Lipidomics

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Abbreviations:

NMR – nuclear magnetic resonance; MS – mass spectroscopy; CE – capillary electrophoresis; BUMA – butanol-methanol method; MTBE – methyl-tert-butyl-ether; EI – electron impact ionization; ESI – electrospray ionization; APCI – atmospheric pressure chemical ionization; APPI – atmospheric pressure photoionization; MALDI – matrix-assisted laser desorption/ionization; TOF – time of flight; PCA – principal components analysis; LDA – Linear discriminant analysis; PLS-DA – partial least squares discriminant analysis; TGs – triacylglycerols; LDL – Low-density lipoproteins; HDL – High-density lipoprotein

1. Introduction

Lipids are important biomolecules involved in many crucial cellular processes. Lipids are the major constituents of biological membranes and are implicated in extra and intracellular signaling processes, where they transduce signal and amplify regulatory cascade. From the chemical point of view, lipids are hydrophobic molecules and can be classified into eight categories: fatty acids; glycerolipids; glycerophospholipids; sphingolipids; sterols; prenol lipids; saccharolipids, and polyketides (Züllig, Trötzmüller, & Köfeler, 2020). In the LIPID MAPS database, there is information about 43636 different lipids divided into previous lipid classes and subclasses (Fahy et al., 2005; Züllig et al., 2020).

In the last decades, the interest in lipids research is increased due to the international recognition of their positive impact on human health through the approved health claims. To better understand the role of lipids in nutrition and health, lipidomics appears as an important tool (Luque de Castro & Quiles-Zafra, 2020).

1.1. Lipidomics: definitions and basic concepts

Lipidomics was initially defined as “the comprehensive analysis of all lipid components in a biological sample” (Luque de Castro & Quiles-Zafra, 2020; Smilowitz et al., 2013). Nowadays, lipidomics can be defined as “the discipline to obtain integral information of all lipids in a biological system concerning cellular signals, membrane architecture, transcriptional and translational modulation cell-cell and cell-protein interactions and response to environmental changes with time” (Luque de Castro & Quiles-Zafra, 2020). The analytical strategies used in lipidomics can be divided into two categories: targeted analysis and untargeted analysis. Targeted analysis requires previous knowledge of the lipids involved in the biological process under study. This is a quantitative or semi-quantitative analysis of a few lipids involved in a metabolic reaction. In this analysis, the most used analytical techniques are liquid or gas chromatography coupled to high resolution mass spectrometer (Luque de Castro & Quiles-Zafra, 2020). Regarding untargeted analysis, this is based on the qualitative or semi-quantitative analysis of many lipids from various chemical and biological classes. Due to the large range of lipids, combined analytical platforms

54 can be used such as nuclear magnetic resonance (NMR) or mass spectroscopy (MS) coupled to
55 liquid or gas chromatography, and capillary electrophoresis (CE). These studies include the
56 calculation of the relative concentration of lipids and their variation between system situations
57 (Luque de Castro & Quiles-Zafra, 2020). The most common workflow (figure 1) in lipidomics
58 includes sampling, sample preparation - lipid extraction, chromatographic analysis coupled mass
59 detector, data collections, and data analysis by chemometrics.

