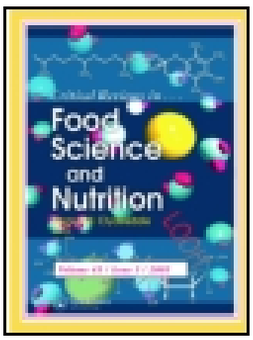

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REVIEW

4-hydroxy-2-alkenals in foods: a review on risk assessment, analytical methods, formation, occurrence, mitigation and future challenges

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ABSTRACT

Undoubtedly, significant advances were performed concerning 4-hydroxy-2-alkenals research on foods, and their formation by double oxidation of polyunsaturated fatty acids. But further studies are still needed, especially on their occurrence in foods enriched with n-3 and n-6 fatty acids, as well as in foods for infants and processed foods. Major factors concerning the formation of 4-hydroxy-2-alkenals were discussed, namely the influence of fatty acids composition, time/temperature, processing conditions, salt, among others. Regarding mitigation, the most effective strategies are adding phenolic extracts to foods matrices, as well as other antioxidants, such as vitamin E. Exposure assessment studies revealed 4-hydroxy-2-alkenals values that could not be considered a risk for human health. However, these toxic compounds remain unaltered after digestion and can easily reach the systemic circulation. Therefore, it is crucial to develop in vivo research, with the inclusion of the colon phase, as well as, cell membranes of the intestinal epithelium. In conclusion, according to our review it is possible to eliminate or effectively decrease 4-hydroxy-2-alkenals in foods using simple and economic practices.

KEYWORDS

4-hydroxy-2-nonenal; 4-hydroxy-2-hexenal; foods; mitigation; risk assessment

Introduction

Lipid oxidation is responsible for important losses across the food chain, with impact on important characteristics of foods, namely odor, flavor and texture (Guéraud et al. 2010; Vieira, Zhang, and Decker 2017). Several compounds are formed during lipid oxidation of polyunsaturated fatty acids (PUFA), namely alkanes, ketones, alcohols, furans and aldehydes. One of the most reactive group of lipid peroxidation products are α,β-unsaturated aldehydes (Esterbauer, Schaur, and Zollner 1991; Guéraud et al. 2010; Guillén and Goicoechea 2008a; Vieira, Zhang, and Decker 2017).

4-hydroxy-2-alkenals are highly reactive compounds formed by at least three functional groups, aldehyde (CHO), alkene (C2 = C3 double bound), and a group that could be a hydroxyl (4-hydroxy-2-alkenals), hydroperoxyl (-OOH), or oxo group (OH at the chiral center C4) (Guéraud et al. 2010; Guillén and Goicoechea 2008b; Sousa, Pitt, and Spickett 2017).

The presence of 4-hydroxy-2-alkenals in foods was early detected by Schauenstein in the 1960s (Esterbauer, Schaur, and Zollner 1991). 4-hydroxy-2-alkenals were considered as the major products from lipid peroxidation, and of these, 4-hydroxy-2-nonenal (HNE) is the most studied, followed by 4-hydroxy-2-hexenal (HHE). HNE and HHE are secondary lipid peroxidation of n-6 and n-3 PUFA, respectively (Esterbauer, Schaur, and Zollner 1991; Guéraud et al. 2010; Spickett 2013). These fatty acids are widely and naturally present in several foods, but in the last years, due to the link of PUFA to health benefits, an increasing trend to enrich foods with these fatty acids or a high intake of food sources rich in PUFA is being observed. This behavior can increase the potential exposure to these toxic compounds. Furthermore, 4-hydroxy-2-alkenals were also found in several body tissues, organs and fluids at different concentrations (Guéraud 2017).

In recent years, HNE and HHE were linked to several diseases, like cancer, atherogenesis, diabetes, chronic inflammation, and neurodegenerative diseases (Alzheimer's or Parkinson's diseases), among others (Csala et al. 2015; Esterbauer, Schaur, and Zollner 1991; Guéraud 2017; Pillon et al. 2012; Sousa, Pitt, and Spickett 2017). Due to the high reactivity and biological activity of these compounds, research on their occurrence in foods has increased, especially on edible oils and fats (Guillén and Uriarte 2012; Han and Csallany 2008; Ma and Liu 2017; Papastergiadis et al. 2014a; Seppanen and Csallany 2001, 2002, 2006), as well as in foods enriched with PUFA (Meynier et al. 2014; Surh and Kwon 2005; Surh, Lee, and Kwon 2007). The findings indicate that there are other food matrices of interest, namely fish and fish products, and milk, infant formulas and dairy products. Other aspects that are increasing the attention of researchers are the formation mechanisms of 4-hydroxy-2-alkenals, their analytical determination (Gabbanini et al. 2015; Papastergiadis et al. 2014b; Uchida, Gotoh, and Wada

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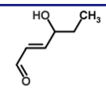
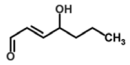
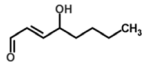
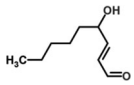
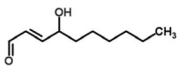
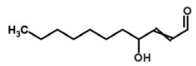
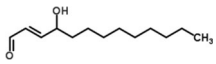
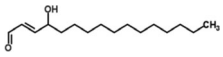
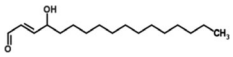
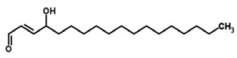
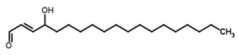
Table 1. Chemical and physical properties of 4-hydroxy-2-alkenals.

Compound	Molecular Formula	Molecular weight	Chemical structure	CAS registry number	Density	Boiling point	Vapor pressure
4-hydroxy-2-hexenal	$C_6H_{10}O_2$	114.143		17427-08-6	1.0 ± 0.1 g/cm^3	233.5 ± 23.0 °C at 760 mmHg	0.0 ± 1.0 mmHg at 25 °C
4-hydroxy-2-heptenal	$C_7H_{12}O_2$	128.17		17427-09-7	1.0 ± 0.1 g/cm^3	243.3 ± 23.0 °C at 760 mmHg	0.0 ± 1.0 mmHg at 25 °C
4-hydroxy-2-octenal	$C_8H_{14}O_2$	142.197		17449-15-9	1.0 ± 0.1 g/cm^3	259.4 ± 23.0 °C at 760 mmHg	0.0 ± 1.2 mmHg at 25 °C
4-hydroxy-2-nonenal	$C_9H_{16}O_2$	156.223		29343-52-0	0.9 ± 0.1 g/cm^3	275.6 ± 23.0 °C at 760 mmHg	0.0 ± 1.3 mmHg at 25 °C
4-hydroxy-2-decenal	$C_{10}H_{18}O_2$	170.25		29389-17-1	0.9 ± 0.1 g/cm^3	291.8 ± 23.0 °C at 760 mmHg	0.0 ± 1.4 mmHg at 25 °C
4-hydroxy-2-undecenal	$C_{11}H_{20}O_2$	184.277		29343-58-6	0.9 ± 0.1 g/cm^3	307.7 ± 25.0 °C at 760 mmHg	0.0 ± 1.5 mmHg at 25 °C
4-hydroxy-2-tridecenal	$C_{13}H_{24}O_2$	212.331		29343-62-2	0.9 ± 0.1 g/cm^3	338.9 ± 25.0 °C at 760 mmHg	0.0 ± 1.7 mmHg at 25 °C
4-hydroxy-2-hexadecenal	$C_{16}H_{30}O_2$	254.411		142449-98-7	0.9 ± 0.1 g/cm^3	383.5 ± 25.0 °C at 760 mmHg	0.0 ± 2.0 mmHg at 25 °C
4-hydroxy-2-heptadecenal	$C_{17}H_{32}O_2$	268.438		142449-99-8	0.9 ± 0.1 g/cm^3	397.8 ± 25.0 °C at 760 mmHg	0.0 ± 2.1 mmHg at 25 °C
4-hydroxy-2-octadecenal	$C_{18}H_{34}O_2$	282.465		142450-00-8	0.9 ± 0.1 g/cm^3	411.8 ± 28.0 °C at 760 mmHg	0.0 ± 2.2 mmHg at 25 °C
4-hydroxy-2-nonadecenal	$C_{19}H_{36}O_2$	296.491		142450-01-9	0.9 ± 0.1 g/cm^3	425.5 ± 28.0 °C at 760 mmHg	0.0 ± 2.3 mmHg at 25 °C

2002) and strategies to reduce their occurrence (Zhu et al. 2009; Zamora et al. 2016; Aladedunye, Matthäus, and Przybylski 2011; Fujisaki, Endo, and Fujimoto 2002).

Our review aims to bring an overview of the recent advances on 4-hydroxy-2-alkenals research in several food matrices, mainly focusing on the factors linked with their formation in foods, analytical methods, occurrence and mitigation strategies. In addition, health effects, exposure and risk assessment (including bioavailability and bioaccessibility data), gaps and future challenges are also discussed.

4-Hydroxy-2-alkenals

4-hydroxy-2-alkenals are formed by the double oxidation of PUFA, being the first step, the decomposition of a hydroperoxide to an alcohol, followed by the formation of a second hydroperoxide that will further undergo β-scission to fragment the fatty acids (Vieira, Zhang, and Decker 2017). HHE is a secondary lipid peroxidation product resulting from the oxidation of docosahexaenoic (C22:6, n-3) and eicosapentaenoic acids (C22:5, n-3), while HNE is generated in the degradation process of n-6 PUFA, such as linoleic (C18:2, n-6) or arachidonic (C20:4, n-6) acids. In addition to the formation of HNE, the aforementioned fatty acids were also identified as precursors of 4-oxo-nonenal (4-HOE) which is a γ-keto aldehyde (Ayala, Muñoz, and Argüelles 2014; Long and Picklo 2010; Spickett 2013). The chemical and physical properties of 4-hydroxy-2-alkenals is shown in Table 1.

