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Review

The novelty of phytofurans, isofurans, dihomom-isofurans and neurofurans: Discovery, synthesis and potential application

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ABSTRACT

Polyunsaturated fatty acids (PUFA) are oxidized *in vivo* under oxidative stress through free radical pathway and release cyclic oxygenated metabolites, which are commonly classified as isoprostanes and isofurans. The discovery of isoprostanes goes back twenty-five years compared to fifteen years for isofurans, and great many are discovered. The biosynthesis, the nomenclature, the chemical synthesis of furanoids from α -linolenic acid (ALA, C18:3 n-3), arachidonic acid (AA, C20:4 n-6), adrenic acid (AdA, 22:4 n-6) and docosahexaenoic acid (DHA, 22:6 n-3) as well as their identification and implication in biological systems are highlighted in this review.

Contents

1. Introduction	49
2. Biosynthesis	50
3. Nomenclature	51
4. Total synthesis of furanoids in the literature	51
4.1. Total syntheses of IsoFs developed by the group of Taber	51
4.2. Total synthesis of NeuroF by the group of Zanoni	52
4.3. Total syntheses of dihomom-IsoFs, NeuroF and PhytoFs by Galano and Oger	52
5. Identification and implication of isofuranoids in biological systems	53
5.1. Preparation for measurements	53
5.1.1. Mammalian samples	53
5.1.2. In plant samples	54
5.2. Mass spectrometry measurement	54
5.2.1. Implications of isofuranoids	55
6. Conclusion	60
References	60

1. Introduction

Reactive oxygen species (ROS) are free radicals generated under physiological conditions, but also under the so-called oxidative

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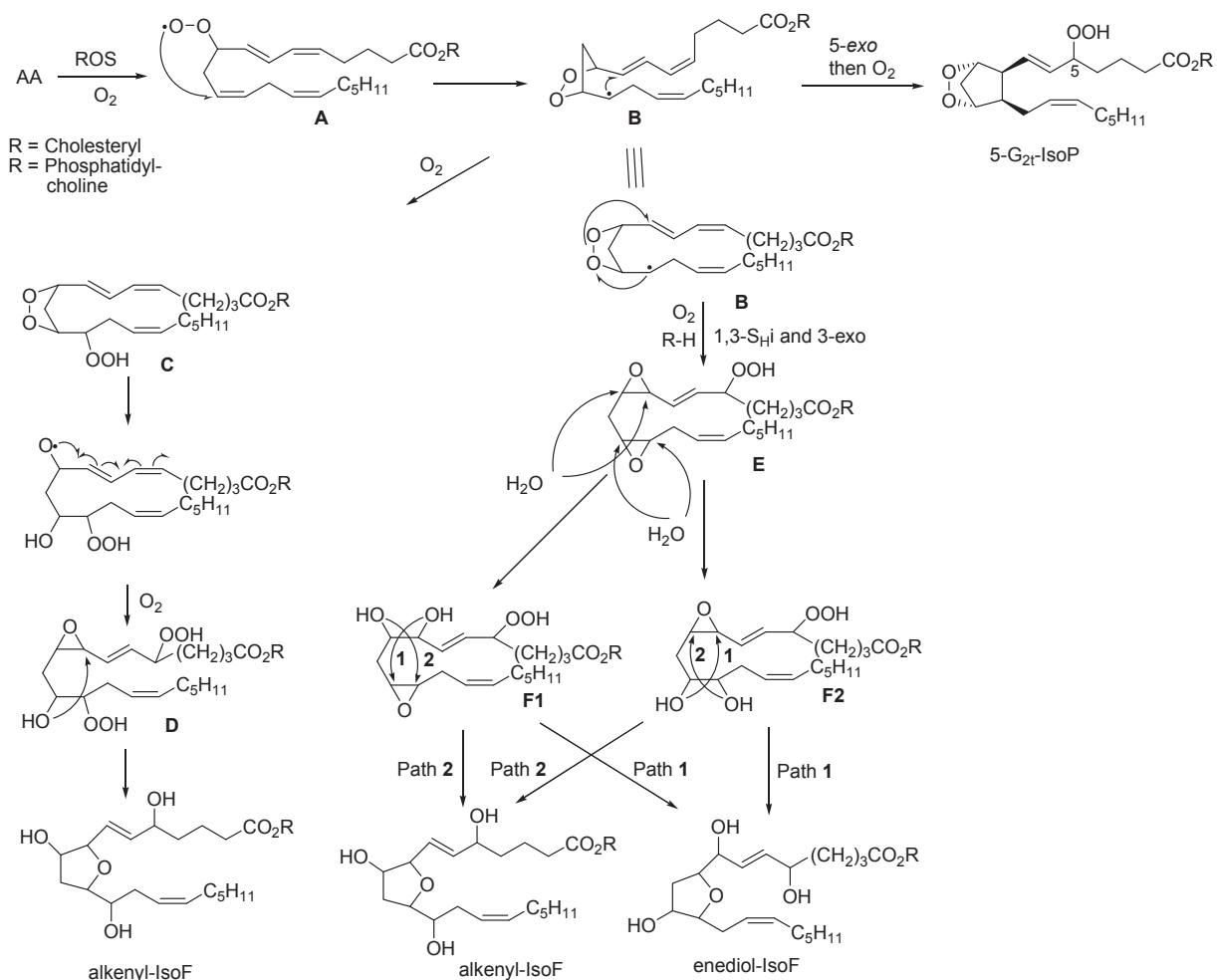
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stress condition (OS) when natural antioxidant defences are overwhelmed by the amount of ROS generated. ROS target oxidation prone polyunsaturated fatty acids (PUFA) to undergo lipid peroxidation (LPO) [1]. The hydroxyl radical ($\cdot\text{OH}$) is believed to be the most reactive radical *in vivo*, however other radicals can also participate in the processes of LPO [2]. However, no contentious exists if ROS can be specific of certain lipid and therefore of certain type of oxygenated metabolites. Furthermore, it is also believed that the non-enzymatic free radical mechanism of LPO that occurs in three phases (initiation, propagation, termination) could be more subtle than anticipated [3,4]. OS and LPO have been clearly associated to several pathologies and diseases, and one particular biomarker of OS was discovered in the nineties and called F_2 -isoprostanes (F_2 -IsoPs). They are generated from autooxidation of arachidonic acid bounded phospholipid (AA, $\text{C}_{20}:4$ n-6) and are circulating in biological fluids as free form mainly. Today they are currently considered as the best marker of OS in biological systems. In the biosynthesis route of IsoPs, an endoperoxide carbon radical (B) undergoes a 5-*exo*-trig cyclization to form the cyclopentane ring of IsoPs [5,6] (Scheme 1). However, at high oxygen tension further transformations can occur and oxygen molecule may react with the endoperoxide carbon radical to generated tetrahydrofuran ring containing compounds. Those new metabolites were termed isofurans (IsoFs). This second route may prevent the IsoPs pathway and limit their formation [7]. It is thus reasonable to measure IsoFs in addition to IsoPs in the case of high oxygen tension injury e.g.

hyperoxia, ischemia-reperfusion etc. Depending on the parent PUFA that at least would possess two skipped diene units (i.e; three alkenes separated by a CH_2), other furanoid metabolites can be formed and were discovered in human tissues or fluids e.g. neurofurans (NeuroFs) from docosahexaenoic acid (DHA, $22:6$ n-3), dihomom-isofurans (dihomo-IsoFs) from adrenic acid (AdA) and in plants like the phytofurans (PhytoFs) from α -linolenic acid (ALA, $\text{C}_{18}:3$ n-3). Those metabolites should be added to the list of metabolites to quantitate OS. The present review aims to provide a concise overview on these furanic PUFA metabolites, from how they are formed *in vivo* and how they are named. We also would like to highlight that these metabolites are not commercially available and need to be prepared by organic chemists before being evaluated. Accordingly, the syntheses developed until now will be presented. Finally, the last part will be focused on the identification of those metabolites and their implication in biological systems. More importantly, this review will also give keys to the researchers in the preparation of the samples and the measurements of such metabolites.

2. Biosynthesis

In 1990, Morrow and co-workers revealed the formation of IsoPs from arachidonic acid esterified as phospholipid, and explained their formation via a radical initiated mechanism [5]. Hydrogen abstraction of the bis-allylic hydrogen atom of AA ester lead to



Scheme 1. Mechanistic explanation for the formation of IsoPs and IsoFs (only one isomer of one series is shown).

isomeric pentadienyl radicals which are oxygenated into penta-dienylperoxyl radicals (**A**) (Scheme 1). Peroxyl radicals **A** can also undergo kinetically controlled 5-*exo* cyclization with suitably positioned alkene units to generate the endoperoxide intermediate **B** (dioxolanylcarbiny radical). Irreversible carbon-centered radical intramolecular attack onto a double bond generates the cyclopentane ring with the appropriate configuration (*cis*) characteristic of the IsoPs.