60

61 ## 2. Lipid extraction

62 Lipids can be extracted from numerous sources like plasma, tissues, cells, foods, and others. There
63 are many validated methods for this proposal: Folch method; Bligh-Dyer method; Butanol-
64 methanol (BUMA) method and methyl-tert-butyl ether method (Lam, Tian, & Shui, 2017;
65 Löfgren et al., 2012; Matyash, Liebisch, Kurzchalia, Shevchenko, & Schwudke, 2008; Ulmer,
66 2015; Z. Wu, Bagarolo, Thoröe-Boveleth, & Jankowski, 2020). Folch is the most used method
67 for lipid extraction from biological samples (Folch, Lees, & Sloane Stanley, 1987). It is
68 characterized by its high sensitivity for phospholipids and neutral lipids. In this extraction method,
69 the sample is mixed with chloroform and methanol (Folch solution). After repeated extractions,
70 Folch solution with water is used to remove non-lipid substances and lipidic compounds are
71 concentrated in the chloroform phase (Folch et al., 1987; Reis et al., 2013; Z. Wu et al., 2020).
72 Bligh-Dyer extraction is a modification of the Folch method, using a chloroform-methanol
73 mixture (designated Bligh-Dyer solution). This method has high recovery rates in comparison to
74 the original method (Bligh & Dyer, 1959; Breil, Abert Vian, Zemb, Kunz, & Chemat, 2017;
75 Patterson, Ducrocq, McDougall, Garrett, & Yost, 2015). Hara-Radin method uses lower toxicity
76 solvents than the previous methods, and the lipids are extracted with a mixture of
77 hexane/isopropanol (3:2 v/v), after a homogenization step. Then, the samples are filtrated and the
78 residues are washed with the same solvent solution (Hara & Radin, 1978). Sodium sulfate solution
79 is used to promote the phase separation, and the hexane lipid phase (upper phase) is collected for
80 further analysis (Hara & Radin, 1978). In the case of the BUME method, the sample is added to

81 a solvent mixture composed of butanol/methanol (3:1 v/v) followed by heptane/ethyl acetate (3:1
82 v/v) with acetic acid. After, the sample is dried under nitrogen stream and the lipid extraction is
83 performed by Folch or Bligh-Dyer methods (Löfgren, Forsberg, & Ståhlman, 2016; Löfgren et
84 al., 2012). Finally, in the MTBE method, lipids are extracted using MTBE and methanol. Water
85 is also added to promote the phase formation. MTBE has a lower density than water, therefore
86 the organic phase containing lipids is formed in the top phase, simplifying the extraction process
87 and increment the recovery rates (Matyash et al., 2008; Z. Wu et al., 2020).

88

89 3. Mass spectroscopy - an important tool in lipidomics

90 Mass spectroscopy is the most used analytical technique in metabolomics, particularly in
91 lipidomics, either for targeted or untargeted approaches. Basically, the lipid molecules are
92 ionized, transferred into the gas phase, and then accelerated as ions by an electric field to be
93 further separated according to their mass/charge ratios in the mass spectrometer (Z. Wu et al.,
94 2020). Mass spectrometers can be divided into four components: sample introduction system; ion
95 source; mass analyzer and mass detector (Z. Wu et al., 2020). Normally, in lipidomics, the most
96 used sample introduction systems are gas and liquid chromatography. The choice of the ion source
97 is depending on the chemistry of the lipids in the study. The available option includes electron
98 impact ionization (EI) or chemical ionization (CI), which are used combined with GC-MS (Lam
99 et al., 2017; Thibon et al., 2015). Electrospray ionization (ESI), atmospheric pressure chemical
100 ionization (APCI), and atmospheric pressure photoionization (APPI) are normally used combined
101 with liquid chromatography systems (Cai, Short, Syage, Potvin, & Curtis, 2007; Donot et al.,
102 2013; Koivusalo, Haimi, Heikinheimo, Kostiainen, & Somerharju, 2001; Lam et al., 2017;
103 Liigand et al., 2017). Finally, for solid samples matrix-assisted laser desorption/ionization
104 (MALDI) is the most common ion source (Lam et al., 2017; Murphy, Hankin, & Barkley, 2009).
105 Concerning the mass analyzer the actual preferable options are triple quadrupole; quadrupole-
106 time-of-flight; Q-linear Ion trap and LIT-Fourier transform ion cyclotron resonance (Bielawski,
107 Szulc, Hannun, & Bielawska, 2006; Del Boccio et al., 2012; He et al., 2007; Marinas et al., 2009;