Among the referred 4-hydroxy-2-alkenals, HNE is the most studied because it easily modifies and causes crosslinking of proteins, resulting in toxic effects. Schiff base formation and Michael addition are the most common chemical reactions leading to the formation of protein adducts. These protein adducts are associated with several cytotoxic consequences, including disruption of cell signaling, inhibition of enzyme activity, change in tertiary structure, altered gene regulation, mitochondrial dysfunction and loss of cytoskeletal formation (Guéraud et al. 2010).

Health concerns, exposure and risk assessment

Under oxidative stress conditions 4-hydroxy-2-alkenals can be endogenously formed. As mentioned before, these compounds are highly reactive, being able to produce adducts and instigating dysfunctions, as well as to inhibit enzymatic activities and protein synthesis, among others (Guillén and Goicoechea 2008b). Cancer, Alzheimer, Parkinson's disease, chronic inflammation, atherogenesis, and diabetes, are examples of diseases that were related with the presence of these hazardous compounds (Csala et al. 2015; Esterbauer, Schaur, and Zollner 1991; Guéraud et al. 2010; Guéraud

2017; Guillén and Goicoechea 2008b; Pillon et al. 2012; Spickett 2013; Vieira, Zhang, and Decker 2017; Zarkovic 2003; Zhong and Yin 2015). HNE has a higher genotoxic potential than HHE and other similar compounds (Eckl and Bresgen 2017). Different concentrations of HNE have been linked with several effects, namely irreversible damage in Ehrlich ascites tumor cells (10-20 μM); DNA fragmentation (≥60 μM); and reduction of CHO and V79 cells survival (≥10 μM) (Eckl and Bresgen 2017; Csala et al. 2015).

The hypothesis that these compounds can be absorbed from the diet was not clear, until studies with animals confirmed that after HNE oral administration, it is absorbed and detected in rat tissues (Kanazawa and Ashida 1998; Oarada, Miyazawa, and Kaneda 1986). Concerning humans, it is known that these compounds remained unaltered after digestion, being available to be absorbed and reach the systemic circulation. Nevertheless, there is a lack of studies concerning this subject, especially with respect to the inclusion of the colon phase, as well as, cell membranes of the intestinal epithelium (Steppeler et al. 2016).

So far, to the best of our knowledge, a safe dose for these aldehydes has not been established. Although, following the recommendations of the European Food Safety Authority, Papastergiadis et al. (2014a) have established a Threshold of Toxicological Concern (TTC) for HNE and HHE of 1.5 μg/kg of body weight/day (European Food Safety Authority 2012).

To estimate the exposure and/or risk assessment of hazard compounds, it is necessary to have reliable data on the occurrence of these compounds and their consumption (Alexander et al. 2012). Although, nowadays, it is also assumed that it is necessary to go a step forward and have data on the bioaccessibility and if possible bioavailability, which in general are obtained after in vitro digestion of foods. This approach aims to simulate physiological conditions in vivo and normally include oral, gastric and small intestine phases, being less frequent to include large intestinal fermentation (Minekus et al. 2014).

According to Surh and Kwon, the total exposure of 4-hydroxy-2-alkenals from vegetable oils, fish, shellfish and fried foods could be 16.1 μg/day, which corresponds to 0.3 μg/kg of body weight/day for an adult with approximately 60 kg of body weight (Surh and Kwon 2005). Consequently, authors have concluded that the value found could not be considered a risk for human health. The results reported by Papastergiadis et al. are in agreement with Surh and Know, except for cured minced meat products, for which consumers can be exposed to HNE levels above 1.5 μg/kg of body weight/day, meaning that the exposure risk may occur if consumers frequently eat this specific category of food (Papastergiadis et al. 2014a).

Goicoechea et al. (2008) have submitted sunflower oil and thermally oxidized sunflower oil to an in vitro digestion model to evaluate if α,β-unsaturated aldehydes, like HNE and HHE, could reach the systemic circulation after ingestion or if these compounds react with other components of food and enzymes. After in vitro digestion, author findings indicate that a significant amount of these toxic compounds persists being available to be absorbed in the gastrointestinal tract, and therefore, they can easily reach the systemic circulation. Later, the same group of researchers have submitted an infant formula with thermodegraded vegetable oil to in vitro digestion and similar results were obtained. After the digestion of foods, the highest percentage of 4-hydroxy-2-alkenals was in the lipidic phase (82.2 and 96.6% for HNE and HHE, respectively) (Goicoechea et al. 2011).

Besides this type of matrices, other studies have applied in vitro digestion to cooked beef, pork, chicken, salmon and herring to evaluate the amounts of 4-hydroxy-2-alkenals (Steppeler et al. 2016; Larsson et al. 2016). Accordingly, with the findings reported by Steppeler et al. higher amounts of HHE were found for salmon than for minced beef after digestion, especially in the intestinal phase. Nevertheless, the amounts of HNE and HHE before in vitro digestion were higher for minced beef than for salmon. Furthermore, the levels of HNE and HHE for salmon were similar in the gastric phase, but were significantly different in the intestinal phase, being the content of HHE approximately three times higher in the intestinal phase than in the gastric. Some reasons were appointed for the different results, namely the content of pro- and antioxidants, salt, muscle type, fatty acids composition and oxygen availability. Furthermore, in what concerns the differences between the digestion phases, authors indicate that the high amounts of HHE in the intestinal phase, are probably due to the emulsifying nature of bile that leads to an increased lipid droplet surface area susceptible to lipid oxidation (Steppeler et al. 2016).

Baltic herring (17% and 4% fat) and farmed salmon with high levels of astaxanthin were investigated regarding the potential health risks of the formed oxidation products during digestion, before and after oven baking (Larsson et al. 2016). After digestion HHE content was higher for raw herring than for raw salmon (3.8-fold and 33-times, in the gastric and intestinal phases, respectively). This is possible due to the higher portion of dark muscle and larger quantity of n-3 PUFA in herring in comparison with salmon. In what concerns the effects of oven baking, higher concentrations of HHE were reported for baked salmon than for raw. On the other hand, for herring (4% fat), the amounts were reduced by approximately 45% after cooking. After gastric digestion, HHE levels were higher for both cooked products than for raw (Larsson et al. 2016). Concerning the intestinal lumen, higher quantities of HHE were reported for baked salmon than for raw, while for herring the amounts remain similar. Although there are some inconsistency within this study and in comparison with other studies, these results herein show that higher quantities of HHE will be available after digestion, especially in the intestines, for cooked salmon than for herring.

Analytical determination

In the early years, the studies focused on the development of analytical methods to evaluate 4-hydroxy-2-alkenals in biological samples, being HNE the mostly determined compound. Therefore, up to now there is not a standardized

methodology for the simultaneous separation, identification and quantification of 4-hydroxy-2-alkenals in foods, especially in complex food matrices, allowing a good recovery. The selection of the method depends on the study objective, sample nature and the compounds under evaluation. A literature review on the applied sample preparation and extraction conditions, as well as the chromatographic settings are shown in Table 2.

Extraction conditions

Most of the analytical methods reported in the literature involve several extraction steps, which sometimes can induce important losses, leading to an underestimation of the actual amount present in the sample (Papastergiadis et al. 2014b). Moreover, it involves the use of large amounts of organic solvents, which is not acceptable especially for routine laboratories since it increases the analysis costs and the results are needed in due time (Gabbanini et al. 2015).

Matera et al. (2012) have studied different derivatization reagents because according to their findings, most of the time, the conversion of HNE into the desired imine, oxime, or hydrazine is generally incomplete, mainly due to the formation of other compounds. 2-(trifluoromethyl)-phenylhydrazine and 2,3,4,5,6-pentafluorophenylhydrazine were considered the best derivatization reagents due to their stability and favorable UV absorbance (Matera et al. 2012).

For most of the reported liquid chromatographic methods, usually butylated hydroxytoluene (BHT) is added directly to the sample or to the extract to reduce or inhibit the oxidation process (Table 2). This step is of utmost importance for the extraction of matrices with a high content in PUFA (Ma and Liu 2017). Afterwards, 2,4-dinitrophenylhydrazine (DNPH) is added for the derivatization step, which is commonly carried out overnight and at room temperature. Although, concerning the preparation of DNPH, it is necessary to be freshly prepared and usually hydrochloric acid (1 M) is applied. Then, several other steps can be used, namely centrifugation, filtration, separation with thin layer chromatography (TLC), evaporation and reconstitution of residues with different organic solvents, being methanol the most used (Aladedunye, Matthäus, and Przybylski 2011; Aladedunye and Przybylski 2011, 2012; Csallany et al. 2015; Grune et al. 2001; Lynch et al. 2008; T. Sakai et al. 2004, Sakai, Shimizu, and Kawahara 2006; Sakai and Kuwazuru 1995; Sakai et al. 1995; Sakai, Kazuhiro, and Eto 2000; Tadashi Sakai et al. 1997; Seppanen and Csallany 2001, 2002, 2004, 2006; Uchida, Gotoh, and Wada 2002; Zanardi et al. 2002). Methods like liquid chromatography coupled to mass spectrometry (LC-MS) have the advantage of not requiring derivatization, and thus the sample may be analyzed with less extraction intermediate steps (Sousa, Pitt, and Spickett 2017; Spickett 2013).

