other substances to exert more excellent characteristics.

Structured lipid compositions containing SA and/or PA alone or in combination with other bioactive fatty acids such as eicosapentaenoic acid and docosahexaenoic acid, exhibit certain physiological activities (Remmereit and Berger 2020). A juniper oil having JA and SA at a ratio of 2.5:1-1:2.5 provides a bioactive lipid formulation that can alleviate or improve one or more diseases or disorders such as inflammation and obesity (Berger and Remmereit 2017).

Apart from the commercial conifer nut oil rich in PA, structured lipid (SL) could be an alternative for further application. Kim and Hill (2006) studied SL through lipase-catalyzed acidolysis of menhaden oil with PA, and it was found that novozym 435 can incorporate PA at both the *sn-1,3* and *sn-2* positions of the glycerol backbone, whereas lipozym RM IM showed high selectivity for *sn-1,3* positions (Kim and Hill 2006). Generally, Δ5-UPIFAs are naturally located at *sn-1,3* positions of the TAG backbones which may lead to their relatively lower bioavailability compared to the *sn-2* positional ones (Hunter 2001). SL was produced from pine nut oil (PNO) and palm stearin (PS), and intentionally incorporated into the *sn-2* position through interesterification with lipozym TL IM, to provide a possible low trans soft margarine fat as an alternative to partially hydrogenated soft margarine fat (Zhu et al. 2012). Fat products containing PA [3.1%–11.6% (w/w)] and linoleic acid [16.1%–35.7% (w/w)] at *sn-2* position were also prepared from pine seed oil (PSO) and fully hydrogenated soybean oil to obtain solid and semi-solid fats, thereby extending the range of applications of the feedstock oils (Cristina et al. 2013).

Even though several functions of Δ5-UPIFAs have been explored, the degree of commercial application is limited,

Table 7. Physiological functions of Δ5-UPIFAs.

Benefits	Targeted object	Models	Administration	Experimental designs	Results	References
Lipid-lowering activity	PA, SA	Male Wistar rats	Oral	I, 5% (w/w) *P. pinaster* seed oil was added. II, 5% (w/w) of a mixture prepared with safflower, oleic acid enriched sun flower, and linseed oils were added in the proportion of 66.5, 31, and 2.5%. III, 5% (w/w) *P. koraiensis* seed oil. IV, 5% (w/w) of a mixture prepared with safflower, oleic acid enriched sunflower, and linseed oils were added in the proportion of 55, 44.7, and 0.3%.	PA and SA can reduce triglycerides and VLDL, and SA has a greater potential to reduce VLDL than PA.	(Asset et al. 1999)
	Δ5-UPIFAs	Female human apolipoprotein B mice		Mices were fed with 20% (w/w) coconut oil, 20% (w/w) sunflower oil or 20% (w/w) maritime pine (*Pinus pinaster)* for trials.	Δ5-UPIFAs can reduce the content of cholesterol, triglyceride, phospholipid and apolipoprotein B.	(Asset et al. 2001)
	SA and JA	Male Sprague Dawley		I, 90% safflower oil and 10% high oleic acid safflower oil were mixed to prepare LA rich fat. II, 85% linseed oil and 15% safflower oil were mixed as ALA rich fat. III, *Biota orientalis*seed oil	JA and SA can inhibit the synthesis and promote the catabolism of triglycerides.	(Ikeda et al. 1992)
	SA	Male Sprague Dawley rats		Four-week-old rats were divided into three groups and fed with experimental feed containing 10% *Torreyanucifera*, corn or soybean oil.	SA can reduce the TAG levels of plasma and liver. The activities of most enzymes involved in fatty acid synthesis were significantly reduced.	(Endo et al. 2007; Endo, Tsunokake, and Ikeda 2009; Pedrono et al. 2018)
	PA	Male ICR mice		The mice were fed with standard diet supplemented with 7.5% corn oil (control group) or 7.5% PInnoThin^{TM} for 8 weeks.	PA can reduce liver weight, plasma triglyceride and total cholesterol of the mice. PA can also decrease the activity of citric acid transport.	(Ferramosca et al. 2008)
	PA	HepG2 cell	_	One group treated the cells with 1 μM HPAFE. In the second group, the cells were treated with the 1 μM LPAFE. In the third group, cells were untreated and used as controls.	The PA can have LDL-reducing properties by enhancing liver uptake of LDL.	(Lee et al. 2004)
				HepG2 cell line was cultured with 50 μM six kinds of fatty acids (palmitic acid, oleic acid, γ-LA, PA, eicosapentaenoic acid, and α-LA).	PA can down-regulate the ACSL3 and ACSL4 adipogenesis pathway and down-regulate the lipid anabolic pathway of HepG2 cells. It can regulate the assembly and secretion of VLDL.	(Lee and Han 2016)
	PA		_	The cultured cells were pretreated with PA (0–25μM) for 12 h and then treated with OA (0.5 μM) for 24 h.	PA prevent lipid accumulation in HepG2 cells via upregulating the AMPK/SIRT1 signaling pathway	(Zhang et al. 2019)
Anti-inflammatory activity	PA, SA	Male Wistar rats	Oral	The cells were treated with 100 μM fatty acids.	PA and SA hadmodification effect on PI and can reduce the AA content in PI	(Tanaka et al. 1999)
	PA, SA	Murine RAW264.7 cells	_	Incubated murine RAW264.7 and rat peritoneal macrophases with PA at a dose of 25 or 50 μM and stimulated with LPS.	Δ5-UPIFAs can reduce the production of anti-inflammatory effects, such as NO, PGE2, IL-6, TNF-α and iNOS.	(Huang et al. 2014)
	SA	Epithelial cells				(Ells et al. 2012)

(*continued*)

Table 7. Continued.