108 J. Wang, Wang, & Han, 2019; B. Wu et al., 2021). Lastly, the mass detectors are used to display
109 the ions separated by mass analyzers as an electric signal, these detectors can be divided into three
110 generations: the first is based on Faraday cups. The second use photo plates to generate an
111 electrical signal. Currently, the third generation of mass detectors uses image current for detection
112 (Z. Wu et al., 2020). This modular instrumentation strategy promotes the combination of different
113 types of sample introduction systems, ion sources, analyzers, and detectors and offers a high
114 degree of freedom of instrumentation to accommodate the challenges of lipidomics. According to
115 these presumptions, five mass spectrometric methods are available for lipidomics: electrospray
116 ionization mass spectroscopy; MALDI mass spectroscopy, GC-MS (these three are the most
117 used), non-aqueous capillary electrophoresis mass spectroscopy, and MALDI image.

118 ### 3.1. Electrospray mass spectroscopy (ESI-MS)

119 ESI-MS is preferably combined with liquid chromatography systems. ESI-MS converts lipids
120 from the condensed phase into isolated ions in the gas phase at atmospheric pressure, whereby
121 the high vacuum of the mass analyzer is achieved. It is a soft ionization process, for this reason,
122 the chemical structure of lipids is rarely disrupted, which is an important advantage. This method
123 is equally suited for free fatty acids or triacylglycerols. Furthermore, due to its high ionization
124 efficiency can detect lipids in very low concentrations (Han et al., 2006; Z. Wu et al., 2020).

125 ### 3.2. Matrix-assisted laser desorption ionization mass spectroscopy (MALDI)

126 It is a soft ionization technique that uses a laser energy absorbing matrix to generate ions from
127 lipids with minimal fragmentation. The principal advantages of this technique are suitability for
128 solid samples, high sensitivity, easy sample handling, salt tolerance, speed, and the capacity to
129 analyze polar lipids with low solubility in organic solvents (Z. Wu et al., 2020). MALDI is
130 applicable to high hydrophobic and non-polar lipids as well as hydrophilic compounds such as
131 phospholipids. However, direct analysis of fatty acids is not possible (Z. Wu et al., 2020).

132 ### 3.3. GC-MS

133 GC-MS systems are frequently operated with quadrupole or TOF detector for high-resolution
134 mass spectroscopy. In this technique, the lipids of interest must be converted by derivatization
135 steps into a vaporizable state before GC-MS analysis. Commonly fatty acids are derivatized with
136 methanol to form fatty acids methyl esters (FAME). In the case of a complex sample, a
137 combination between two or more GC columns can be used to promote a clear lipids separation;
138 in this case the technique is designated GCxGC-MS, however, is an expensive technique (Mazur,
139 Zenkevich, Artaev, Polyakova, & Lebedev, 2018; Z. Yu et al., 2017).

140

141 **4. Data analysis**

142 Lipidomic mass spectroscopy approaches generate a large amount of data. Due to the vast type
143 of data generated, the lipidomic studies are intrinsically linked to the statistical methods
144 (chemometrics). The treatment of the lipidomic data can be carried out from the application of
145 univariate tests for comparison between two populations or applying multivariate tests for
146 exploration and classification of more than two types of populations (Checa, Bedia, & Jaumot,
147 2015).

148 4.1. Univariate analysis

149 Univariate tests are easily interpretable and a good option for preliminary screenings for potential
150 molecular features and quantitative changes in individual lipids. The classical workflow of
151 univariate tests begins a normality test to verify a standard distribution of the univariate parameter.
152 If data are normally distributed a t-test or ANOVA can be applied for means comparison between
153 two groups. However, in the case of not normally distributed data, non-parametric tests, like
154 Wilcoxon-Mann-Whitney and Kruskal-Wallis are used for univariate analysis (Checa et al., 2015;
155 Z. Wu et al., 2020). For univariate analysis and interpretation of the large lipidomic data sets,
156 open databases have been established, for example, LIPID MAPS, LipidBlast, METLIN,
157 LipidomeDB, KEGG, SOFA, and others (Chen et al., 2017; Fahy, Sud, Cotter, & Subramaniam,
158 2007; O'Donnell, Ekroos, Liebisch, & Wakelam, 2020; B. Wu et al., 2021).