On the other hand, for gas chromatography coupled to mass spectrometry (GC-MS), the sample requires derivatization, which can be considered a limiting and crucial step. Regarding gas chromatographic methods, solid phase extraction cartridges (Surh and Kwon 2005; Surh, Lee, and Kwon 2007; Surh, Lee, and Kwon 2010; Viau et al. 2016) are frequently used, as well as centrifugation, evaporation and reconstitution of the residue (Table 2). Other authors, applied solid phase micro extraction, using a fiber coated with divinylbenzene/carboxen/polydimethylsiloxane (Goicoechea et al. 2008; Guillén and Goicoechea 2009). For derivatization *N,O*-bis(trimethylsilyl)trifluoroacetamide (BSTFA) is the most applied (Surh and Kwon 2005; Surh, Lee, and Kwon 2010; Surh, Lee, and Kwon 2007; Michalski et al. 2008; LaFond et al. 2011; Papastergiadis et al. 2014a, 2014b; Viau et al. 2016). Derivatization of samples is an important step for GC-MS analysis because it contributes to increase the volatility and thermal stability of the molecules (LaFond et al. 2011).

Chromatographic methods

The most frequent methodologies reported in the literature for food analysis of 4-hydroxy-2-alkenals (Table 2) are: high-performance liquid chromatography (HPLC) coupled to ultraviolet (UV) (Aladedunye, Matthäus, and Przybylski 2011; Aladedunye and Przybylski 2012; Aladedunye and Przybylski 2011; Alghazeer and Howell 2008; Csallany et al. 2015; Gasc et al. 2007; Grune et al. 2001; Han and Csallany 2008; Han and Csallany 2009; Lynch et al. 2008; T Sakai and Kuwazuru 1995; T Sakai et al. 1995; Tadashi Sakai et al. 1997; T. Sakai et al. 2004; T. Sakai, Shimizu, and Kawahara 2006; Tadashi Sakai, Kazuhiro, and Eto 2000; Seppanen and Csallany 2006; Seppanen and Csallany 2004; Seppanen and Csallany 2002; Seppanen and Csallany 2001; Munasinghe et al. 2003; Uchida, Gotoh, and Wada 2002); fluorescence (FL) (Liu, Miao, and Toyo'oka 1996) or mass spectrometry (MS) detection (Csallany et al. 2015; Douny et al. 2015; Alghazeer and Howell 2008; Gabbanini et al. 2015; Zanardi et al. 2002) and gas chromatography mass-spectrometry (GC-MS) (Goicoechea et al. 2008; Guillén and Goicoechea 2009; LaFond et al. 2011; Michalski et al. 2008; Papastergiadis et al. 2014a, 2014b; Petersen et al. 2013; Surh and Kwon 2003, 2005, Surh, Lee, and Kwon 2007, 2010; Takamura and Gardner 1996; Viau et al. 2016). Other techniques have also been reported, such as: liquid chromatography with electrochemical detection (Goldring et al. 1993), gas chromatographic analysis with electron capture detection (GC-ECD) (Santaniello et al. 2007), ^{1}H-NMR spectroscopy (Guillén and Goicoechea 2008a; Guillén and Ruiz 2008b); headspace with solid phase extraction and gas chromatography coupled to mass spectrometry detection (HS-SPME-GC-MS) (Guillén and Uriarte 2012).

Regarding the choice of analytical columns in HPLC-UV or HPLC-MS, silica-based reversed-phase C18 columns have been widely used (Table 2). The combination of different proportions of organic solvents, namely acetonitrile (Aladedunye, Matthäus, and Przybylski 2011; Aladedunye and Przybylski 2012; Aladedunye and Przybylski 2011; Csallany et al. 2015; Douny et al. 2015; Alghazeer and Howell 2008; Gabbanini et al. 2015; Gasc et al. 2007; Liu, Miao, and Toyo'oka 1996; Lynch et al. 2008; Uchida, Gotoh, and Wada 2002) or methanol (Csallany et al. 2015; Grune et al. 2001; Han and Csallany 2008; Han and Csallany 2009; Seppanen and Csallany 2001; Seppanen and Csallany 2002;

Table 2. Sample preparation, extraction and chromatographic conditions applied for the determination of 4-hydroy-2-alkenals in several food matrices.

Food matrices	Samples preparation/ Extraction	Compounds	Chromatographic conditions	Validation data	References
Minced pork and beef; Fish; smoked fish	5 g of sample was mixed with 25 mg of BHT; add 50 mL of 1 M HCl containing 2.5 mmol DNPH to the sample (keep it in the dark for 2 h at 4 °C); add 150 mL of dichloromethane; evaporated and redissolved in 0.2 mL of chloroform; DNPH derivatives were separated into a silica gel column by washing with n-hexane/chloroform (2:1 v/v) and eluted with chloroform; then, chloroform was evaporated, and the residue was redissolved in 0.5 mL of methanol	HNE	System: HPLC-UV-vis Column: Ultrasphere C18 (250 × 4.6 mm i.d., 5.0 μm particle size) Run time: - Mobile phase (gradient elution): (A) sodium citrate (30 mM); (B) acetate buffer (pH 4.75) Flow rate: 1 mL/min Injection volume: - Column temperature: 40 °C Detection (λ): 365 nm	–	Sakai et al. 1995, 1997, Sakai, Kazuhiro, and Eto 2000, 2004, Sakai, Shimizu, and Kawahara 2006; Sakai and Kuwazuru 1995
Soybean and sesame oils	0.25 – 1.0 g of oil samples and 1.0 mL of the freshly prepared solution (0.25%, w/v) trichloroacetic acid, 150 pg/mL BHT and 0.5 mM 7-nitro-2,1,3-benzoxadiazole in MeOH-H_2O (8:2, v/v)); vortex for 1 min and then heated at 60 °C for 10 min; cooled on ice and centrifuged at 3000 *g* for 10 min at 5 °C; inject the supernatant	HNE	System: HPLC-Fluorescence Column: Inertsil ODS-80A (150 × 4.6 mm i.d., 5.0 μm particle size) Run time: 40 min Mobile phase (gradient elution): (A) MeOH; (B) CH_3CN Flow rate: 1 mL/min Injection volume: 5 μL Column temperature: Emission (λ): 540 nm Excitation (λ): 490 nm	Range: 0-250 nM; LOD: 10 fmol	Liu, Miao, and Toyo'oka 1996
Soybean seeds	–	HNE	System: GC-MS Column: SPB-1 (30 m x 0.32 mm i.d. x 0.25 μm film thickness) Run time: - Mobile phase: 160 to 200 °C at 4 °C/min followed by 200 to 300 °C at 10 °C/min Flow rate: 2 mL/min Injection mode: - Injection volume: - Injector temperature: 260 °C Detector temperature: 310 °C Ion source temperature: - Ionization energy: -	–	Takamura and Gardner 1996
Eggs	1 g of egg yolk with 1 mL of DNPH solution (1.8 mM in 1 M HCl) in presence of BHT in final concentration of 10 mM; keep it for 2 h in the dark and for 1 h in an ice bath; extract three times with 4 mL of dichloromethane; centrifuge at 900 × *g*, and evaporated at 35 °C; transfer for vials with 1.0 mL of dichloromethane into small vials; extract three times with 5 mL each of methanol and evaporate to dryness; dissolve the residue with 1 mL of methanol	HNE	System: HPLC-UV-vis Column: Supelcosil (150 × 4.0 mm i.d., 5.0 μm particle size) Run time: - Mobile phase: MeOH/H_2O (80:20, v/v) Flow rate: 1 mL/min Injection volume: - Column temperature: - Detection (λ): -	–	Grune et al. 2001
Beef	Samples were immediately reacted with an equal volume of DNPH solution (50 mL of DNPH in 100 mL of HCl (1 N)); keep it in the dark for 2 h at room temperature; extract three times with dichlorometane; evaporate and redissolve in 200 μl of chloroform; sample was applied to a TLC plate; elute three times with methanol; evaporate and redissolve in 200 μl of methanol	HNE	System: HPLC-UV-vis Column: Spherical C18 (250 × 4.6 mm i.d., 5.0 μm particle size) Run time: - Mobile phase (gradient elution): (A) H_2O/ACN/THF (60/30/10, v/v/v); (B) H_2O/ACN (20/80, v/v) Flow rate: 1 mL/min Injection volume: 35 μL	–	Lynch et al. 2008

(*continued*)

Table 2. Continued.