However, another fate of dioxolanylcarbiny radical **B** was revealed by Fessel and coworkers in 2002. It relies on the diradical character of O₂ and its high reactivity toward carbon-centered radicals, and permits to access IsoFs [7]. Interestingly, early *in vitro* experiment revealed the importance of oxygen concentration on the preferential generation of IsoFs versus IsoPs, and while the IsoPs concentration reached a plateau at 21% oxygen, the concentration of IsoFs increased further as the oxygen tension increased. This observation also proved to be true for organs of known different oxygen tension like liver versus lung.

The mechanism of formation was closely investigated and so far three biosynthetic pathways are proposed for the formation of IsoFs. From those and by competitive pathways, two types of IsoFs are synthesized, namely the alkenyl-IsoFs and the enediol-IsoFs, each of them present as a mixture of five regioisomeris and diastereomers. As shown in Scheme 1, we represented two out of the three biosynthetic pathways which all agree with the *in vitro* isotopic labelling experiments [6].

It is believed that these pathways diverge from dioxolanylcarbiny radical **B**. In the first one, oxygen trapping can lead to hydroperoxy-1,2-dioxolane **C** (Scheme 1, bottom left), which undergoes after single electron reduction of the peroxide bridge, 3-*exo* cyclization and oxygen trapping, the dihydroperoxy epoxide **D**. Further regioselective intramolecular nucleophilic ring opening of the epoxide by the hydroxyl group and final reduction of the hydroperoxide gives rise to the four regioisomeric alkenyl isofuran classes as mixture of diastereomers.

Another route (Scheme 1, bottom right) is also possible directly from dioxolanylcarbiny radical **B** after a facile 1,3-S_HI reaction, 3-*exo* cyclization and oxygenation to lead diepoxyhydroperoxide **E**. After hydrolysis to form the regioisomeric epoxy diols **F1** and **F2**, intramolecular nucleophilic ring opening of the epoxide by a suitably positioned hydroxy group leads to alkenyl-IsoFs according to path 1 and to enediol-IsoFs by path 2.

After the initially discovered isofuranoid derived AA, analogous compounds from other PUFAs were discovered like the NeuroFs in 2008 by FitzGerald [8] from DHA, as depicted in Table 1. Later, the combined groups of Galano/Durand and Lee discovered the dihom-IsoFs derived from AdA in pig brain [9]. Recently, this joint collaborative effort also identified the PhytoFs from ALA identified in seed and nuts [10]. It is obvious that other isofuranoids are probably generated from other PUFA and are still to be discovered.

3. Nomenclature

Biosynthetically related to IsoPs and containing a furan ring, those metabolites were naturally labeled as IsoFs. As depicted in the biosynthesis part, IsoFs are produced by three different

pathways, and lead to the formation of two classes of IsoFs, called alkenyl and enediol. There are thus eight different isofuran regioisomers (four alkenyl and four enediol), which can exist as 16 diastereomers, for a total of 256 enantiomerically-pure metabolites. A systematic nomenclature allows the differentiation of each isomeric structures and established by Taber, Fessel and Roberts [11]. This nomenclature is based on the relative orientation of the side chains and substituents. Furthermore, this nomenclature system was validated by IUPAC.

Several rules must be followed for the generation of a name from a structure (Fig. 1). First, it is noted that: the carbon number one is the carbon atom of the acid function (COOH) and the first ring carbon atom as well as the carbon bearing the hydroxyl groups (OH) on the side chain have, by default, (*S*) configuration. Secondly, the relative orientation of the alkyl chains must be defined: *syn* (*S*) or *anti* (*A*), prior to the relative orientation of the ring hydroxyl group and the adjacent alkyl chain: *cis* (*C*) or *trans* (*T*). Then, the carbon number of the *E* double bond should be addressed as a superscript after the Δ sign, followed by the carbon number of the first ring carbon atom. The abbreviation IsoF, for isofuran, is appended thereafter and reveals the parent PUFA: IsoF (AA and EPA), PhytoF (ALA), dihom-IsoF (AdA) and NeuroF (DHA).

Great care should be paid to the configuration of the first ring carbon atom and the carbon atoms bearing hydroxyl groups on the side chain (*S* by default). If the configuration of the hydroxyl is inverted, the carbon number followed by “*epi*” is used as a prefix. If the first ring carbon atom configuration is reversed, the prefix “*ent*” is used and all the hydroxyl groups on the side chains are considered (*R*) as default.

4. Total synthesis of furanoids in the literature

The total synthesis of furanoid PUFA metabolites is of great interest because little is known about their biochemistry. Thus, four synthetic strategies were developed by organic chemists. The first one was described by Taber et al. in 2004 through a diol epoxide benzenesulfonate cyclization and permits the synthesis of AA derived IsoFs both alkenyl and enediol type [12–14]. More recently, Zanoni's group used Trost asymmetric alkylation for the first synthesis of a neurofuran [15]. Finally, the Galano and Oger group developed in parallel two strategies toward furanoid derivatives based on Borhan cyclization or a one-pot Payne rearrangement/5-*exo*-tet cyclisation, giving access to enediol and alkenyl furanoids derived from ALA, AdA and DHA [9,10,16]. So far, none of the isofuranoids synthesized are commercially available but can be available from the Zanoni and Galano/Oger/Durand teams (isofuranoids from Taber were kindly given to the Montpellier group).

4.1. Total syntheses of IsoFs developed by the group of Taber

The first strategy developed by Taber in 2004 gave access to the alkenyl furans and relied on an epoxide intermediate **1** where the lateral chains are introduced later on the synthesis (Scheme 2A) [12].

The epoxide moiety **1** comes from an epoxide opening cyclization where the benzenesulfonate unit is substituted (compound **2**).

Table 1
Summary of the number of oxygenated furan metabolites of PUFAs generated.

PUFA	Metabolite abbreviation	Number of lateral chain structures	Number of regioisomers	Number of isomers
AA	IsoF	4	8	256
AdA	Dihomo-IsoF	4	8	256
ALA	PhytoF	4	4	128
DHA	NeuroF	8	16	512

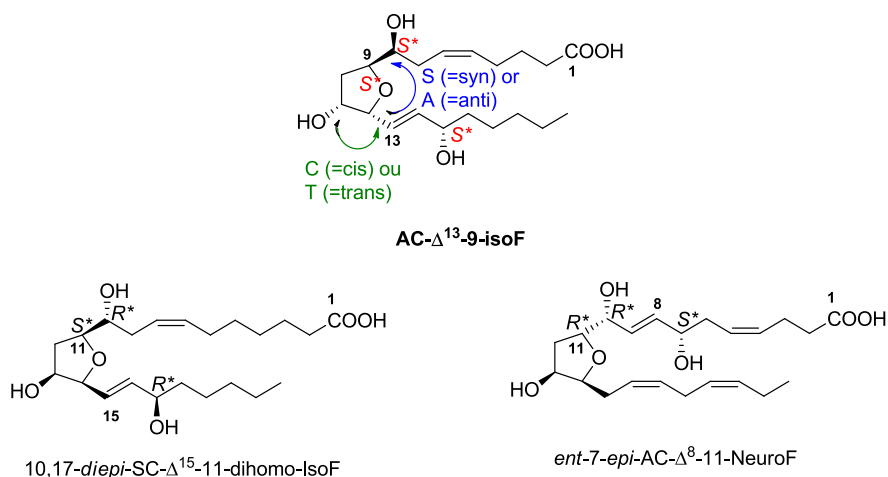
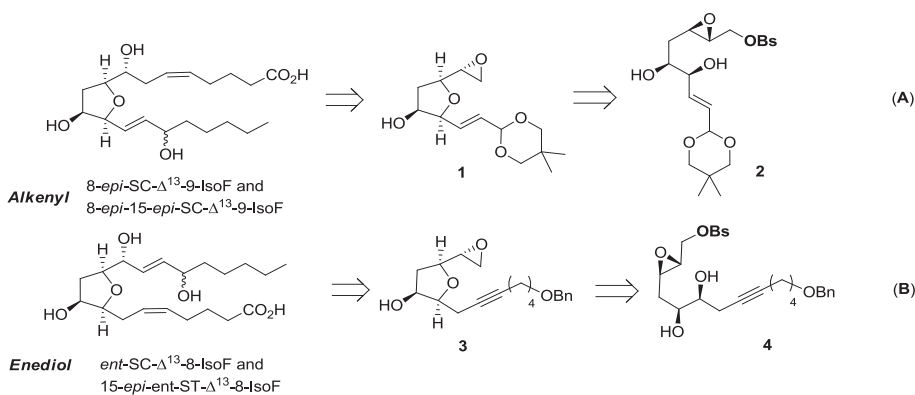


Fig. 1. Nomenclature of the furan derived from PUFA.



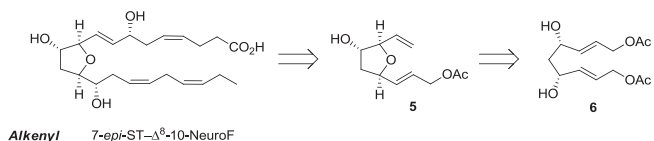
Scheme 2. Taber synthetic strategies.