4.2. Multivariate analysis

A multivariate approach can include for example a principal components analysis (PCA); linear discriminant analysis (LDA); cluster analysis and partial least squares discriminant analysis (PLS-DA). PCA is the most used multivariate method, it is based on the reduction of the data dimension, defining principal components, which retain the most relevant information from original data (Checa et al., 2015). In the case of mass spectroscopy data, the original multidimensional variables; retention time, m/z ratio, and mass signal intensities are redefined based on individual influence on the data differences as principal components explaining the maximum variation of the data (Z. Wu et al., 2020). LDA uses a data dimension reduction like PCA, aiming the projection of the data set into lower dimensional space based on a prior supervised group separation. Contrarily to PCA (unsupervised method), LDA requires previous knowledge of the data groups. LDA identifies the variables that maximize the separations between the groups instead of those that mostly explains the variance (Biancolillo & Marini, 2018; Checa et al., 2015; Z. Wu et al., 2020). Cluster analysis is used to characterize groups of data hierarchically or non-hierarchically to identify similarities. The hierarchical cluster analysis results in a dendrogram that reflects the relation of objects. Similar objects will be clustered in the same class, simultaneous be apart from other dissimilar objects (Z. Wu et al., 2020). Lastly, PLS-DA is an important tool when more variables than samples are considered. PLS-DA is a regression method, for this reason, PLS-DA has two data matrices, one corresponding to an experimental data (X) and the other corresponding to a vector or a matrix (Y) (Checa et al., 2015). The purpose of this method is to link X and Y using a small number of relevant factors, which represent the maximum covariance between X and Y. From PLS-DA models diverse information can be extracted, plot scores, and loadings generated to provide information similar to PCA (Checa et al., 2015). Moreover, PLS-DA includes information about the classes during the model building that can help the interpretation of the results. From the PLS weight vector, variables' importance can be calculated to facilitate the feature selection (Chong & Jun, 2005).

186 **5. Lipidomics applications**

187 Lipidomics is a relatively young research area, however, its applicability can be extended to many
188 research fields such as food science, clinical diagnosis, and environmental toxicology
189 (Aristizabal-Henao, Ahmadireskety, Griffin, Ferreira Da Silva, & Bowden, 2020; B. Wu et al.,
190 2021; Z. Wu et al., 2020). In this section, the principal application of lipidomics in food science
191 and clinical research will be summarized.

192 5.1. Applications in Food Science

193 In food research lipidomic can be applied in several fields, such as identification of food origin,
194 detection of food adulteration, and assessment of food safety and quality(B. Wu et al., 2021).
195 Food origin is considered an essential indicator of food quality by consumers. The information
196 regarding a food product normally includes the plant or animal strains; geographical origin;
197 planting or rearing method (for example farmed, organic), processing and storage conditions. For
198 food label validation, particularly in the case of suspected products, robust and effective analytical
199 methods are required, and in this field lipidomic strategies play an important role (B. Wu et al.,
200 2021). The identification of the species, variety, or cultivar present in a food product is the starting
201 point on food traceability. In this regard, targeted lipidomics focusing on several lipid classes or
202 minor lipids can be used for varieties identification. This strategy has been successfully applied
203 in edible oils, meat, marine foods, cereals, and grapes (Table 1) (Ferreira et al., 2019; Hammann,
204 Korf, Bull, Hayen, & Cramp, 2019; Ng et al., 2018; Pérez-Navarro et al., 2019; X. Yu, Chen, Li,
205 Wang, & Shen, 2019; Zhou et al., 2018). For example, a study published by Ferreira et al. (2019)
206 reported varied discrimination in coconut oil from 15 different varieties and commercial samples,
207 using ESI-MS and chemometrics (Ferreira et al., 2019). In this study, the authors concluded that
208 the triacylglycerols (TGs) are composed mainly of medium-chain fatty acids, but their content
209 varies between samples. Samples from tall cultivars presented the ion m/z 577 and m/z 605
210 (specific fragmentations of TGs with medium-chain fatty acids), and differences of m/z 605 and
211 m/z 633 were observed in the parents and hybrids (Ferreira et al., 2019). In a recent study, 155
212 agricultural food products belonging to 23 plant families were characterized using untargeted