Food matrices	Samples preparation/ Extraction	Compounds	Chromatographic conditions	Validation data	References
			Column temperature: - Detection (λ): 378 nm		
Soybean oil	3 g of sample reacted with 6 mL of DNPH solution; incubate overnight at room temperature in the dark with shaking at ~120 rpm; extract three times with 10 mL HPLC-grade methanol/water (75:25, v/v); separate by centrifugation at 1360 × *g* for 10 min; extract three times with 10 mL dichloromethane and separated by centrifugation; dichloromethane was evaporated until the sample size was ~1 mL; aliquots (500 μL) of the DNPH derivatives were applied to silica gel TLC plates ; combined methanol extracts were centrifuged at 1360 × *g* for 15 min; supernatant fractions were concentrated to 2.0 mL	HNE; HHE; HOE	System: HPLC-UV-vis Column: - Run time: 40 min Mobile phase: MeOH/H_2O (75:25, v/v) Flow rate: 0.8 mL/min Injection volume: - Column temperature: - Detection (λ): 378 nm	LOD: 1 ng	Seppanen and Csallany 2001
Soybean oil	2 g of sample reacted overnight at room temperature with 5 mL of DNPH reagent (10 mg recrystallized DNPH with 20 mL 1 N HCl); extract first with methanol/water (75:25 v/v) (3 × 10 mL) and then dichloromethane (3 × 10 mL); elute from the TLC plates with methanol (3 × 10 mL); and evaporate to 1 mL	HNE; HHE; HOE HNE	System: HPLC-UV-vis Column: Ultrasphere ODS C18 (250 × 4.6 mm i.d., 5.0 µm particle size) Run time: 45 min Mobile phase (gradient elution): (A) MeOH; (B) H_2O Flow rate: 0.8 mL/min Injection volume: 100 µL Column temperature: - Detection (λ): 378 nm System: HPLC-MS Column: HP-5MS (30 m x 0.25 mm i.d. x 0.25 µm film thickness) Run time: 10 min Oven ramp: 100 to 230 °C at a rate of 35 °C/min, followed by 10 min hold at 230 °C; then increase 50 °C/min until 300 °C Carrier gas: Helium Flow rate: 1 mL/min Injection mode: splitless Injector temperature: - Detector temperature: - Ion source temperature: - Ionization energy: - Range m/z: -	–	Seppanen and Csallany 2002, 2004, 2006
Soybean oil and porcine liver	100 mg of sample was added to an HNE-methanol solution (2.91 nmol and 0.29 nmol) in 1% volume; methanol was removed from the oil sample under a nitrogen stream, and the sample was then extracted twice with 2 mL of distilled water containing 0.1% BHT; extracts were combined and centrifuged at 3,000 × *g* for 10 min; extracts from oil samples were combined and immediately reacted with an equal volume of DNPH reagent (3.5 mg of DNPH dissolved in 10 mL of 1 M HCl); store in the dark at room temperature for 2 h; after the reaction, the pH of the reaction mixture was adjusted to 7.0 with 1 N NaOH and made to 10 mL with NaCl solution; 1 g of porcine liver sample was added to an HNE-	HNE	System: HPLC-UV-vis Column: ODS (250 × 4.6 mm i.d., 5.0 µm particle size) Run time: - Mobile phase: ACN/H_2O (40 :60, v/v) Flow rate: - Injection volume: - Column temperature: - Detection (λ): 223 nm	Range: 0-0.8 nmol/mL; r^2: 0.9938	Uchida, Gotoh, and Wada 2002

	methanol solution (2.91 nmol and 0.29 nmol); sample was homogenized with 2 mL of methanol containing 0.1% BHT for 2 min; the homogenate was centrifuged at 3000 × *g* for 10 min, and the supernatant was collected; the residue was re-extracted by the same procedure, and the supernatant was collected; the supernatants were combined and made to 10 mL with NaCl solution				
Pork products	10 g of sample were homogenized in 20 mL of distilled water by an Ultra-Turrax ; homogenate was centrifuged for 10 min at 5000 rpm and 4 °C; upper liquid phase was filtered by a filter paper, and the residue was further homogenized in 20 mL of distilled water; centrifugation at 5000 rpm and 4 °C for 10 min; the joint upper filtered phases (≈35 mL) were loaded on an SPE C18 end-capped cartridge previously equilibrated with 3 mL of methanol and 3 mL of distilled water; residual lipid substances were eluted by 15 mL of petroleum ether; residual water was eliminated by anhydrous sodium sulfate; after filtration and evaporation to dryness by a steam of nitrogen, the sample was dissolved in 1 mL of methanol acidified by 0.1% formic acid and filtered through a 0.45 ím PTFE filter	HNE	System: HPLC-MS Column: LiCrospher (250 × 4.0 mm i.d., 5.0 μm particle size) Run time: - Mobile phase: MeOH (0.1% formic acid) Flow rate: 0.8 mL/min Injection volume: - Column temperature: - Ionization mode: - Detection mode: - Range m/z: - Source temperature: -	Range: 0.1-10 mg/kg; LOD: 0.005 mg/L	Zanardi et al. 2002
Ham, bacon and sausage	Ground meat (5 g) was mixed with 25 mg BHT; HCl (50 mL of 1 mol/L) containing 2.5 mmol DNPH was added to the BHT mixture; reaction was carried out in the dark for 2 h at room temperature; DNPH derivatives were extracted three times with 150 mL dichloromethane; dichloromethane extract was evaporated and the residue was redissolved in 0.2 mL chloroform; the solution was applied to a disposable silica gel extraction column, which had been pre-equilibrated with n-hexane/chloroform (2:1 v/v); the resulting chloroform eluate was evaporated in vacuum and the residue was redissolved in 0.5 mL methanol	HNE	System: HPLC-UV-vis Column: Ultrasphere C18 (250 × 4.6 mm i.d., 5.0 μm particle size) Run time: - Mobile phase (gradient elution): (A) sodium citrate (30 mM); (B) acetate buffer (27.7 mM, pH 4.75) Flow rate: 1 mL/min Injection volume: - Column temperature: 40 °C Detection (λ): 365 nm	–	Munasinghe et al. 2003
Olive and perilla oils	The oil sample (1 g) was extracted twice with 10 mL distilled water containing 0.1% of BHT; each extraction was performed on a horizontal shaker at 150 rpm for 20 min; the combined aqueous phases were centrifuged at 3000 g for 20 min; the aqueous phase was subjected to further extraction with 20 mL chloroform three times; the chloroform extract was concentrated to 1 mL in a rotary evaporator below 30 °C at reduced pressure and concentrated again using a gentle stream of nitrogen.	HOE, HNE and HHE	System: GC-MS Column: FFAP (30 m x 0.20 mm i.d. x 0.33 μm film thickness) Run time: 25 min Oven ramp: 120 °C (hold 10 min), then increased to 220 °C at a rate of 5 °C/min Carrier gas: Helium Flow rate: 0.7 mL/min Injection mode: - Injection volume: - Injector temperature: - Detector temperature: - Ion source temperature: 230 °C Ionization energy: 70 eV Range m/z: -	–	Surh and Kwon 2003
Vegetable oils, fish, bivalve mollusks, infant formulas, raw cereal, vegetable	BHT (0.01 g per sample) was added to 5 g of the homogenized sample, and then 10 mL distilled water were added; the homogenate was centrifuged at 5000 x *g* (4 °C for 10 min); the aqueous phase	HHE; HNE	System: GC-MS Column: SPB-5 (50 m x 0.20 mm i.d. x 0.33 μm film thickness) Run time: 20 min	**HHE**: LOD (5 μg/kg); LOQ (9 μg/kg) **HNE**: LOD (3 μg/kg); LOQ (5 μg/kg)	Surh and Kwon 2005; Surh, Lee, and Kwon 2010, 2007

(*continued*)

Table 2. Continued.

Food matrices	Samples preparation/ Extraction	Compounds	Chromatographic conditions	Validation data	References
powder, snacks, fish sticks, tuna cans, and baby foods	was filtered; the residue was further centrifuged in 10 mL distilled water; after the second centrifugation, the combined aqueous filtrates were applied to SPE C18 cartridge pre-equilibrated with 3 mL methanol followed by 3 mL distilled water; after loading the sample to the SPE cartridge, residual lipid substances were washed off by 15 mL petroleum ether; 4-hydroxy-2-alkenals were eluted using 2 mL of methanol; evaporation to dryness and redissolved in 500 mL acetonitrile; BSTFA (200 mL) was added and the mixture was heated at 70 °C for 15 min for derivatization		Oven ramp: 120 °C (hold 5 min), increased at a rate of 5 °C/min to 160 °C and then 15 °C/ min to 200 °C (hold 5 min) Carrier gas: Helium Flow rate: 0.7 mL/min Injection mode: split Injection volume: 2 μL Injector temperature: 210 °C Detector temperature: 220 °C Ion source temperature: 230 °C Ionization energy: 70 eV Range m/z: -		
Chicken meat, beef meat, liver pâté, blood sausage	Samples (1–2 g) were homogenized in 30 ml water with an Ultra-Turrax for 2 min and then centrifuged (10,000 rpm/10 min); the residue was homogenized and centrifuged again; the supernatants were pooled and lipophilic compounds were extracted twice with 60 ml of dichloromethane; organic phases were evaporated under reduced pressure; the extract was defatted using a partition between 16 ml isooctane saturated acetonitrile and 4 ml acetonitrile saturated isooctane; acetonitrile was evaporated under reduced pressure and the dry extract containing HNE was dissolved in 500 μL acetonitrile/water (50:50, v/v)	HNE	System: HPLC-UV-vis Column: ODS2 Spherisorb (100 × 4.6 mm i.d., 5.0 μm particle size) Run time: - Mobile phase: ACN/H_2O (50:50, v/v) Flow rate: 1 mL/min Injection volume: - Column temperature: - Detection (λ): 221 nm	–	Gasc et al. 2007
Butter, corn and soybean oils	1 g of sample was reacted overnight at room temperature with 5 ml freshly prepared DNPH reagent; the DNPH derivatives were extracted with 10 ml of methanol/water (75:25, v/v) from the oil repeat two more times and the methanol: water extracts were combined; re-extracted with 10 ml of dichloromethane three times from the combined methanol/water extracts and concentrate to about 1 ml; apply to two TLC plates; extract three times with 10 ml of methanol; evaporate to the exact volume of 1 ml	HNE	System: HPLC-UV-vis Column: Ultrasphere ODS (250 × 4.6 mm i.d., 5.0 μm particle size) Run time: 35 min Mobile phase (gradient elution): (A) MeOH; (B) H_2O Flow rate: - Injection volume: - Column temperature: - Detection (λ): 378 nm	–	Han and Csallany 2008
Mackerel	1 g of sample was shaken with 20 ml of deionized water containing 1 mL/L of BHT; repeat three times; 30 ml of sample was collected and centrifuged at 5000 × *g* for 5 min; aqueous layer was applied onto a disposable ODS column conditioned and equilibrated with 3 mL of methanol and 3 ml of deionized water; petroleum ether (15 mL) was added; 4-HNE was eluted with 2 mL of methanol; evaporate off the petroleum ether and redissolve the dry sample in 1 mL of methanol acidified	HNE	System: HPLC-UV-vis-MS Column: Spherisorb S5ODS2 (250 × 4.6 mm i.d., 5 μm particle size) Run time: 10 min Mobile phase: ACN/H_2O (40:60, v/v) Flow rate: 1.0 mL/min Injection volume: 50 μL Column temperature: - Detection (λ): 220 nm Ionization mode: electrospray Detection mode: positive Range m/z:- Source temperature: -	Range: 0.1-5.0 mg/kg; r^2: 0.9868	Alghazeer and Howell 2008
Deep-frying fat, sunflower oil	Extraction of the volatile and semivolatile components of the headspace of the oil and fat samples (5 g in a 20 mL vial) was accomplished automatically using a CombiPAL autosampler; the fiber used was coated	HNE	System: GC-MS Column: VF-1MS (60 m x 0.25 mm i.d. x 0.25 μm film thickness) Run time: -	–	Goicoechea et al. 2008