Benefits	Targeted object	Models	Administration	Experimental designs	Results	References
				The cells were treated with 50 μM SA.		
	SA	Murine microglial BV-2 cells	_	Macrophages were treated with different concentrations of SA (0, 10, 25, 50 or 100 μM).		(Chen et al. 2012)
	SA, PA		_	The cells treated with 50 μM PA or SA and stimulate by LPS (0.1 μg/mL).		(Chen et al. 2015)
Anti-proliferative activity	JA	Swiss 3T3 cells	_	Swiss 3T3 cells were pre-loaded with LA, EPA, JA, SA, respectively, which then stimulated with mitogenic neuropeptide bombesin	JA has anti-proliferative activity.	(Morishige et al. 2008)
Enhancing insulin sensitivity	PA	Male C57BL/6 mice	Injection	The mice were treated with PA (100 mg/kg) or PNO (1 g/kg). And corn oil (1 g/kg) was injected into the abdominal cavity of mice in the control group.	PA can enhance the secretion of glucose dependent insulin and promote effective glucose metabolism.	(Christiansen et al. 2015)
Anti-tumor activity	PA	MDA-MB-231 cell line	_	The cell lines were cultured in DMEM medium with different concentrations (50 or 100 μM) of DHA, EPA, GLA or PA.	PA can decrease the invasiveness of cells and inhibit cell movement.	(Chen et al. 2011)
Anti-microorganism activity	FAMA and FAMB	*Plasmodium falciparum*, *Mycobacterium tuberculosis* and *Escherichia coli*	_	The microorganisms were treated with FAMA (a 1:2 mixture of 23:2-5,9 and 24:2-5,9) and FAMB (a mixture of 24:2-5,9, 25:2-5,9 and 26:2-5,9 at 3:3:2).	23:2-5,9 is an active ingredient that inhibits EcFabI and MtFabI, MtFabI, IC_{50}, 9.4 μg/mL and EcFabI, IC_{50}, 0.5 μg/mL.	(Tasdemir et al. 2007)
	16: 2-5, 9	*Pseudomonas aeruginosa*, *Escherichia coli*, *Staphylococcus aureus*and*Streptococcus faecalis*group D	_	The fatty acids and microorganisms were incubated overnight. And the minimum inhibitory concentration (MIC) was determined.	(5Z,9Z)-5,9-hexadecadienoic acid showed moderate antibacterial activity against Gram-positive bacteria *Staphylococcus aureus* (MIC80μM) and *Streptococcus faecalis* (MIC200μM).	(Carballeira et al. 2002)

and only a few studies provide detailed underlying molecular mechanisms to elucidate how exactly Δ5-UPIFAs exert different activities. Most of the published studies on Δ5-UPIFAs associated with the health benefits did not describe their composition used in animal and clinical experiments. Therefore, further studies should emphasize the association between composition and health benefits of Δ5-UPIFAs to help develop more diversified functional Δ5-UPIFAs products for targeted health benefits and to accelerate their future applications.

8. Conclusions

In this review, we summarized the past achievements of Δ5-UPIFAs in terms of the sources, biosynthesis and metabolism, analysis, preparation, and potential benefits. Δ5-UPIFAs can be synthesized from simple and common compounds. Moreover, a small amount of high-purity Δ5-UPIFAs can be prepared by chromatographic technique. In addition, the selectivity of the enzyme can also enrich the target Δ5-UPIFAs to a certain extent, so as to prepare for subsequent experiments. Though several researches have been done to prepare Δ5-UPIFAs, how to produce Δ5-UPIFAs environmentally-friendly (enzymatic synthesis) and efficiently with the aid of green chemistry still remains to be improved. Moreover, bioengineering seems to be another promising way for producing Δ5-UPIFAs. Δ5-UPIFAs could be of potential benefits in improving insulin sensitivity, lowering the risk of tumor disease, reducing inflammation and lowing the level of lipids. However, most studies have been performed on cell lines or in rodent models with only limited human research. A long research gap exists in investigating the benefits of Δ5-UPIFAs in humans when applied as functional foods or nutraceuticals. Thus, it will be important to conduct well-designed human trials of Δ5-UPIFAs to better identify their beneficial effects in humans in order to clarify their roles in improving human health, tap new resources, develop structured lipide rich in Δ5-UPIFA, enhance their delivery, so as to accelerate their applications in food, pharmaceutical, and cosmetic industries.

Disclosure statement

The authors declare no conflict of interest.

Funding

This work was supported by the National Natural Science Foundation of China (No. 31772001, 31971699, U20A2049); Zhejiang Province Key R&D Program (No. 2019C02069, 2019C02019 and 2021C02001), the Zhejiang Provincial Academy Cooperative Forestry Science and Technology Project (2021SY11); the Selective Breeding of New Cultivars in Torreya grandis (2016C02052-12); the Zhejiang provincial ten thousand people plan (2019R52025).

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