213 lipidomics and 51 lipid subclasses were identified (Matsuzawa et al., 2021). Another example, in
214 a study published by Li and their co-author, UPLC-Q-Exactive Orbitrap MS, was used to identify
215 the lipid profile of different milk samples from different sources (Li et al., 2017). In this study 10
216 samples from goat milk, 10 samples from soymilk, and 10 samples from bovine milk were used,
217 and the lipids were extracted by Folch and Bligh and Dyer methods, and analyzed by UPLC-Q-
218 Exactive Orbitrap MS. The authors conclude that soymilk is rich in phospholipids, goat milk in
219 medium-chain fatty acids and omega-3 fatty acids, and bovine milk in ceramides, TGs, and
220 diglycerol. These parameters can be used as biomarkers for these milk types (Li et al., 2017).
221 Geographical origin is also an important criterion for consumers. In this area MS-based lipidomics
222 presents an important contribution, particularly, in the case of goat milk, saffron meat, and olive
223 oil (Hou et al., 2018; Liu, Guo, Zhao, Qin, & Zhang, 2020; Man et al., 2021; Mi et al., 2018;
224 Rubert, Lacina, Zachariasova, & Hajslova, 2016). In the case of olive oil, fatty acids and TGs
225 were characterized by targeted MS-based lipidomics for identification of the geographical origin.
226 For this propose the most common chemometrics tools used were PCA or PLS-DA (Peršurić,
227 Saftić, Mašek, & Kraljević Pavelić, 2018). In a study published by Liu et al, 50 goat milk samples
228 in different lactation stages and from different China regions were analyzed by UPLC-MS, to
229 discriminate the geographical origin and lactation phase. Four lipids markers were achieved for
230 this discrimination, ceramides, glycerophosphocholine, phosphatidylinositol, and sphingomyelin
231 (Liu et al., 2020). Linked to the geographical origin determination it is also possible to establish
232 the identification of possible food adulterations. It is a serious problem in many countries, and the
233 most common practices include the substitution by low-grade but similar ingredients or the
234 addiction of exogenous material to mask inferior quality. One of the most known food
235 adulterations is the addiction of refined olive oil in extra virgin olive oil. MS-based lipidomics is
236 a good strategy to discriminate the olive oil categories. In this case, phospholipid molecules could
237 be considered as a promising marker to detect olive oil adulterations (Criado-Navarro, Mena-
238 Bravo, Calderón-Santiago, & Priego-Capote, 2019). This strategy can be applied to identify
239 frauds in other food products, for example, in a study published by Righetti et al. untargeted
240 lipidomics using UPLCQ-TOF-MS are used to discriminate the common wheat and durum wheat,

241 after unsupervised and supervised statistical tools applications (Righetti et al., 2018). Lipids are
242 unstable components in many foodstuffs, whose changes are a major cause of deterioration. For
243 example, the final products of lipid oxidation are one of the main causes of the deterioration,
244 moreover, these compounds are also linked to many diseases. Lipidomic MS methods, can be
245 used to quantify the amount of these compounds present in foods. Song and their colleagues create
246 a method using LC-MS/MS for quantification of 9,10-epoxyoctadec-12-enoic acid and 9,10-
247 dihydroxy-12-octadecenoic acid in meat products (Song et al., 2016). This method is based on a
248 methanol extraction without a derivatization process. Before LC-MS/MS a clean-up step was
249 performed with C18 SPE cartridges. In MS/MS-specific fragments are monitored (m/z 277.1 and
250 m/z 201.3) (Song et al., 2016).