Thanks to Sharpless epoxidation and dihydroxylation reactions, all stereogenic centers are controlled and may be changed by a simple modification of the chiral ligand. Moreover, even if the SC structure is obtained herein, a Mitsunobu reaction on epoxide **2** may allow the change of configuration of the hydroxyl group on the furan and thus the synthesis of ST structures. This strategy permitted the synthesis of the 8-*epi*-SC- Δ^{13} -9-IsoF and its epimers on the 15th carbon atom, in 16 steps and 0.8% global yield.

The same cyclization key step was used for the synthesis of enediol structures. However, the cyclization substrate had to be slightly modified to get the *ent*-SC- Δ^{13} -8-IsoF and its epimer on the 15th carbon (**Scheme 2B**) in 21 steps and in 1.7% global yield [13].

4.2. Total synthesis of NeuroF by the group of Zanoni

The discovery of the NeuroFs in 2008 led by Zanoni group to develop a new strategy for the synthesis of furanoids by making the first total synthesis of one NeuroF, named 7-*epi*-ST- Δ^8 -10-neurofuran (**Scheme 3**) [15].



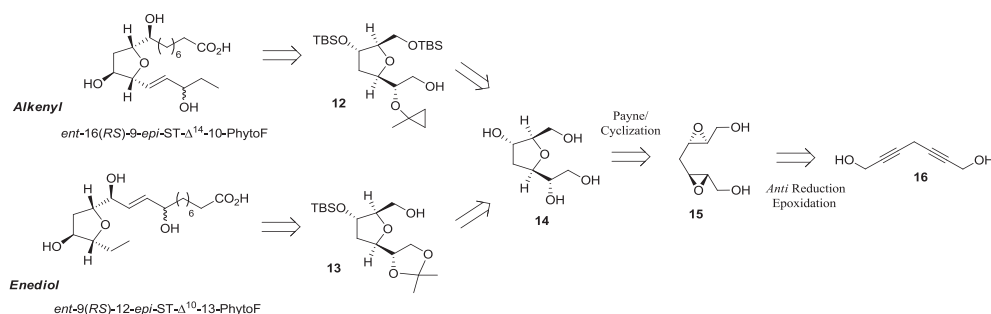
Scheme 3. Zanoni synthetic strategy.

The key step of the strategy is a diastereo- and enantioselective cyclization using Tsuji-Trost alkylation, starting from diene-diol **6**, to obtain the furan intermediate **5** in good yield (86%) and good enantiomeric excess (96%). This intermediate **5** allowed the insertion of the lateral chains by Julia-Kocienski coupling and Wittig reaction, to access to the 7-*epi*-ST- Δ^8 -10-neurofuran in 18 steps and 1.6% global yield.

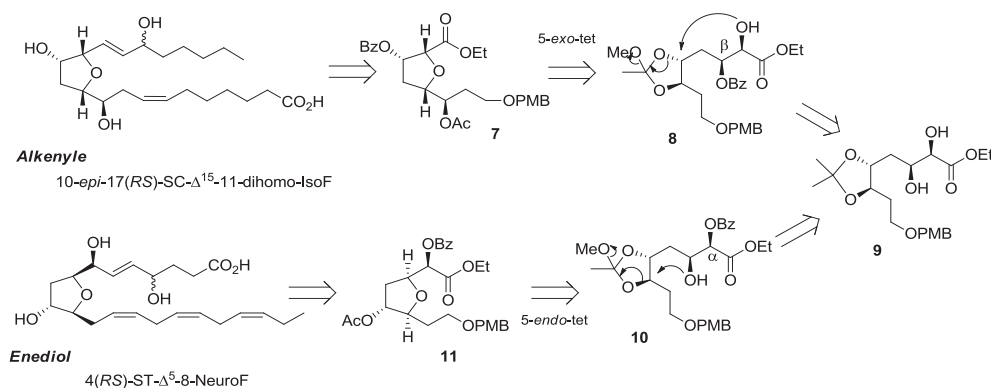
4.3. Total syntheses of dihomo-IsoFs, NeuroF and PhytoFs by Galano and Oger

The recent advances in the field of non-enzymatic lipid metabolites prompted our group to develop synthetic strategies to access all PUFA furan metabolites, even those not yet discovered at that time, such as PhytoFs derived from ALA or dihomo-IsoFs derived from AdA. Thus, two strategies were developed in parallel to access those metabolites. In the first strategy the key step is an intramolecular cyclization of a hydroxyl onto an orthoester (**Scheme 4**) [9] whereas a Payne rearrangement followed by a basic mediated intramolecular 5-*exo*-tet cyclization is used in the second one (**Scheme 5**) [10].

The strategy goes through intermediate **9**, common to the alkenyl and enediol classes. The regioselective monoprotection of the hydroxyl group in α (compound **10**) or β (compound **8**) of the ester **9** allows either a 5-*exo*-tet cyclization (compound **7**) or a 5-*endo*-tet cyclization (compound **11**). Then, the lateral chains are introduced by Wittig or Horner-Wadsworth-Emmons reactions,



Scheme 4. Galano-Oger first strategy.



Scheme 5. Galano-Oger second strategy.

leading to the enediol or alkenyl furanoids.

This strategy permitted the first syntheses of dihom-IsoFs derived from Ada: the 10-epi-17(RS)-SC- Δ^{15} -11-dihomo-IsoF and the 7(RS)-ST- Δ^8 -11-dihomo-IsoF, as well as the first enediol-NeuroF, the 4(RS)-ST- Δ^5 -8-NeuroF, in 21–23 steps and 1.5–2% global yield [9,16].

The second strategy allows the acquisition of both alkenyl and enediol furanoids through common intermediate **14**. The bis-epoxide **15** is easily obtained from diyne-diol **16** by a *trans*-reduction of the diyne followed by a Sharpless bis-epoxidation. Thus, subjected to basic conditions (KOH, 80 °C), the bis-epoxide leads to a Payne rearrangement followed by an epoxide opening and a 5-*exo*-tet cyclization (compound **14**).

The orthogonal protections of the hydroxyl moieties and regioselective deprotection lead to two intermediates (compounds **12** and **13**), needed for the lateral chains insertion by Wittig or Horner-Wadsworth-Emmons reactions.

This strategy permitted the first syntheses of PhytoFs derived from ALA: the ent-16(RS)-9-epi-ST- Δ^{14} -10-PhytoF, the ent-16(RS)-13-epi-ST- Δ^{14} -9-PhytoF and the ent-9(RS)-12-epi-ST- Δ^{10} -13-PhytoF, in 20 steps and 1% global yield [10].

5. Identification and implication of isofuranoids in biological systems

The synthesis of these novel compounds allows us to expand their measurement in biological samples. To date, the most robust measurement of the isofuranoids is by gas-chromatography-mass spectrometry (GC-MS) or liquid chromatography tandem mass spectrometry (LC-MS/MS). These instruments rely on the mass-to-charge ratio (*m/z*) and additional transition ion in the case for LC-MS/MS. The disadvantage of GC-MS is the requirement of a derivatizing process and inability to separate isomeric compounds with

same molecular weight. Nevertheless, it should be noted the sensitivity of isofuranoids determination also depends on the preparation of the samples. In the literature, not all methods of isofuranoids and other oxidized lipid product measurement are in alignment between researchers; this depend on how lipid portion of the samples are extracted prior to purification by thin layer chromatography (TLC) or solid phase extraction (SPE) for example which are well reported [17–19].

5.1. Preparation for measurements

5.1.1. Mammalian samples

The detection and quantification of isofuranoids from tissues and/or cells mandatorily require the initial step of sample extraction. To avoid and minimize potential *ex vivo* generation of lipid metabolites via either enzymatic or non-enzymatic pathways, a small amount of BHT and indomethacin are commonly added into the sample before any homogenization procedures [18,20]. Accurate quantities of specimens are often weighed in a highly precise balanced weighing machine, usually based on wet weight. Alternatively, in the case of sampling from cell culture system, the estimated number of cells is determined using various cell counting methods before adding in cell lysis buffer and lipid extracting solvent. It is important to note that having a fully homogenized sample is one of the critical steps in quantifying isofuranoids accurately, as the received shearing force are often closely related to the amount of cellular components being released during the process.

Homogenization of samples is usually performed under physical disruption of tissues with the use of sharp blades or small silica beads and even ultrasound [21]. While these homogenization methods are well recognized, blade homogenizer is widely adopted possibly due to its lower cost and maintenance. The heat generated

during homogenization is one of the main concerns by general users. Although the rotor-stator homogenizer is efficient in producing high shearing force upon the sample, large amount of heat is also generated from the fast turning rotor and transferred to the homogenizing sample through the attaching blade. Exposing samples to high levels of heat during preparation might contribute to the formation of artefacts, or *ex vivo* generation of molecules, which ultimately could lead to false positive-negative results. To reduce the amount of heat retained in the sample, one generally uses ice-cold solvents during homogenization as well as cooling the outer surface of the sample tube with ice-cold water or ice itself. In addition, it is encouraged to wash or blend-pass through homogenizing blade with methanol and MilliQ water in between samples, even though they might be from the same treatment group. This additional step prevents cross contamination and maximizes the confidence of gaining highly accurate quantitation in the later stage.