	with DVB/CAR/PDMS (50/30 μm film thickness, 1 cm long), which was inserted into the headspace of the sample and was maintained for 60 min (50 °C, 250 rpm)		Oven ramp: 50 °C (5 min hold), increased to 290 °C at 4 °C/min (hold 30 min) Carrier gas: Helium Injection mode: splitless Injector temperature: - Detector temperature: - Ion source temperature: -		
Infant formulas and human milk	500 μl of milk or 200 mg of powders was spiked with 15–20 ng of both internal deuterated standards; 50 μl citric acid (0.15 M) and 1 mL of O-pentafluorobenzyl hydroxylamine (50% in PIPES buffer, pH 6.5) were added; after vortexing, the reaction took place under nitrogen for 30 min at room temperature; the following reagents were then successively added, with vortexing for 1 min between each addition: methanol (2.5 mL), hexane (5 mL), and 98% H_2SO_4 (60 μl); after centrifugation at 1800 rpm for 5 min, upper phases were transferred into 4 mL conical glass tubes; after evaporation under nitrogen, 100 μl of BSTFA was added; the reaction took place at 60 °C for 30 min, after which samples were transferred into vials for injection	HNE; HHE	System: GC-MS Column: HP-1MS (30 m x 0.25 mm i.d. x 0.25 μm film thickness) Run time: - Oven ramp: 2 min at 57 °C, then increased to 180 °C at 20 °C/min, followed by an increase to 280 °C at 4 °C/min Carrier gas: Helium Flow rate: 1 mL/min Injection mode: splitless Injector temperature: 260 °C Detector temperature: - Ion source temperature: 130 °C Ionization energy: 70 eV Range m/z: 50-800	–	Michalski et al. 2008
Cod liver oil	Vials containing 1 g of oil were introduced into a water bath (50 °C); sample equilibration (15 min); a fiber coated with DVB/CAR/PDMS (50/30 μm film thickness), was inserted into the headspace of the sample and was maintained for 60 min	HHE	System: GC-MS Column: HP-5MS (60 m x 0.25 mm i.d. x 0.25 μm film thickness) Run time: - Oven ramp: 50 °C (5 min hold), increased to 280 °C at 4 °C/min (hold 2 min) Carrier gas: Helium Injection mode: splitless Injector temperature: 220 °C Detector temperature: 280 °C Ion source temperature: 130 °C	–	Guillén and Goicoechea 2009
Fatty acids methyl esters; Mozzarella cheeses	1 g of FAMEs was reacted overnight at room temperature with 5 ml DNPH; DNPH derivatives were extracted with 10 ml of methanol/water (75:25, v/v); repeat two more times and combine the methanol/water extracts; DNPH derivatives were re-extracted with 10 ml of dichloromethane three times from the combined methanol/water extracts; concentrate to about 1 ml; apply on two TLC plates and developed in dichloromethane; extract three times with 10 mL of methanol; combine methanol extracts and centrifuge at 1,360 x *g* for 15 min; supernatant fractions were concentrated to the exact volume of 1 mL; 100 g of cheese and 200 ml of hexane; blend for 10 min; filtration; 10 ml of hexane extracts were reacted overnight with 5 mL DNPH at room temperature; .DNPH derivatives were extracted with 10 mL of methanol:/water (75:25, v/v); repeat two more times and combine the extracts; extract four times with 10 mL of dichloromethane; evaporate to about 1 mL the combined dichloromethane extracts	HNE; HHE; HDE; HOE	System: HPLC-UV-vis Column: Ultrasphere ODS (250 × 4.6 mm i.d., 5.0 μm particle size) Run time: 40 min Mobile phase (gradient elution): (A) MeOH; (B) H_2O Flow rate: - Injection volume: - Column temperature: - Detection (λ): 378 nm	–	Han and Csallany 2009, 2012
Corn-soy oil blend		HNE		–	LaFond et al. 2011

(*continued*)

Table 2. Continued.

Food matrices	Samples preparation/ Extraction	Compounds	Chromatographic conditions	Validation data	References
	1 g of oil with 5.0 µl of a 0.606 µg/µL solution of $^{2}H_{2}$-HNE in methanol (*3 ppm in the oil) was added; vortex mixed for 1 min; HNE was extracted two times (10 mL of distilled water; shaken for 20 min; and centrifuge for 15 min at 3000 x *g*; pool the aqueous layers and mix with 1 mL of a methanolic solution containing 25 mg/mL PFBHA and 0.1% (w/v) BHT, and sonicate for 1 h; HNE-oxime was then extracted into pentane (2 × 10 mL); pentane extract was evaporated to dryness at 40 °C; 200 µl of 10% TMCS in BSTFA was added; mixture was heated at 90 °C for 1 h; 1.0 ml of dichloromethane was added		System: GC-MS Column: Sac-5 (30 m x 0.25 mm i.d. x 0.25 µm film thickness) Run time: 35 min Oven ramp: 40 °C (5 min); increase to 300 °C at a rate of 10 °C/min (hold 20 min) Carrier gas: Helium Flow rate: 1 mL/min Injection mode: - Injector temperature: - Detector temperature: - Ion source temperature: 250 °C Ionization energy: 70 eV		
Canola oil	Novel procedure: 200 mg of oil in isooctane was loaded into column with conditioned to 5% water silica gel; non-polar fraction was eluted with 10 mL of 15% diisopropyl ether in hexane; polar fraction was eluted with four 5 mL portions of methanol; combined polar fractions were subsequently concentrated to the volume of 5 mL; then centrifuged at 2300 rpm for 5 min, and the supernatant analyzed for HNE DNPH method: 1 g of oil was mixed with 2 mL of DNPH and incubated overnight at room temperature in the dark; the oil was extracted three times with 5 mL methanol/water (75:25, v/v); centrifugation at 2500 rpm for 10 min; extract three times with dichloromethane (5 mL each); centrifuge and evaporate the dichloromethane extract until the sample size 1 mL; the sample was separated on a silica gel TLC plate using dichloromethane; the band corresponding to HNE–DNPH was extracted three times with methanol (10 mL each); the combined methanol extract was centrifuged at 2500 rpm for 10 min; the supernatant was concentrated to 2.0 mL Solvent extraction: 200 mg of sample dissolved in 2 mL isooctane; homogenized with 5 mL of methanol/water (8:2, v/v); extraction was repeated three times and the combined extract was centrifuged at 2500 rpm for 5 min; sample volume was reduced to 5 mL; solution was centrifuged, and the supernatant was analyzed	HNE	System: HPLC-UV-Vis Column: C18 Novapak (300 × 3.9 mm, i.d., 4 µm particle size) Run time: 35 min Mobile phase: ACN/H_2O (30:70, v/v) Flow rate: 0.75 mL/min Injection volume: 20 µL Column temperature: - Detection (λ): 223 nm	–	Aladedunye, Matthäus, and Przybylski 2011; Aladedunye and Przybylski 2011, 2012
Sunflower, high-oleic sunflower, rapeseed, and high-oleic rapeseed oils; palm olein and French fries	5 g of oil in 10 mL glass headspace vials; fully automated extraction using a GERSTEL MPS-2 autosampler configured for auto-DHS injection; after each oil had incubated in the sample bottle for 5 min at 120 °C, dynamic sampling was performed by connecting a tube to the outlet of the sample bottle and applying a flow of nitrogen at 50 mL/min for 30 min; the desorption of the trapped volatile compounds was carried out for 15 min using a Gerstel thermal desorption unit-2 thermal desorption unit mounted on top of the CIS-3 PTV injector; for	HNE	System: GC-MS Column: VF-624MS (30 m x 0.18 mm i.d. x 0.32 µm film thickness) Run time: - Oven ramp: 60 °C (1 min), then 5° C/min to 90 °C and 30 °C/min to 240 °C Carrier gas: Helium Flow rate: 1.5 mL/min Injection mode: split Injector temperature: - Detector temperature: -	Range: 1-65 µg/mL	Petersen et al. 2013