251 5.2. Lipidomics in clinical research

252 Lipids are involved in the genesis and progression of many diseases. Lipidomics has a key
253 function for monitoring metabolic imbalances in various diseases (Table 2). In clinical research,
254 the most well-known lipids are LDL, HDL and, triglycerides used as markers of cardiovascular
255 risk, and lipidomic studies have shown that cardiovascular diseases are associated with only a
256 small fraction of the lipid-derived risk factors. The use of lipidomics can, therefore, help to
257 redefine cardiovascular risk for patients, complement the lipid plasma screening panels currently
258 used, as well as improve predictive models (Kolovou, Kolovou, & Mavrogeni, 2015). For
259 example, the presence of oxidized phospholipids on apo-B100 lipoproteins predicts
260 cardiovascular problems, additionally, the presence of abnormal levels of oxylipins are linked to
261 cardiovascular and cerebrovascular events (Solati & Ravandi, 2019). Another study with 900
262 adolescents identified novel glycerophosphocholines (namely PC16:0/2:0 and PC14:1/0:0) in
263 serum using LC-ESI-MS, these compounds are strongly associated with the risk of cardiovascular
264 problems in young people (Syme et al., 2016). A non-targeted lipidomic profile of 1028
265 individuals identified lysophosphatidylcholines 18:1, 18:2, monoglyceride 18:2, and
266 sphingomyelin 28:1 as risk factors of coronary heart disease (Ganna et al., 2014).

267 Type 2 diabetes is a complex metabolic disorder associated with insulin resistance and β-cell
268 dysfunctions. Lipidomic studies conducted in this area showed that this pathology is associated
269 with the increased levels of acetylcarnitine, free fatty acids, TGs, diglycerols, and cholesteryl
270 esters, and a decrease in unsaturated phosphatidylcholines (Nie, Xing, Chen, Hu, & Nie, 2019).

271 In clinical research, another key topic is the discovery of obesity-related biomarkers, as it is
272 known obesity is a problem at a global level. In this case, human and animal models have been
273 used coupled with lipidomics to achieve possible biomarkers. MALDI-TOF MS is the preferable
274 analytical platform to establish the lipid profile of different samples, such as liver, brain, and
275 adipose tissues. The determination of the differences in the lipid composition induced by a high-
276 fat diet, depending on the location of the fat, can reveal certain patterns related to the accumulation
277 of lipids not described until now. The adipose tissue analysis by mass spectroscopy reveals a
278 strong impact of high-fat diets on the lipid composition of adipose tissue (Ibáñez, Mouhid,
279 Reglero, & Ramírez De Molina, 2017). In the case of liver samples, lipidomics shows an increase
280 in fatty acids availability. This fact can lead to the increased esterification into TGs, which may
281 result from the increased influx of plasma-free fatty acids and increased *de novo* free fatty acids,
282 and decreased β-oxidation (Yetukuri et al., 2007). Another study shown upregulated levels of
283 short and medium-chain triglycerides and ceramides in liver tissues of obese mice, using
284 UPLC/MS as an analytical strategy (Yetukuri et al., 2007). Moreover, a study with high-fat diet-
285 induced obese mice demonstrates that sphingomyelins, glycerophospholipids, and cholesteryl
286 ester species are altered in obese mice (Eisinger et al., 2014). In this study, phosphatidylinositol
287 34:1 strongly correlates with fasting glucose and proinsulin levels. Phosphatidylcholines 26:0,
288 40:2, and 40:5, which are induced in obesity, correlate with cholesterol. PC 38:4 and PC 40:6 are
289 positively correlated with fasting glucose (Eisinger et al., 2014). A clinical study with 50 obese
290 boys, shown a significant difference in the plasma lysophosphatidylcholines profiles between
291 obese and normal-weight adolescents (Y. Wang et al., 2019). These lysophosphatidylcholines
292 belong to the unsaturated classes (Y. Wang et al., 2019).