Isofuranoids extraction from different types of tissues using this rotor-stator homogenizer however, is not always reproducible. The extraction of total lipids from tissues is generally based on two established methods, i.e. Folch and Bligh/Dyer. These two methods commonly utilized organic solvents (Folch solution: chloroform:methanol, 2:1; Bligh/Dyer: chloroform:methanol, 1:2) for their lipid extraction [22,23]. Mammalian tissues, e.g. kidney and liver, tend to be hardened when directly exposed to organic solvents, increasing the difficulty in successive homogenization. Other tissue types, like the brain, are comparatively softer even in the presence of organic solvents, but brain homogenates tend to stick to the homogenizing blade, inducing great loss if they were not recovered. Hence, multiple washes of the homogenizing blade with extracting solution are needed and should then be pooled together to maximize the tissue recovery. To overcome the structural changes of tissues in organic solvents, some co-workers [24,25] have successively extracted total lipids by homogenizing the tissues first in PBS, and followed by adding appropriate organic solvents. Alternatively, some other researchers have reported to extract lipids by first grinding freshly harvested tissues into powder form in the presence of liquid nitrogen, and subsequently weigh accurate portion for lipid extraction using organic solvents [26,27]. These two alternative methods provided a detour to total lipid extraction by organic solvents from tissues after they were fully disrupted.

Depending on the target species (e.g. isofuranoids) that are of interest in the study, extracted total lipids from specimens are further processed before quantitative analysis. For example, when determining the levels of isofuranoids, which are mostly esterified as phospholipids, those are usually liberated by adding high molar alkaline in organic solvent, e.g. 1 M KOH in methanol. These are then purified from polar lipid species via TLC or SPE columns. Finally, the extracts are subjected to qualitative and quantitative analysis commonly via GC-MS or LC-MS/MS. An additional step of derivatization is necessary when using GC-MS [28,29]. Isofuranoids have not been investigated in urine samples, but like for IsoPs they are probably excreted as free forms and conjugated forms (glucuronidates). A validated method for IsoPs was described by Medina et al. [30].

5.1.2. *In plant samples*

Phytofurans (PhytoFs) are newly found oxygenated metabolites produced by non-enzymatic peroxidation of α -linolenic acid (ALA) in plants. It was found that PhytoFs are detected in nuts and seeds by LC-MS/MS recently [10]. It is essential for us to extract lipids from the samples properly in order to measure PhytoFs accurately. Three different methods, Soxhlet extraction and solvent extraction have been commonly used by researchers to extract lipids from the

nuts and seeds. It is noted that no matter which method is used for lipid extraction, ground dry nuts and seeds samples should be used since ground samples have a higher surface area which gives better yield and also that dry weight is a better reference for comparisons between different samples [31–34].

Soxhlet extraction method extracts the total oil from nuts and seeds with chemical solvent such as petroleum ether [34] or hexane [33,35] in a Soxhlet apparatus for 5–6 h. Afterwards, the solvent is evaporated either under nitrogen gas or in a vacuum evaporator [34,35]. Oil is extracted from the samples through repeating washing with chemical solvent by reflux in Soxhlet apparatus so the solvent can be used repeatedly and the whole extraction process is automatic. Also, depending on the size of the Soxhlet apparatus, the amount of samples used for the extraction can vary from less than 10 g to 100 g [34] allowing the extraction of lipids from samples with low lipid content. However, this method takes a long period of time to extract the lipids from the samples and some of the researchers argued that the heating process during reflux in Soxhlet extraction may cause degradation of lipids that are prone to peroxidation such as PUFA, especially DHA [36].

For solvent extraction, the most common method used is Folch extraction method [22]. Briefly, the ground samples are homogenized with Folch solution, followed by agitation using orbital shaker at room temperature. The major advantage of Folch extraction is that less solvent is needed for small amount of samples (1 g in 20 ml of solvent mixture) while for Soxhlet extraction, the amount of solvent used depends on the Soxhlet extractor; the latter requires 100 ml of solvent for 1 g sample extraction. Folch extraction method also require less preparation time than Soxhlet extraction, in which the whole process only takes approximately 1–2 h while Soxhlet extraction takes at least 5 h to complete the extraction. Although the time used in Folch extraction is shorter than Soxhlet extraction, the extract weight is even higher than the one extracted by Soxhlet extraction when same amount of sample were used for the extractions [33]. Furthermore, there are no significant difference between the fatty acids composition of the extract extracted by both methods [33].

It could be surmised that different extraction methods do not affect the lipids composition of the extracts. Extraction method can be chosen based on the nature of your samples. For nuts and seeds having high percentage of lipids, Folch extraction should be used since a small amount of sample (0.5 g–1 g) is sufficient to extract the highest amount of oil extract. For nuts and seeds having low percentage of lipids, Soxhlet extraction should be used in order to handle large amount of samples (>100 g) with less solvent.

5.2. *Mass spectrometry measurement*

In early reports, when IsoFs were first identified [7], GC-MS was the tool of choice by researchers. It requires negative ion chemical ionization and monitors a single ion, 585 in the case of IsoFs and 609 for NeuroFs. However, there is a drawback with the application of GC-MS as it is unable to identify the isomeric forms therefore only the 'total' of the isomers could be determined. Nevertheless, due to the increasing interest in other isofuranoids such as PhytoFs from ALA, dihomono-IsoFs from AdA and NeuroFs from DHA as well as the isomers, the application of LC-MS/MS is currently preferred [18,19]. Multiple reaction monitoring (MRM) is highly sensitive and commonly employed in LC-MS/MS. The technical aspects of GC-MS and LC-MS/MS are well described in several reviews [18,19]. LC-MS/MS approach however, requires the infusion of pure standards to identify the corresponding transition mass ions for the lipid of interest (Table 2). Currently, there are no commercialized standards for isofuranoids ready for researchers, hence the syntheses by organic chemists [9,10,12–16].

Table 2

Precursor and product ions of isofuranoids determined by gas chromatography-mass spectrometry (GC-MS) and liquid chromatography tandem mass spectrometry (LC-MS/MS).

Analyte			
GC-MS		m/z	
IsoFs		585	
NeuroFs		609	
LC-MS/MS		Q1 m/z	Q3 m/z
IsoFs		369	193
7(RS)-ST- Δ^8 -11-dihomo-IsoF		397	201
10- <i>epi</i> -17(RS)-SC-D ¹⁵ -11-dihomo-IsoF		397	221
16(R,S)-13- <i>epi</i> -ST- Δ^{14} -9-PhytoF		343	201
NeuroFs (total)		393	193
4(RS)-ST- Δ^5 -8-NeuroF		393	123

m/z: mass-ion-ratio; Q1: precursor ion; Q3: transition ion.

5.2.1. Implications of isofuranoids

Initially, the measurement of IsoFs and NeuroFs were used to determine the extent of oxidative stress with variable oxygen tension in different models such as fish, rodents, pig, lamb neonates and human (Table 3) and thereafter as potential biomarker (Table 4) related to the function of vital organs. As described below, we investigated the types of isofuranoid measurements conducted related to the vital organs and its potential role in the future.

5.2.1.1. Oxygen tension. In vitro peroxidation of AA revealed a plateau from 21% to 100% of oxygen tension for the generation of IsoPs, contrary to the constant increase of IsoFs. Concentration of IsoFs was two-fold higher than IsoPs, and the ratio of IsoFs to IsoPs in brain and kidney (two highly oxygenated organs) was the inverse of that of liver, with levels of IsoFs exceeding those of IsoPs by 2.0- to 2.3-fold. These early data suggests that the ratio levels of esterified IsoFs and F₂-IsoPs may be able to serve as an index of tissue oxygenation in various organs (Fig. 2).

Complications can arise with more unsaturated precursors like DHA. Indeed, Song et al. showed no difference in NeuroFs concentration at 100% oxygen tension, probably due to the greater capabilities of the hydroperoxide NeuroF intermediates to generate dioxolane derivatives [37] therefore limiting the total amount of NeuroF.

It is evident that a dynamic augmentation or surgical intubation of oxygen in the biological systems potentially increase the formation of isofuranoids compared to isoprostanoids. Fessel et al. [7] found that IsoFs were predominantly generated above 21% oxygen tension in bronchoalveolar lavage (BAL) fluid (Table 3). The IsoFs levels were also high in lung tissues of hyperoxic mice but was a transient phenomenon whereas the levels remained high in lung tissues of genetic mice lacking in *Cyp1a1* gene [38,39]. However, no change in IsoFs was found in lung tissues of lamb exposed to hyperoxia [40]. IsoFs and NeuroFs were elevated in neonates of low gestational age and preterm babies respectively when they were resuscitated at extremely high inspired oxygen level [41,42]. Moreover, in surgical patients requiring tourniquet procedure did not show alteration of IsoFs in the plasma at differential inspired oxygen concentrations [43]. Differential oxygen tension was also found in the brain where certain parts of the brain have higher oxygen tension (white matter) than others (grey matter) [44,45]. Noticeably this difference is described in Tables 2 and 3 in which hyperoxic environment elevated IsoFs and NeuroFs in brain tissues of newborn pigs and IsoFs in cognitively unimpaired transgenic mice [46,47]. In the marine ecosystem, hypoxia-hyperoxia is a natural event but could be altered in abnormal environment such as climate change or pollution. It was found hyperoxia did not

elevate IsoFs and NeuroFs in marine fish muscles despite its high abundance in DHA. Interestingly, hyperoxia acutely augmented dihom-IsoFs in these fish and was predominant in male gender (Table 3) [48].