	all experiments, desorption was in split mode (split ratio 1:10) using helium at a flow rate of 15 ml/min; the thermal desorption unit-2 was programmed from 40 to 300 °C at 720 °C/min with a final time of 5 min		Ion source temperature: 230 °C Ionization energy: - Range m/z: -		
Potato crisps, beef, vegetable oils, infant formula, cookies, soya based products, meat based products, cheese, walnuts, and fish	Oils: 500 mg sample were mixed with 0.5 ml of hexane; vortex for 1 min; add 2 ml of water/methanol (60:40, v/v) and vortex; centrifuge at 2,000 × *g* for 2 min; 1 ml of the aqueous portion was subjected to derivatization Infant formulas: QuEChERS were applied; 500 mg sample was mixed with 2 ml of water; vortex for 1 min; 3 ml of acetonitrile was added; vortex 1 min; add 0.3 g of NaCl and 1 g of MgSO4 and mix 1 min; centrifuge at 3,600 × *g* for 5 min; 2 mL of the supernatant was collected and evaporated prior to derivatization Potato crisps: 500 mg of sample mixed with 5 ml of 1.66 M H2SO4/methanol (60:40, v/v); vortex 2 min; centrifuge at 3,600 × *g* for 5 min; the aqueous phase was filtered; 2.5 mL of the filtrate was subjected to derivatization; Extracts were mixed with 1 mL of methanolic solution of 4 mg/ml PFBHA; samples were incubated for 1 h at 40 °C; extract two times with 2 mL of pentane and dried over sodium sulfate prior to collection; rotary evaporator at 30 °C; the remaining oximes were transferred to a glass vial with 200 μL of pentane; evaporate; for silylation, 20 μL of 10 % TMCS in BSTFA and 80 μL of pyridine were added; the mixture was vortexed for 1 min	HNE; HHE	System: GC-MS Column: HP-5MS (30 m x 0.25 mm i.d. x 0.25 μm film thickness) Run time: 30 min Oven ramp: 50 °C (held 1 min), increased to 150 °C at a rate of 10 °C/min, from 150 to 200 °C at a rate of 3 °C/min and finally up to 250 °C at a rate of 40 °C/min Carrier gas: Helium Flow rate: 0.8 mL/min Injection mode: splitless Injection volume: 1 μL Injector temperature: 200 °C Detector temperature: - Ion source temperature: - Ionization energy: 70 eV Range m/z: -	**HNE**: r^2 (0.9625-0.9995); LOD (4.2-32 ng/g); LOQ (12.8-97.3 ng/g) **HHE**: r^2 (0.9966 – 0.9994); LOD (4.2-10.4 ng/g); LOQ (12.7-31.1 ng/g)	Papastergiadis et al. 2014a, Papastergiadis et al. 2014b
French fries, vegetable oils	1 g of the oil extracted were mixed with 5 mL DNPH overnight at room temperature; DNPH derivatives were extracted from the oil with methanol/water (75:25, v/v) and with dichloromethane; DNPH derivatives were then separated by TLC with dichloromethane; elute from the TLC plates with methanol and evaporate to 1 mL	HNE	System: HPLC-UV-Vis Column: Ultrasphere ODS (250 × 4.6 i.d., 5 μm particle size) Run time: 40 min Mobile phase: MeOH/ H_2O (50:50, v/v) Flow rate: 0.8 mL/min Injection volume: 10 μL Column temperature: - Detection (λ): 378 nm System: HPLC-MS Column: Ultrasphere ODS (250 × 4.6 i.d., 5 μm particle size) Mobile phase (gradient elution): (A) H_2O; (B) ACN with 0.1% formic acid Ionization mode: electrospray Detection mode: negative Range m/z:50-1000 Source temperature:120 °C	–	Csallany et al. 2015
Linseed oil	2 g of oil was mixed with 200 μL BHT (1 mg/ml in chloroform), 100 μL MeMDA (10 ng/μL) and 1,900 μL water/ethanol (50:50, v/v); vortex 15 s; centrifuge at 3,700 x *g* for 5 min; repeat the extraction with 2 ml water/ethanol (50:50, v/v); filtrate the supernatant; DNPH prepared by 250 μL DNPH solution (0.05 M in ethanol/HCl 12 M 9:1 (v/v)) was added to 750 μL of	HNE; HHE	System: HPLC-MS Column: Atlantis T3 C18 (150 × 2.1 mm i.d., 3 μm particle size) Run time: - Mobile phase (gradient elution): (A) ACN; (B) CH_3COOH (pH 3.55) Flow rate: 0.25 mL/min	**HNE**: range (0.06-3.0 mg/kg); r^2 (0.9938); LOD (0.14 mg/kg); LOQ (0.7-3.0 mg/kg) **HHE**: range (0.06-3.0 mg/kg); r^2	Douny et al. 2015

(*continued*)

Table 2. Continued.

Food matrices	Samples preparation/ Extraction	Compounds	Chromatographic conditions	Validation data	References
	extract in an injection vial and reaction took place for 2 h at 60 °C		Injection volume: 20 μL Column temperature: 40 °C Detection (λ): - Ionization mode: electrospray Detection mode: negative Range m/z:249-293 (HNE) ; 293-335 (HHE) Source temperature: -	(0.9953); LOD (0.02 mg/kg); LOQ (0.06-2.34 mg/kg)	
Oils of avocado, blackcurrant, apricot kernel, echium, sesame, and wheat germ	3 ml of oil were mixed with 3 ml of acetonitrile; vortex 30 s; centrifuge at 2500 x *g* for 2 min; 1 ml of upper phase were mixed with 100 ml of 20 mM pentafluorophenylhydrazine solution and 10 ml of 22 mM TFA in acetonitrile, then shake; after 10 min the supernatant was withdrawn and analyzed	HNE	System: UHPLC-MS-MS Column: Hypersil Gold (50 × 2.1 mm i.d., 1.9 μm particle size) Run time: 8 min Mobile phase (gradient elution): (A) H_2O (0.1% formic acid); (B) CH_3CN (0.1% formic acid) Flow rate: 0.25 mL/min Injection volume: 2 μL Column temperature: 25 °C Detection (λ): - Ionization mode: electrospray Detection mode: positive Range m/z:315-336 Source temperature: -	Range: 11-21362 ng/g; r^2: 0.9974; LOD: 3.4 ng/g; LOQ: 11 ng/g	Gabbanini et al. 2015
Rapeseed, sunflower, kiwiseed and tuna oils	15 − 100 mg of oil were acidified with H2SO4 and extracted with hexane, using SPE silica cartridge (500 mg; 6 ml) previously equilibrated with 5 ml ethanol and 5 ml hexane; lipids were eluted by 5 ml hexane, and the oxime derivatives with 5 ml hexane/diethyl ether (50/50; v/v); the hydroxyl group was converted into trimethylsilylether during a 30 min treatment at 60 °C with BSTFA	HNE; HHE	System: GC-MS Column: HP-5MS (30 m x 0.25 mm i.d. x 0.25 μm film thickness) Run time: - Oven ramp: 57 °C, increased to 180 °C at 20 °C/min, and to 280 °C at 4 °C/min Carrier gas: Helium Flow rate: - Injection mode: splitless Injection volume: - Injector temperature: 260 °C Detector temperature: - Ion source temperature: 130 °C Ionization energy: 70 eV Range m/z: -	**HNE**: range (0.1-1.5 μg/g); LOQ (0.5 nmoles/g) **HHE**: range (0.1-1.5 μg/g); LOQ (0.7 nmoles/g)	Viau et al. 2016

Seppanen and Csallany 2004; Seppanen and Csallany 2006; Zanardi et al. 2002) with ultrapure water (aqueous phase) are the most used composition for the mobile phases.

In the GC-MS methods for 4-hydroxy-2-alkenals separation and quantification in food matrices, the most frequently applied columns are fused silica capillary columns coated with (5%-phenyl)-methylpolysiloxane (Guillén and Goicoechea 2009; LaFond et al. 2011; Papastergiadis et al. 2014a; Papastergiadis et al. 2014b; Surh and Kwon 2005; Surh, Lee, and Kwon 2010, 2007; Viau et al. 2016), being 30 m and 0.25 µm the most used length and film thickness of the columns, respectively.

Conditions linked with the formation and mitigation of 4-hydroxy-2-alkenals in foods

Up to now, different authors have dedicated their research to the influence of several factors on the formation of 4hydroxy-2-alkenals in foods, namely the importance of fatty acids composition of foodstuffs; effect of air, temperature and/or time; and processing technology for vegetable oils manufacture, among others. However, other mitigation approaches have also been studied, such as the addition of phenolic acids extracts, vitamin E and modified atmospheres (Aladedunye, Matthäus, and Przybylski 2011; Fujisaki, Endo, and Fujimoto 2002; Zamora et al. 2016; Zhu et al. 2009; Alghazeer and Howell 2008).

Fatty acids composition

Surh, Lee, and Kwon (2007) have studied the occurrence of HHE and HNE in 56 commercially available polyunsaturated fortified foods, including infant formulas (n = 12), baby foods (n = 7), children-friendly soymilks (n = 5), cereals/vegetable powders (n = 5), yoghurts (n = 5), children-friendly milks (n = 7), fruit juices (n = 2), fish sticks (n = 4), tuna cans (n = 3) and snacks (n = 6). Moreover, authors have studied the time dependence of 4-hydroxy-2-alkenals levels at 10 and 30 days after opening infant formulas and baby foods (Surh, Lee, and Kwon 2007). It was verified that for infant formulas and baby foods low correlations were observed between PUFA and 4-hydroxy-2-alkenals occurrence, while for the other foods there was no correlation, which were attributed to the presence of antioxidant/prooxidant constituents in the analyzed foods (Surh, Lee, and Kwon 2007).

Later, this study was extended to 73 Korean foods, namely sesame oils (n = 20), perilla oils (n = 18), olive oils (n = 3), corn oils (n = 3), soybean oils (n = 3), pepper seed oils (n = 2), sunflower oil (n = 1), safflower oil (n = 1), rice bran oil (n = 1), fish (n = 11) and shellfish (n = 10) (Surh, Lee, and Kwon 2010). However, the results were quite different from the previous ones because HNE concentration was strongly and significantly correlated with n-6 PUFA ($r = 0.512$, $p < 0.01$) and with PUFA ($r = 0.462$, $p < 0.01$). On the other hand, weak correlations were observed between HHE and n-3 PUFA ($r = 0.086$, $p = 0.607$) (Surh, Lee, and Kwon 2010).