293 Lately, in cancer research, for example, a lipidomic study identified many different phospholipid
294 species that are differentially expressed in non-small cell lung carcinomas. In this study, the
295 authors verify a decrease in sphingomyelins levels, and an increase in specific
296 phosphatidylinositols (Marien et al., 2015). Another study demonstrates that occurs a reduction
297 in lipoprotein cholesterol and apolipoprotein A1 and B in patients with colorectal cancer (Yang
298 et al., 2013). In the same study, the authors verified that the level of free cholesterol is high in
299 these patients (Yang et al., 2013). Lipidomic are also used for the identification of lipidic profile
300 differences between kidney tumors and surrounding normal tissue. In this study, the results show
301 a significant difference between total concentrations of phosphatidylethanolamines,
302 sphingomyelins, and lysophosphatidylcholines classes, which are decreased in tumor samples
303 (Cífková et al., 2015). Furthermore, palmitic acyl is associated with the proliferation and
304 arachidonic acyl with inflammatory processes in the organism, and these effects are associated
305 with the tumor progression (Cífková et al., 2015). In this field, lipidomic studies can be a valuable
306 tool in the diagnosis and the development of novel therapies (Z. Wu et al., 2020).

307

308 ## 6. Conclusion

309 Lipids have an important role in many biological processes and are an essential nutrient impacting
310 human health. Moreover, lipids can be used as a biomarker for food quality and food processing
311 control. In this regard, lipidomics appears as an important tool, in many research fields. In the
312 case of food science, lipidomics has high importance, particularly for food traceability, which can
313 help in food origin determination and fraud detection. In clinical research, lipidomics is applied
314 as a good diagnostic complementary tool. In fact, many years ago lipids were used as clinical
315 biomarkers for cardiovascular diseases, and recently this applicability is enlarged for other
316 pathologies.

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323

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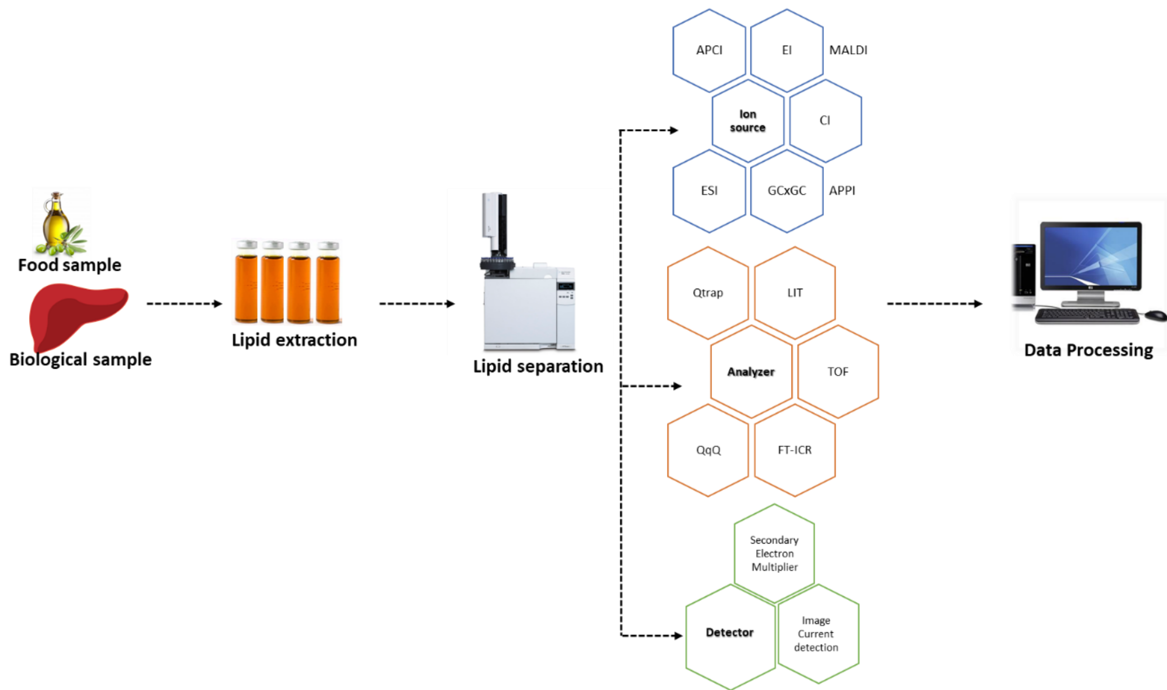
574 Figure 1- Lipidomics workflow