5.2.1.2. Brain. As described by Skinner and Feng [44,45], different oxygen tension exists between the white and grey matter where the latter is higher. In relation to this, recently de la Torre et al. observed dihom-IsoFs, namely 7(RS)-ST- Δ^8 -11-dihomo-IsoF and 17(RS)-10-*epi*-SC- Δ^{15} -11-dihomo-IsoF had the most noticeable level in rat and preterm pig brains [9,16]. This also corresponded to a previous finding in which adrenic acid was more concentrated in the white matter compared to the grey matter. This finding is important as one needs to be cautious when measuring isofuranoids as it is assumed the brain being rich in DHA, NeuroFs would be the variable metabolite among the isofuranoids determined (Table 4).

Regardless to the oxygen tension, IsoFs and NeuroFs were determined in brain tissues of neurodegenerative diseases as well as neurological related injuries (Table 4). It was shown that IsoFs were higher than the controls in the *substantia nigra* of Parkinson's disease and dementia but not in multiple system atrophy and Alzheimer's disease (AD) [49]. However, IsoFs and NeuroFs were shown to be higher in the brain cortex of AD transgenic mice compared to the wild-type [8]. Also kainic acid induced epilepsy seizure in adult rats elevated IsoFs levels in the hippocampus and dentate gyrus compared to baseline [50]. IsoFs in the cerebrospinal fluid from traumatic brain injury and subarachnoid hemorrhage patients were also augmented compared to the controls [51] but melatonin supplementation appeared to reduce IsoFs in the cortex tissue of hypoxia-ischemic injury in rat [52]. Viewing these reports, there seem to be a lack of studies in association of diseases and injuries of the brain and the isofuranoid determinations. It would be valuable to determine the different isofuranoids in the brain tissues by differentiating the white and grey matters and specifically frontal, parietal, temporal and occipital cortexes, and cerebellum and brain stem in normal and disease models to see if there is any distinction between them.

5.2.1.3. Heart. The heart pumps oxygenated blood for circulation and this efficacy depends on the rhythm and status of the heart muscle. Measurement of IsoFs related to the heart (Table 4) were mainly focused in plasma and urine of cardiac bypass (CBP) surgery patients [53] in which it was high compared to the controls. Among CBP patients, those with acute kidney injury showed higher IsoFs levels than just CBP patients. However, treatment with acetaminophen to CPB prior to surgery alleviated urinary IsoFs compared to baseline indicating reduction of oxidative stress by acetaminophen [54]. Recently, plasma and urine IsoFs were determined in post-operative atrial fibrillation patients (PoAF) and it was observed that mitochondrial abnormality appeared to favor the generation of IsoF in PoAF condition [55]. Only one study determined IsoFs, NeuroFs and dihom-IsoFs in heart tissues [16]. It was found among the isofuranoids, dihom-IsoFs in particular the isomer 7(RS)-ST- Δ^8 -11-dihomo-IsoF predominated. Interestingly in the same report, NeuroFs namely 4(RS)-ST- Δ^5 -8-NeuroF was higher in the heart tissue compared to the brain whereas no difference was observed for IsoFs; perhaps this is due to greater dynamics of oxygen tension in the heart compared to the brain, and the higher unsaturation of DHA compared to AA which are more prone to peroxidation. Further, Roy et al. [56] recently observed some anti-arrhythmic event in mice cardiac cells by 4(RS)-ST- Δ^5 -8-NeuroF at high dose, indicating its potential role in the cardiovascular system.

5.2.1.4. Kidney. Reports of IsoFs detection in the renal function

Table 3

Concentration of isofurans, dihomom-isofurans and neurofurans in different biological samples intentionally induced with hyperoxia.

Study	Sample and concentration	Outcome	Reference
Isofurans (IsoFs)			
Brain			
Cognitively unimpaired Alzheimer's transgenic (TG) mice exposed to air (A) or hyperoxia (H).	<i>Hippocampus non-TG</i> [†] : 8 (A) and 10 (H) ng/g after 3 h; 5 ng/g (A) and 8 ng/g (H) after 24 h. <i>Hippocampus TG</i> [†] : 10 (A) and 10 ng/g (H) after 3 h; 8 (A) and 28 (H) ng/g after 24 h. <i>Neocortex non-TG</i> [†] : 4 (A) and 10 (H) ng/g after 3 h; 5 (A) and 5 (H) ng/g after 24 h. <i>Neocortex TG</i> [†] : 7 (A) and 6 (H) ng/g after 3 h; 8 (A) and 27 (H) ng/g after 24 h.	IsoFs increased in hippocampus and neocortex of TG mice at 24 h after a single hyperoxia exposure.	[47]
Newborn pigs exposed to 0, 21, 40 and 100% oxygen.	<i>Prefrontal cortex tissue</i> : 14.69 ± 3.0 (0%), 36.70 ± 10.5 (21%), 48.99 ± 21.1(40%) and 55.99 ± 22.6 (100%) ng/g.	Dose-dependent increase of IsoFs in brain.	[46]
Lung			
Mice	<i>Mouse</i> : 210 ± 30 pg/mL bronchoalveolar lavage fluid; 24 (air) vs 90 (>98% O ₂) pg mg protein lung tissue. <i>Lung tissue</i> : no data shown.	The formation of IsoFs becomes increasingly favored as oxygen tension increased above 21%.	[7]
Lamb exposed to room air or 95% O ₂ or 95% O ₂ plus nitric oxide (5–10 ppm).		There was no difference in lung tissue IsoFs between treatments.	[40]
Extremely low gestational age neonate (ELGAN) resuscitated with a lower (30%) or higher (90%) fraction of inspired oxygen (F _I O ₂) with and without bronchopulmonary dysplasia (BPD).	<i>30% resuscitation</i> : no data provided. <i>90% resuscitation</i> : 8 ng/mg Cr (BPD) and 4 ng/mg Cr (no BPD) in urine day 7 after birth.	Oxidative stress increased significantly in the 90% oxygen group compared to 30% oxygen group first week after birth.	[41]
Mice lacking <i>Cyp1a1</i> gene (more susceptible to hyperoxic lung injury) and C57BL/6J wild-type (WT) mice under hyperoxia environment for 24–72 h.	<i>Lung tissue WT</i> [†] : 0.85 (air) and 1.8 (24 h) ng/mg protein. <i>Lung tissue Cyp1a1^{-/-}</i> : 0.75 (air), 1.1 (24 h) and 0.95 (48 h) ng/mg protein.	WT lung had increased IsoFs after 24 h hyperoxia exposure and <i>Cyp1a1^{-/-}</i> mice lung had increased IsoFs after 48–72 h. Thereafter, the concentrations returned to 'air' level.	[38]
Mice lacking <i>Cyp1a1</i> gene (more susceptible to hyperoxic lung injury) and C57BL/6J wild-type (WT) mice in room air (A) or exposed to >95% oxygen for 24, 48, or 72 h	<i>Lung tissue WT</i> [†] : 0.9 (A), 1.4 (24 h), 0.5 (48 h) and 0.7 (72 h) ng/mg protein. <i>Lung tissue Cyp1a1^{-/-}</i> : 0.8 (A), 0.7 (24 h), 1.2 (48 h) and 1.0 (72 h) ng/mg protein. <i>Liver tissue WT</i> [†] : 0.5 (A), 0.6 (24 h), 0.5 (48 h) and 0.75 (72 h) ng/mg protein. <i>Liver tissue Cyp1a1^{-/-}</i> : 0.5 (A), 0.6 (24 h), 0.5 (48 h) and 0.7 (72 h) ng/mg protein. <i>Liver tissue Cyp1a1^{-/-}</i> : 0.7 (A), 0.4 (24 h), 0.8 (48 h) and 0.82 (72 h) ng/mg protein.	Hyperoxia leads to lung injury but it is transient in WT but not in <i>Cyp1a1^{-/-}</i> mice.	[39]
Others			
Planned surgical patients with isolated upper-limb trauma requiring the pneumatic arterial tourniquet procedure and inspired with 30, 50, or 80% oxygen concentrations.	<i>Plasma</i> : 6.11 (30%), 6.65 (50%) and 5.94 (80%) nmol/L prior oxygen inspiration i.e. baseline; 6.02 (30%), 5.70 (50%) and 4.62 (80%) nmol/L 15 min after inspired oxygen.	Different concentration of inspired oxygen did not alter IsoFs.	[43,51]
Marine fish muscles	<i>Male</i> [†] : 0.75 (baseline) vs 1.8 (hyperoxia) ng/g muscle <i>Female</i> [†] : 3.0 (baseline) vs 1.8 (hyperoxia) ng/g muscle	Hyperoxia did not alter IsoFs concentration in fish muscles.	[48]
Preterm babies resuscitated with inspired oxygen (F _I O ₂).	<i>Control urine</i> : 211 ± 239 (PD 1), 547 ± 651 (PD7) and 167 ± 188 (PD 28) signal units of UPLC-MS/MS per mL <i>BPD urine</i> : 455 ± 188 (PD 1), 612 ± 548 (PD7) and 198 ± 219 (PD 28) signal units of UPLC-MS/MS per mL	Increased IsoFs during the first week after birth.	[42]
Dihomo-isofurans (Dihomo-IsoFs)			
Others			
Marine fish	<i>Male</i> [†] : 0.80 (baseline) vs 3.1 (hyperoxia) ng/g muscle. <i>Female</i> [†] : 0.5 (baseline) vs 0.8 (hyperoxia) ng/g muscle.	Hyperoxia significantly elevated dihomom-isofurans, namely 10- <i>epi</i> -17(RS)-SC- Δ^{15} -11-dihomom-isofuran in male fish muscle.	[48]
Neurofurans (NeuroFs)			
Brain			
Newborn pigs exposed to 0, 21, 40 and 100% oxygen.	<i>Prefrontal cortex tissue</i> : 6.07 ± 2.0 (control), 6.84 ± 2.7 (21%), 13.98 ± 7.8 (40%) and 15.81 ± 10.1 (100%) ng/g.	Dose-dependent increase of NeuroFs in brain.	[46]
Others			
Preterm babies resuscitated with inspired oxygen (F _I O ₂).	<i>Control urine</i> : 16 ± 26 (PD 1), 44 ± 38 (PD7) and 23 ± 28 (PD 28) signal units of UPLC-MS/MS per mL <i>BPD urine</i> : 20 ± 18 (PD 1), 54 ± 44 (PD7) 34 ± 19 (PD 28) signal units of UPLC-MS/MS per mL	Augmented NeuroFs by hyperoxia was reduced after one-week PD.	[42]
Marine fish	<i>Male</i> [†] : 5.0 (baseline) vs 10.0 (hyperoxia) ng/g muscle <i>Female</i> [†] : 10.0 (baseline) vs 4.0 (hyperoxia) ng/g muscle	Hyperoxia did not alter NeuroFs concentration fish muscles.	[48]