In 2009, Han and Csallany (2009) have studied fatty acid methyl esters (methyl stearate, methyl oleate and methyl linoleate) thermally treated (0 to 6 h at 185 °C) to follow the formation of 4-hydroxy-2-octenal (HOE), HNE, HHE and 4-hydroxy-2-decenal (HDE), as well as 2,4-decadienal which is a precursor of HNE (Han and Csallany 2009). According to their results, methyl stearate and methyl oleate did not produce any lipophilic aldehyde under study. However, HHE was found in higher concentrations especially in methyl linolenate, while HNE was detected in higher amounts in methyl linoleate. On the other hand, authors have concluded that 2,4-decadienal was not found to be a precursor of lipophilic aldehydes in thermally oxidized fatty acids (Han and Csallany 2009).

In 2013, Petersen et al. (2013) have evaluated volatile compounds in different frying oils, being the highest amount of HNE reported for sunflower oil in comparison with other oils (high-oleic sunflower oil, rapeseed oil, high-oleic rapeseed oil and palm olein), which was probably due to their fatty acids profile, but also due to the presence of antioxidants (Petersen et al. 2013).

Csallany et al. (2015) have analyzed the HNE content of French fries acquired in six different fast-food chains and have correlated the observed HNE amounts with the fatty acids profile (Csallany et al. 2015). Linoleic acid content in the fat extracted from French fries varied between 24.7 and 54.9% of total fatty acids and the samples with a higher content of linoleic acid also contained a higher amount of HNE. Nonetheless, one exception was reported, since for two of the samples of French fries with higher amounts of linoleic acid (52.3 and 54.9% of total fatty acids), the content of HNE was 2.35 and 0.68 µg/g of fat, respectively. This was probably due to differences in the length of heating time and temperature of the oil used to fry (Csallany et al. 2015).

Temperature/time

HNE formation along different periods of time (2, 4, 6, 8 and 10 h) at the same frying conditions (185 °C) was studied (Seppanen and Csallany 2002). After 6 h HNE concentration reached the maximum value (42.5 µg/g of oil). Then, in the following 4 h of heating the amount of HNE decreased, which was probably due to the degradation of HNE in other compounds. Another interesting aspect of this study was that authors have investigated the relationship between HNE formation and the presence of tocopherols. Apparently, the presence of tocopherols in soybean oil restricts the formation of these aldehydes in the first hours of heating (Seppanen and Csallany 2002).

Seppanen and Csallany (2006) have investigated the effect of intermittent and continuous heating on the formation of HNE, HHE, HOE and HDE in soybean oil, and for both treatments, over a total of 5 h, a similar increase was observed. With respect to the applied methods, it is very important to take into account that the total heating time and temperature was similar for intermittent (185 ± 5 °C; 1 h/day; during 5 days) and continuous (185 ± 5 °C; 6 h) heating, however the treatments were different concerning

the equipment and the volume of oil used (Seppanen and Csallany 2006).

Han and Csallany (2008) investigated the temperature dependence of HNE formation in vegetable oils and butter. Samples of corn and soybean oils (higher contents of linoleic acid) and butter (lower contents of linoleic acid) were heated for different periods at 190 °C (recommended frying temperature) and 218 °C. It was found that HNE amounts after 30 min of heating at 218 °C were 3.7, 4.9 and 8.7 times higher than at 190 °C, for soybean, corn and butter oil, respectively (Han and Csallany 2008). It is important to enhance, that 218 °C is not a real frying temperature, being these results correlated with extreme frying conditions. Furthermore, these experiments were conducted in the absence of food, which highly influences the frying temperature, since after food immersion the frying temperature decreases.

Primary and secondary oxidation products of corn oil were studied in samples stored (up to 121 months) at room temperature (between 20 a 25 °C), under different air-oil volume ratios and different air-oil contact surfaces (Guillén and Goicoechea 2009). For samples of corn oil stored for longer periods of time (103 and 121 months) under the same air-oil contact surfaces conditions, but with different air-oil volume ratios (0.3; 1.321; 6.230; 7.090), it was found that the increase of 4-hydroxy-2-alkenals amounts was correlated with a higher air-oil volume ratio (Guillén and Goicoechea 2009).

In 2012, the effects of heat treatment on the formation of HNE in natural low-moisture part-skim and imitation Mozzarella cheeses have been studied (Han and Csallany 2012). The samples of cheese were heated at 204 °C (30 and 60 min) and at 232 °C (15 and 30 min), since these temperatures are commonly applied at home and in commercial applications. Regarding the comparison between natural and imitation Mozzarella cheeses, Han and Csallany (2012) concluded that the HNE formations were much higher in imitation cheeses than in natural cheeses, which was expected since the imitation cheeses are manufactured with vegetable oils usually rich in linoleic acid that is a precursor of HNE.

Besides the temperature during frying, another important aspect is the presence or absence of foods, since it can significantly contribute to the changes that occur in the oil/fat. Therefore, LaFond et al. (2011) have evaluated the formation of HNE in a corn-soy oil blend submitted to frying conditions with or without food products. Authors have reported that the frying oil (without food) have a higher concentration of HNE than the frying oil (with food), which is in disagreement with other studies (Seppanen and Csallany 2004; Seppanen and Csallany 2002; LaFond et al. 2011). LaFond et al. (2011) justify their results based on the type of experiment carried out (volume-to-surface ratios), since there are differences between a small-scale bench top experiment and a pilot plant scale experiment.

Concerning the time of frying and the occurrence of HNE, Petersen et al. (2013) have subjected sunflower oil, high-oleic sunflower oil, rapeseed oil, high-oleic rapeseed oil, and palm olein to frying (170 ± 2 °C) up to 36 h. It was possible to observe that until 20 h of deep-frying, sunflower oil contained significantly more 4-HNE compared to high-oleic sunflower oil. Moreover, after 27 h of deep-frying palm olein showed the lowest amount of HNE (Petersen et al. 2013).

Ma and Liu (2017) have performed accelerated oxidation tests (60 °C for 30 days and 180 °C for 5 h) on palm, corn, rapeseed, camellia and linseed oils, to study the effect on HNE and HHE occurrence. Higher amounts of HNE and HHE occur when oils were subjected to 60 °C for 30 days than for 180 °C for 5 h, except for corn oil, suggesting that the relationship between temperature and time could highly influence lipid oxidation reactions.

Sodium chloride

Sodium chloride (NaCl), commonly known as salt, is frequently added to foods for sensorial purposes, as well as to reduce microbial activity. Nevertheless, salt may promote lipid oxidation, namely because: (i) it is able to disrupt cell membrane integrity facilitating the access of oxidizing agents to lipid substrates; (ii) it contributes to the liberation of iron ions; and (iii) it inhibits the activity of antioxidant enzymes such as catalase, glutathione peroxidase and superoxide dismutase (Hernández, Park, and Rhee 2002; Lee, Mei, and Decker 1997; Mariutti and Bragagnolo 2017).

In 2004, Sakai et al. have performed a study on the effects of 1% and 2% NaCl on HNE formation in minced pork and beef, during 10 days of storage at 0 °C (Sakai et al. 2004). For pork, HNE content increased only after 7 and 10 days of storage when 2% of NaCl was used, while for beef, HNE content increased in all samples during storage (Sakai et al. 2004). Later, the effect of boiling of pork meat without (control sample) and with 1% or 2% of NaCl on HNE formation was studied, and researchers concluded that neither boiling nor NaCl have effect on the concentration of HNE. Moreover, they also evaluated the effect of storage time and observed that the HNE content of the control sample increased significantly after 3-days and was much higher than in the samples that contained NaCl (Sakai, Shimizu, and Kawahara 2006). The results reported herein are controversial and it is not possible to evaluate whether the content of NaCl can be linked with 4-hydroxy-2-alkenals increase in this type of foods.

Phenolic acids

For several years, synthetic antioxidants have been widely utilized to retard lipid oxidation in foods. Nevertheless, several concerns have arisen due to their possible toxicity. Therefore, the demand for natural antioxidants has increased, and consequently the research concerning the potential of several plant foods as natural sources of antioxidants has also emerged (Costa et al. 2015). Phenolic compounds are naturally present in different foods, namely aromatic plants, fruits, vegetables, coffee, tea, wine and chocolate, among others. To retard the oxidation reactions, it is common to prepare extracts from these matrices to be

further added to other food products and increase their antioxidant properties (Maqsood et al. 2014).

So far, few studies were conducted aiming to evaluate mitigation strategies for HNE and HHE reduction. For instance, Zhu et al. have studied the effectiveness of 21 natural polyphenols as scavenging agents of HNE and eight of these compounds effectively scavenged HNE (13.4 − 90.1%). The most effective was phloretin, followed by theaflavin-3,3′-digallate, epigallocatechin-3-gallate, epicatechin-3-gallate and epigallocatechin (Zhu et al. 2009).

The capacity of phenolic acids (ferulic, caffeic, dihydrocaffeic, gallic and vanillic acids) to improve frying performance of canola oil was evaluated (Aladedunye and Przybylski 2011). Frying experiments were conducted at 180 °C during 2 h and canola oil was fortified with 500 mg/kg of each phenolic acid. All the phenolic compounds significantly contributed to HNE decrease, because the control sample after 2 h of frying reached 11.2 mg/kg of HNE, while for the samples enriched with phenolic acids the HNE amount varied between 4.9 and 7.1 mg/kg (Aladedunye and Przybylski 2011). In this research study, phenolic acids were also combined with the sterol and the tocopherol fractions of canola oil. The reported results support a positive interaction between phenolic acids and endogenous canola sterols, probably due to the formation of steryl ferulates (γ-oryzanol) during frying (Aladedunye and Przybylski 2011).