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578 Tables

579 Table 1 – Examples of applications of lipidomics in food science.

Food matrix	Samples Preparation	MS Analytical Approach	Chemometric Tool	Reference
Coconut oil	Aqueous extraction; direct dilution	HR-ESI-MS	PCA, ICA	(Ferreira et al., 2019)
Flowers/fruits/seeds/stems and leaves	MTBE/methanol 3:1 v/v	LC-MS QTOF	PCA	(Matsuzawa et al., 2021)
Milk	Folch; Bligh and Dyer methods	UPLC-Q-extractive orbitrap MS	PLS-DA	(Li et al., 2017)
Olive oil	Direct dilution; esterification	MALDI-TOF	PCA; PLS; PLS-DA	(Peršurić et al., 2018)
Milk	Folch method	UPLC- Q-extractive orbitrap MS	PLS-DA LDA	(L. Wang et al., 2020)
Wheat	Methanol/dichloromethane 50:50 v/v	UHPLC-HRMS	PCA; OPLS-DA	(Righetti et al., 2016, 2018)
Meat	Methanol and SPE (C18)	HPLC MS/MS QqQ		(Song et al., 2016)
Olive oil/flaxseed oil/grapeseed oil/margarine/ pineapple	Acetone/BHA 99.995:0.005 v/w	UHPLC-ESI-QqQ-MS/MS	ANOVA; Tukyes test	(Medina et al., 2020)
Saffron	Ethanol/water 70:30 v/v	UPLC-HRMS-QTOF	PCA; OPLS-DA	(Rubert et al., 2016)
Beef	Bligh and Dyer method	UPLC-orbitrap-MS	PCA; PLS-DA; OPLS-DA	(Man et al., 2021)

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583 Table 2- Examples of applications of lipidomics in clinical research

Disease	Type of Sample	MS Analytical Approach	Chemometric Tool	Reference
Cardiovascular disease	Serum	LC-ESI/MS	Linear mixed regression	(Syme et al., 2016)
Coronary heart disease	Serum	UPLC-Q-TOF-MS	Mendelian randomized analysis	(Ganna et al., 2014)
Obesity	Liver tissue	UPLC triple-TOF-MS	PCA; PLS/DA	(Yetukuri et al., 2007)
Obesity	Serum	UPLC/MS	Mann Whitney U test	(Eisinger et al., 2014)
Obesity	Plasma	LC-ESI-MS/MS	Mann Whitney U test; PLS-DA	(Y. Wang et al., 2019)
Lung cancer	Tissue	ESI MS/MS	Mann Whitney U test; Benjamini Hochberg false discovery rate; PCA; LDA	(Marien et al., 2015)
Kidney cancer	Tissue	HILIC-HPLC/ESI-MS	PCA; OPLS	(Cífková et al., 2015)

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