Values are mean ± SD; [†]estimate from graphical display; Cr: creatinine; Se: selenium; PD: postnatal day; UPLC-MS/MS: ultra performance tandem mass spectrometry; CSF: cerebrospinal fluid; CCl₄: carbon tetrachloride.

Table 4

The generation of isofurans, diholo-isofurans, phytofurans and neurofurans in different biological samples related to vital organs that were unintended to generate hyperoxia condition in the study.

Study	Sample and concentration	Outcome	Reference
Brain			
IsoFs			
Parkinson's disease (PD), multiple system atrophy (MSA), dementia with Lewy body (DLB) disease and Alzheimer's disease (AD) patients.	<i>Brain substantia nigra tissue</i> ^{1,5} (control), 10 (PD), 10 (DLB), 4 (MSA) and 3 (AD) ng/g.	IsoFs increased preferentially in PD and DLB patients and may be related to the relative intracellular hyperoxia due to mitochondrial dysfunction.	[49]
Alzheimer disease <i>Tg 2576</i> transgenic mice	<i>Cortex tissues</i> : 10.5 (control) vs 14.3 (<i>Tg 2576</i>) ng/g. <i>Cerebellum</i> ¹ : 80 (control) vs 100 (<i>Tg 2576</i>) ng/g.	IsoFs are elevated in <i>Tg 2576</i> brain.	[8]
Kainic acid-induced with seizure (<i>status epilepticus</i>) in adult rats.	<i>Hippocampus</i> ¹ : 1.25 ng/g tissue before seizure, and 4.5 ng/g tissue (16 h) and 3.25 (48 h) ng/g tissue after seizure. <i>Dentate gyrus</i> ¹ : 1.0 ng/g tissue before seizure, and 6.75 ng/g tissue (16 h) and 3.25 (48 h) ng/g tissue after seizure. <i>Cerebellum</i> ¹ : 0.75 ng/g tissue before seizure, and 1.0 ng/g tissue (16 h) and 1.25 (48 h) ng/g tissue after seizure.	Increased IsoFs after seizure induction in hippocampus and dentate gyrus. No change in cerebellum. The hippocampal IsoFs correlated with mitochondrial oxidative stress and reoxygenation after a transient hypoxia.	[50]
Comatose severe aneurysmal subarachnoid hemorrhage (aSAH) and traumatic brain injury (TBI) patients.	<i>CSF</i> ¹ : 0.50 (control) and 2.10 nmol/L (aSAH) patients; 0.50 (control) and 1.80 nmol/L (TBI) patients.	IsoFs measurement could assist in management of comatose-neurological care patients.	[43,51]
Rat with cerebral hypoxia-ischemia (HI) injury and melatonin (Met) treatment.	<i>Cerebral right cortex tissue</i> : 0.11 (control), 1.56 (HI) and 0.84 HI-Met ng/mg.	Melatonin protected brain injury.	[52]
Preterm pig brain cortex	<i>Cortex tissue</i> ¹ : 2 ng/g (prefrontal) and 6 ng/g (medial prefrontal cortex)	Higher IsoFs concentration in medial prefrontal cortex tissues.	[9]
Rat brain	<i>Brain tissue</i> ¹ : 3.5 ng/g	No difference in the level of IsoFs between brain and heart.	[16]
Dihomo-IsoFs			
Preterm pig brain cortex	<i>Cortex tissue</i> ¹ : 11 (prefrontal) and 16 (medial prefrontal cortex) ng/g of 17(RS)-10- <i>epi</i> -SCΔ ¹⁵ -11-dihomo-Isofuran.	17(RS)-10- <i>epi</i> -SCΔ ¹⁵ -11-dihomo-IsoF was identified in brain tissue.	[16]
Rat brain	<i>Brain tissue</i> ¹ : 280 ng/g of 7(RS)-ST-Δ ⁸ -11-dihomo-Isofuran and 3 ng/g of 17(RS)-10- <i>epi</i> -SCΔ ¹⁵ -11-dihomo-Isofuran.	Among the isofuranoids, 7(RS)-ST-Δ ⁸ -11-dihomo-IsoF predominated in the brain tissue.	[16]
NeuroFs			
Alzheimer disease <i>Tg 2576</i> transgenic mice.	<i>Cortex tissues</i> : 156.2 (wild-type) vs 99.3 (<i>p47^{phox}</i>) ng/g; 109.1 (wild-type) vs 173.2 (<i>Tg 2576</i>) ng/g.	Elevated levels of NeuroFs are found in <i>Tg 2576</i> brain cortex.	[8]
Preterm pig brain cortex	<i>Cortex tissue</i> ¹ : 3 ng/g (prefrontal) and 5 ng/g (medial prefrontal cortex).	No difference between brain tissue types.	[9]
Rat brain	<i>Brain tissue</i> ¹ : 0.2 ng/g (total neurofuran) and 0.02 ng/g (4(RS)-ST-Δ ⁵ -8-Neurofuran).	Neurofuran was synthesized and identified in heart and brain tissues.	[16]
Heart			
IsoFs			
Cardiac surgery patients with developed acute kidney injury (AKI)	<i>Plasma</i> : 32 (baseline), 58 (post cardiac bypass), 57 (ICU) and 30 (day 3 post-operation) pg/mL ¹ ; 37.7 ± 5.7 (control) vs 27.4 ± 5.4 (AKI) pg/mL. <i>Urine</i> : 2.7 (baseline), 5.5 (post cardiac bypass), 8.5 (ICU) and 4 (day 3 post-operation) ng/mL Cr ¹ ; 4.2 ± 0.5 (smokers) vs 2.5 ± 0.3 (non-smokers) ng/mL Cr among the patients; 3.3 ± 0.5 ng/mL Cr (control) vs 2.2 ± 0.3 (AKI) ng/mL Cr.	Hemoprotein induced in AKI increased lipid peroxidation.	[74]
Elective cardiac bypass (CPB) surgery children patients treated with acetaminophen or placebo prior to surgery.	<i>Acetaminophen plasma</i> ¹ : 85 (baseline) and 120 (post CPB) pg/mL. <i>Acetaminophen urine</i> ¹ : 15 pg/mg Cr (baseline) and 30 (post CPB) ng/mg Cr.	Compared to placebo (data not provided), acetaminophen attenuated the IsoFs increase in plasma during CPB.	[53]
Rat heart	<i>Heart tissue</i> ¹ : 3.0 ng/g	No difference in the level of IsoFs between brain and heart.	[16]
Cardiac bypass (CPB) surgery patients treated with acetaminophen or placebo prior to surgery.	<i>Placebo plasma</i> ¹ : 68.7 (baseline) and 109.5 (60 min CPB) pg/mL. <i>Acetaminophen plasma</i> ¹ : 61.7 pg/mL (baseline) and 80 (60 min CPB) pg/mL. <i>Placebo urine</i> ¹ : 2.78 ng/mg Cr (baseline) and 8.33 (post-CPB) ng/mg Cr. <i>Acetaminophen urine</i> ¹ : 3.11 ng/mg Cr (baseline) and 9.22 (post-CPB) ng/mg Cr.	Plasma IsoFs peaked 60 min during CPB and returned to baseline level at postoperative day 1. Urine IsoFs increased with time and returned to baseline at postoperative day 1.	[54]
Postoperative atrial fibrillation (PoAF) patients.	<i>No PoAF plasma</i> ¹ : 60 (baseline), (end of surgery) and 55 (day 2 post operation) pg/mL. <i>PoAF plasma</i> ¹ : 68 (baseline), 68 (end of surgery) and 60 (day 2 post operation) pg/mL. <i>No PoAF urine</i> ¹ : 2 (baseline), 6 (end of surgery)	Mitochondrial dysfunction and elevated oxygen concentration favored the development of IsoFs in PoAF.	[55]

(continued on next page)

Table 4 (continued)

Study	Sample and concentration	Outcome	Reference
	and 2.5 (day 2 post operation) ng/mg Cr. <i>PoAF urine</i> ¹ : 2 (baseline), 9 (end of surgery) and 2.8 (day 2 post operation) ng/mg Cr.		
Dihomo-IsoFs			
Rat heart	<i>Heart tissue</i> ¹ : 140 ng/g of 7(<i>RS</i>)-ST-Δ ⁸ -11-dihomo-Isofuran and 3 ng/g of 17(<i>RS</i>)-10- <i>epi</i> -SCΔ ¹⁵ -11-dihomo-Isofuran).	Among the isofuranoids, 7(<i>RS</i>)-ST-Δ ⁸ -11-dihomo-IsoF predominated in the heart tissue.	[16]
NeuroFs			
Rat heart	<i>Heart tissue</i> ¹ : 0.2 ng/g (total neurofuran) and 0.11 ng/g (4(<i>RS</i>)-ST-Δ ⁵ -8-Neurofuran).	Higher 4(<i>RS</i>)-ST-Δ ⁵ -8- neurofurans level in heart tissue than the brain was found.	[16]
Arrhythmia of mice cardiac cells	4(<i>RS</i>)-ST-Δ ⁵ -8-Neurofuran reduced arrhythmia.	Higher concentration (1 μM) of 4(<i>RS</i>)-ST-Δ ⁵ -8-Neurofuran compared to lower concentration (0.1 μM) showed anti-arrhythmic effect in cardiac cells.	[56]
Kidney			
IsoFs			
Severe sepsis patients with and without renal failure or hepatic failure or coagulation failure.	<i>Plasma without renal failure</i> ¹ : 250 pg/mL. <i>Plasma with renal failure</i> ¹ : 1250 pg/mL. <i>Plasma with coagulation failure</i> ¹ : 1500 pg/mL.	IsoFs are highly associated with renal in critically ill patients with severe sepsis.	[61]
Maintenance hemodialysis therapy patients supplemented with or without antioxidant (mixed tocopherol + α-lipoic acid) for 6 months.	<i>Placebo plasma</i> : 0.89 ± 1.9 (baseline) and 0.79 ± 1.36 (6 months) ng/mL. <i>Antioxidant plasma</i> : 0.69 ± 1.31 ng/mL (baseline) and 0.65 ± 0.90 (6 months) ng/mL.	No improvement of oxidative stress by antioxidant supplementation to hemodialysis patients.	[57]
End- stage renal disease patients receiving hemodialysis.	<i>Baseline</i> : <0.25 - ≥ 0.73 ng/mL plasma. <i>6-month after hemodialysis</i> : <0.26 - ≥ 0.73 ng/mL plasma.	Higher level of IsoFswas associated with increased resistance to erythropoiesis stimulating agents.	[58,59]
End- stage renal disease patients receiving hemodialysis supplemented with 4 weeks of pomegranate juice and 4 weeks of pomegranate extract.	<i>Plasma</i> : Not reported.	Supplementation had no effect on the level of IsoFs.	[58,59]
Supplementation of coenzyme Q10 to hemodialysis patients.	<i>Plasma</i> : 141 ± 67.5(before) and 72.2 ± 37.5 (after) pg/mL supplementation.	Plasma IsoFs concentrations decreased after coenzyme Q10 supplementation.	[60]
Liver			
IsoFs			
Rat, mouse and human.	2 (-CCl ₄) vs 30 ng/g (+CCl ₄) liver tissue.	The formation of IsoFs by CCl ₄ in liver.	[7]
Severe sepsis patients with and without hepatic failure.	<i>Plasma without hepatic failure</i> ¹ : 500 pg/mL. <i>Plasma with hepatic failure</i> ¹ : 1300 pg/mL.	IsoFs are highly associated with hepatic failure in critically ill patients with severe sepsis.	[61]
NeuroFs			
Mice with or without CCl ₄ treatment, <i>p47^{phox}</i> knock-out mice	<i>Liver tissues</i> : 141.3 (control), and 412.2 (1 h) and 1330.6 (2.5 h)ng/g post CCl ₄ injection.	Acute increase of NeuroFs after CCl ₄ treatment in liver.	[8]
Lung			
IsoFs			
Mice with cytoplasmic domain mutation (BMPR2 ^{R899X}).	<i>Lung tissue</i> ¹ : 11 ng/g (control) and 21 ng/g (BMPR2 ^{R899X}).	Mice with cytoplasmic domain mutation show increased IsoFs levels.	[64]
Transgenic mice with low levels of tissue factor (LTF) and wild type (WT) exposed to lipopolysaccharide (LPS), and patients alveolar hemorrhage	<i>WT lung tissue</i> ¹ : 1.0–1.5 (-LPS) and 1.5–2.1 (+LPS) ng/g. <i>LTF lung tissue</i> ¹ : 1.0–2.8 (-LPS) and 3.0–5.0 (+LPS) ng/g. <i>Human bronchoalveolar lavage</i> ¹ : increment with sampling aliquot, 50, 60, 150, 150 pg/mL.	Tissue factor deficiency potentially increased intraalveolarhaemorrhage and LPS leading to enhanced IsoFs levels.	[62]
Lung transplant recipients with primary graft dysfunction (PGD) and controls (non-PGD).	<i>Non-PGD plasma</i> : 31.9 pg/mL. <i>PGD plasma</i> : 39.7 pg/mL. <i>PDG with non-smoker lung plasma</i> : 34.6 pg/mL. <i>PDG with smoker's lung plasma</i> : 66.9 pg/mL.	IsoFswas higher in patients with PGD after smoker lung transplantation compared with patients without PGD development.	[63]
Others			
IsoFs			
Rat and human.	<i>Rat</i> : 3.3 ± 0.3 ng/mL urine; 334 ± 80 pg/mL plasma; 0.32 (control) vs 0.05 (n-acetylcysteine) vs 0.12 (α-lipoic acid) ng/mL plasma ¹ ; <i>Human</i> : 5.8 ± 1.0 ng/mL urine; 71 ± 10 pg/mL plasma.	IsoFs detectable in different samples.	[7]
Mice low-high iron proximal colon after azoxymethane (AOM) or saline (control) treatment.	<i>Control</i> ¹ : 5.5 (low iron) and 2.5 (high iron) ng/g. <i>AOM</i> ¹ : 2.8 (low iron) and 3.0 (high iron) ng/g.	Iron overload did not affect colon tissue levels of IsoFs.	[65]
Severe sepsis patients with and coagulation failure.	<i>Plasma without coagulation failure</i> ¹ : 300 pg/mL. <i>Plasma with coagulation failure</i> ¹ : 1500 pg/mL.	IsoFs are highly coagulation failure in critically ill patients with severe sepsis.	[61]
Spinal (SA) or general (GA) anesthesia in ischemia/reperfusion of the leg surgery	<i>Plasma GA</i> : 8.80 (baseline), 8.70 (2 h), 8.80 (3.5 h) and 5.80 (24 h) nmol/L. <i>Plasma SA</i> : 8.80 (baseline), 8.30 (2 h), 5.50 (3.5 h) and 5.40 (24 h) pmol/L.	IsoFs were lower in patients undergoing SA compared with patients undergoing GA.	[66]
Normal pregnancy versus pre-eclampsia.	<i>Plasma from maternal blood</i> ¹ : 2.00 (normal) and 2.50 (pre-eclampsia) nmol/L. <i>Plasma from cord blood</i> ¹ : 12.00 (normal) and 14.00 (pre-eclampsia) nmol/L.	Significant elevation of IsoF in maternal plasma but not cord blood.	[67]

Table 4 (continued)

Study	Sample and concentration	Outcome	Reference
Age macular degeneration (AMD) patients.	<i>Non-AMD plasma</i> : 0.15 ± 0.18 ng/mL. <i>AMD plasma</i> : 0.22 ± 0.38 ng/mL.	Potential role of IsoFs in AMD pathogenesis.	[68,69]
Age macular degeneration (AMD) patients before and after antioxidant supplementation.	<i>Non-AMD plasma</i> : 0.20 ± 0.072 (before) and 0.17 ± 0.048 (after) ng/mL. <i>AMD plasma</i> : 0.24 ± 0.20 (before) and 0.25 ± 0.29 (after) ng/mL.	Antioxidant failed to reduce IsoFs in AMD.	[68,69]
Canadian Inuits	<i>Plasma</i> : 20.86 (18.90–23.02 range) pg/mL	IsoFs correlated with visceral adiposity and smoking was related to lower plasma levels of IsoFs due to lower ambient levels of tissue oxygen among smokers.	[70]
Canadian Inuits	<i>Plasma from blood with</i> : <200 Se µg/L, 29.98 pg/mL IsoFs; ≥200 and < 340 Se µg/L, 17.53 pg/mL IsoFs; ≥340 Se µg/L, 18.14 pg/mL IsoFs.	Plasma IsoFs decreased with increasing tertiles of blood Se.	[71]
Storage of leukoreduced human red blood cells (RBC).	<i>Supernatant of stored RBC[†]</i> : 5 days: 75 pg/mL; 12 days: 90 pg/mL; 40 days: 170 pg/mL; 47 days: 190 pg/mL. <i>Supernatant washing of RBC[†]</i> : 55 pg/mL (pre-wash) vs 17 pg/mL (post-wash). <i>Serum</i> : 0.015–147 nmol/L range.	Linear increase in IsoFs in RBC supernatant with storage time but decreased if washed.	[72]
Infants with normal gestational period Dihomo-IsoFs Pine nut, walnut, chia seed and flax seed	<i>Nuts and seeds[‡]</i> : 0.3 (pine), 9 (walnut), 6 (chia) and 0.7 (flax) ng/g of <i>ent-16-(RS)-13-epi-ST-Δ¹⁴-9-PhytoF</i> and <i>Δ¹⁴-9-Phytofuran</i> .	No outcome.	[73]
NeuroFs Infants with normal gestational period	<i>Serum</i> : 0.013–14.5 nmol/L range.	<i>ent-16-(RS)-13-epi-ST-Δ¹⁴-9-PhytoF</i> predominated in walnut and chia seeds.	[10]
		No outcome.	[73]

Values are mean ± SD; [†]estimate from graphical display; Cr: creatinine; Se: selenium; PD: postnatal day; UPLC-MS/MS: ultra performance tandem mass spectrometry; ICU: intensive care unit.

mainly focused on hemodialysis patients (Table 4) in particular to reduce oxidative stress. It was shown antioxidant supplementation of mixed tocopherols with α-lipoic acid or pomegranate juice had no effect on the IsoFs level in plasma but supplementation of co-enzyme Q10 decreased plasma IsoFs concentration in hemodialysis patients [57–60]. However, in critically ill patients with sepsis and renal failure showed multiple increase in IsoFs concentration in plasma compared to severe sepsis patients [61]. To date, no reports on dihom-IsoFs and NeuroFs levels in relation to kidney disorders were found.

5.2.1.5. *Liver*. Liver is the main organ for the synthesis, metabolism and storage of PUFA. Despite this, only a handful of studies on isofuranoids and liver function were found in the literature (Table 4), probably because the liver oxygen tension is the lowest of all organs, therefore the isoprostanoids should predominate (ref Fessel 2002). It was shown severe sepsis patients with hepatic failure had higher levels of plasma IsoFs compared to severe sepsis patients [61]. Moreover, an acute increase of NeuroFs was identified in mice liver when treated with carbon tetrachloride [8].

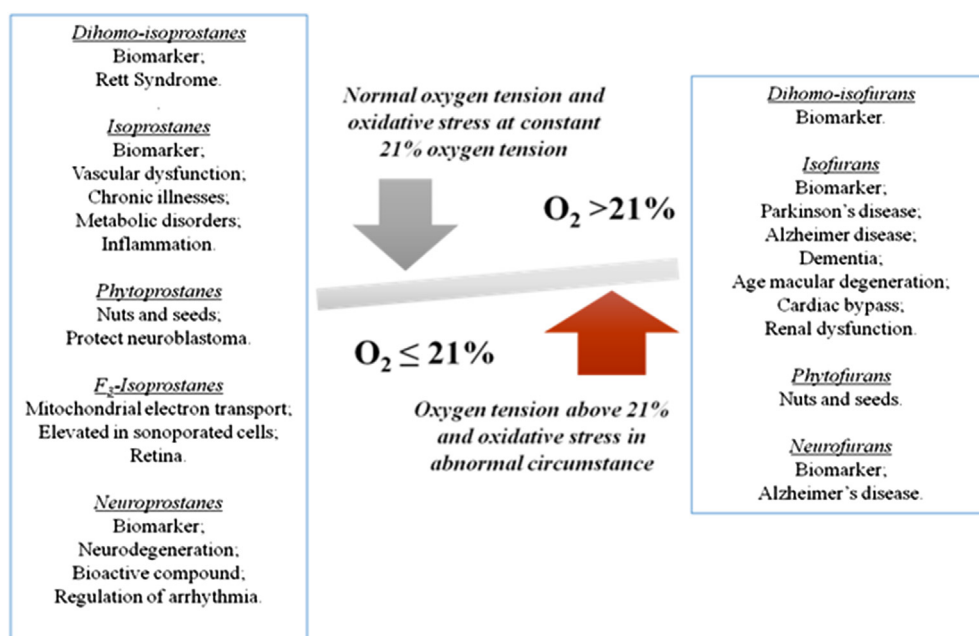


Fig. 2. Summary of isoprostanoids and isofuranoids identified and its association to diseases: Isofuranoids formation from arachidonic acid shows constant increase with oxygen tension (up to 100%) compared to the plateau reached by isoprostanoids at 21% of oxygen tension, in vitro experiment. Can this fact be translated to specific tissues, organs, diseases and pathologies for more accurate biomarkers of oxidative stress?

5.2.1.6. *Lung*. When determining isofuranoids in the biological systems, one area of interest is undoubtedly the lung since it is the main organ for oxygen delivery to the circulatory system. It was found that alveolar hemorrhage induces IsoFs formation in human bronchoalveolar lavage [62], and that plasma of patients undergoing lung transplant with primary graft dysfunction (PGD) had higher IsoFs level especially those receiving a smoker's lung compared to non-PGD and PGD [63]. Lung tissues of transgenic mice with low levels of tissue factor had higher IsoFs compared to the wild-type when exposed to inflammation such as lipopolysaccharide (LPS) [62] and mice with cytoplasmic domain mutation had higher IsoFs in the lung tissue compared to the controls [64]. Surprisingly, no reports were identified for dihom-IsoFs and NeuroFs in relation to biological systems where oxygen concentrations were not intentionally induced as explained above.

5.2.1.7. *Others*. Aside from the function of vital organs, reports of isofuranoids levels were made in different systems. It was shown iron overload in mice did not alter IsoFs in the colon tissues compared to controls [65]. Moreover, the use of spinal anesthesia reduced plasma IsoFs whereas general anesthesia augmented IsoFs level in surgical procedure for leg ischemic-reperfusion compared to baseline [66]. High blood pressure showed an effect on IsoFs concentration, where maternal blood of pre-eclampsia showed higher IsoF levels compared to normal pregnancy [67]. It was also found plasma IsoFs may have potential role in the development of age macular degeneration (AMD) in adults [68] but, antioxidant supplementation was unable to reduce IsoFs in AMD patients [69]. Plasma IsoFs was found to have an association with visceral adiposity and smoking in Canadian Intuits but appears to decrease in plasma IsoFs with increased blood selenium concentration [70,71]. In a more recent study, IsoFs levels in red blood cells increased with storage time but interestingly a washing procedure of the red blood cells reduced the IsoFs level and were not further induced during storage [72]. Serum NeuroFs of infants of normal gestational period was determined lately [73] and PhytoFs in nuts and seed were measured, which showed some variable levels depending on the type [10].

6. Conclusion

It is clear from this report (Fig. 2) that isofuranoids are an important group of compounds in biological systems. They exist due to increased or dynamic change of oxygen tension *in vivo* in the vital organs, and the gauging of isofuranoids may be necessary in critical illnesses or surgical procedures, and monitoring neurodegenerative diseases for better management of the patients – an elevation or reduction by intervention could indicate the health status of the patients. Isofuranoids are a group of novel compounds which in the future could be promising oxidative stress biomarkers due to their stability and abundance especially in the brain and heart but may also have a biological role in particular ones from n-3 PUFA that needs to be studied explicitly.

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