To mitigate the formation of HNE in mackerel, green tea extracts (250 and 500 mg/kg) were used and the occurrence of HNE was studied during 26 weeks at −10 °C against control samples (Alghazeer and Howell 2008). The results indicated that HNE amounts were higher in the control samples (0.45 mg/kg) than for the samples of mackerel with green tea.

Vitamin E

Vitamin E is a fat-soluble vitamin that comprises eight enantiomers, namely α-, β-, γ-, δ-tocopherols, and α-, β-, γ-, δ-tocotrienols. It is recognized that dietary supplementation of vitamin E (α-tocopherol) enhances the inclusion of the antioxidant into the phospholipid membrane where PUFA are located, and therefore decreases lipid peroxidation and increases the stability (Brewer 2011). Therefore, Grune et al. have studied the influence of supplementation of the diet of Leghorn laying hens with different concentrations of vitamin E (0 to 160 IU/kg) on the occurrence of cytotoxic lipid peroxidation products (malondialdehyde, HNE and HHE) during the production and storage of n-3 PUFA enriched eggs (Grune et al. 2001). After 4 weeks of storage, it was notable that a diet supplemented with vitamin E ($\geq$ 80 IU/kg) was effective and no difference in the content of HNE and HHE was observed. Furthermore, these findings enhance that with these doses of vitamin E, it is possible to protect against oxidation, high contents of n-3 and n-6 PUFA in eggs (Grune et al. 2001).

Aladedunye and Przybylski have tested several combinations of tocopherol isomers during frying with canola oil triacylglycerols on the mitigation of HNE (Aladedunye and Przybylski 2012). The different combinations of tocopherols were obtained by isolation from rice bran and canola oils. The vitamin E fraction of rice bran oil was composed by 822.2 ± 57 mg/g of vitamin E, being γ-tocopherol the most abundant isomer (505.1 mg/g). On the other hand, the other fraction, that was obtained from canola oil, had a lower total vitamin E content (791.1 ± 40 mg/g), but seven enantiomers of vitamin E were quantified and the most abundant was γ-tocotrienol (317.4 mg/g). Results indicated that the HNE content significantly decreased in the presence of tocopherols (from 11.7 to 6.2 mg/kg). Nevertheless, concerning the different vitamin E fractions no significant differences were observed in the amounts of HNE formed (Aladedunye and Przybylski 2012).

Modified atmospheres

One other feasible approach to reduce lipid oxidation is the use of modified atmospheres, namely by the reduction of oxygen or by adding carbon dioxide (Hotchkiss, Werner, and Lee 2006). Aladedunye et al. have evaluated the effect of carbon dioxide blanketing on the formation of HNE in French fries in comparison with common frying procedures (Aladedunye, Matthäus, and Przybylski 2011). To perform this test, authors have used a fryer with 8 L capacity, that was heated at 185 °C, 7 h/day for 7 days, and every day eight batches (400 g) of frozen French fries were prepared using canola oil. The carbon dioxide was delivered into the fryer at a flow rate of 2.5 L/min, 10 min prior to frying, and after finishing each frying day, the flow of carbon dioxide was kept until the frying oil reached 100 °C. The reported results indicate that HNE was reduced by about 62%, when frying under carbon dioxide was applied (Aladedunye, Matthäus, and Przybylski 2011). This is a very interesting approach, although questionable if it is feasible at a large-scale industry, namely concerning cost effectiveness.

Another proposed approach, by Fujisaki et al. was to use a low oxygen atmosphere to reduce the formation of volatile aldehydes (Fujisaki, Endo, and Fujimoto 2002). Nevertheless, these experiments were carried out at a laboratory scale and did not use equipment usually applied under realistic frying conditions. Although authors concluded that if the atmosphere concentration of oxygen is kept below 2%, it is possible to significantly decrease the presence of these hazardous compounds, namely HNE. Once more, it was not evaluated if this technique is reasonable for an industrial scale, as well as the economic viability and impact.

4-Hydroxy-2-alkenals occurrence in foods

During the last forty years, several types of foodstuffs were studied regarding the occurrence of these toxic compounds (Tables 3–7). Some difficulties were encountered during this review, namely because of the presentation of data in graphics (Uchida, Gotoh, and Wada 2002; Aladedunye and Przybylski 2012; Grune et al. 2001; Michalski et al. 2008; LaFond et al. 2011; Petersen et al. 2013); impossibility to convert results in order to perform comparisons with other

Table 3. Occurrence of 4-hydroxy-2-alkenals in edible oils.

			HNE (mg/kg)		HHE (mg/kg)		
Food matrices	Conditions	Country of origin	Mean	SD	Mean	SD	Reference
Soybean oil A	as purchased	Japan	4.06E-09	1.27E-09	–	–	Liu, Miao, and Toyo'oka 1996
Soybean oil B	as purchased	Japan	5.62E-09	5.47E-10	–	–	Liu, Miao, and Toyo'oka 1996
Sesame oil A	as purchased	Japan	9.33E-08	4.69E-10	–	–	Liu, Miao, and Toyo'oka 1996
Sesame oil B	as purchased	Japan	1.23E-07	8.44E-10	–	–	Liu, Miao, and Toyo'oka 1996
Soybean oil	8h; 185 °C	United States	2.45	0.15	0.17	0.02	Seppanen and Csallany 2001
Soybean oil	6h; 180 °C	United States; Industry	42.50	–	–	–	Seppanen and Csallany 2002
Olive oil	as purchased	Korea; household	2.667E-06	7.218E-07	–	–	Surh and Kwon 2003
Perilla oil	as purchased	Korea; household	3.343E-07	1.875E-08	–	–	Surh and Kwon 2003
Frying oil A	as purchased	Korea; local market	1.968E-07	3.437E-08	–	–	Surh and Kwon 2003
Frying oil B	as purchased	Korea; local market	2.062E-07	3.437E-08	–	–	Surh and Kwon 2003
Frying oil C	as purchased	Korea; local market	3.437E-07	6.562E-08	–	–	Surh and Kwon 2003
Soybean oil	as purchased	United States; local grocery store	5.76	1.10	–	–	Seppanen and Csallany 2004
Soybean oil	5h; 185 °C	United States; local grocery store	4.39	0.41	–	–	Seppanen and Csallany 2004
Soybean oil	1h; 185 °C	United States; local stores	2.27	0.22	0.40	0.08	Seppanen and Csallany 2006
Soybean oil	1h; 185 °C; storage 1 day	United States; local stores	2.85	0.65	0.14	0.01	Seppanen and Csallany 2006
Soybean oil	2h; 185 °C	United States; local stores	2.01	0.28	1.23	0.46	Seppanen and Csallany 2006
Soybean oil	2h; 185 °C; storage 1 day	United States; local stores	1.91	0.36	0.26	0.03	Seppanen and Csallany 2006
Soybean oil	3h; 185 °C	United States; local stores	2.62	0.13	0.30	0.07	Seppanen and Csallany 2006
Soybean oil	3h; 185 °C; storage 1 day	United States; local stores	3.22	0.17	0.44	0.07	Seppanen and Csallany 2006
Soybean oil	4h; 185 °C	United States; local stores	3.33	0.15	0.94	0.14	Seppanen and Csallany 2006
Soybean oil	4h; 185 °C; storage 1 day	United States; local stores	3.58	0.56	0.37	0.09	Seppanen and Csallany 2006
Butter oil	3h; 190 °C	United States; retail stores	1.85	–	–	–	Han and Csallany 2008
Butter oil	5 min; 218 °C	United States; retail stores	2.13	–	–	–	Han and Csallany 2008
Butter oil	30 min; 218 °C	United States; retail stores	6.71	–	–	–	Han and Csallany 2008
Corn oil	1h; 190 °C	United States; retail stores	5.46	–	–	–	Han and Csallany 2008
Corn oil	5 min; 218 °C	United States; retail stores	5.93	–	–	–	Han and Csallany 2008
Corn oil	30 min; 218 °C	United States; retail stores	15.48	–	–	–	Han and Csallany 2008
Soybean oil	1h; 190 °C	United States; retail stores	3.73	–	–	–	Han and Csallany 2008
Soybean oil	5 min; 218 °C	United States; retail stores	8.08	–	–	–	Han and Csallany 2008
Soybean oil	30 min; 218 °C	United States; retail stores	10.72	–	–	–	Han and Csallany 2008
Perilla oil	Traditional produced	Korea; local shops	0.07	0.03	0.25	0.19	Surh, Lee, and Kwon 2010
Perilla oil	Industrial produced	Korea; industry	0.07	0.04	0.22	0.35	Surh, Lee, and Kwon 2010
Sesame oil	Traditional produced	Korea; local shops	0.28	0.15	0.06	0.02	Surh, Lee, and Kwon 2010
Sesame oil	Industrial produced	Korea; industry	0.10	0.09	0.08	0.05	Surh, Lee, and Kwon 2010
Frying oil ω-3 enriched	5h; 170 °C	Belgium	3.75	0.02	0.52	0.01	Papastergiadis et al. 2014a
Frying oil		Belgium	3.76	0.02	0.18	0.01	Papastergiadis et al. 2014a
Colza oil		Belgium	0.11	–	0.15	–	Papastergiadis et al. 2014a
Extra virgin olive oil A		Belgium	0.07	–	0.16	–	Papastergiadis et al. 2014a
Extra virgin olive oil B		Belgium	0.12	–	0.14	–	Papastergiadis et al. 2014a
Extra virgin olive oil C		Belgium	0.06	–	0.10	–	Papastergiadis et al. 2014a
Plant oils	as purchased	Belgium; supermarket chains	0.04	–	0.02	–	Papastergiadis et al. 2014a
Linseed oil	60 °C; 12 days of storage	Belgium	63.92	–	9.44	–	Douny et al. 2015
Linseed oil	60 °C; 24 days of storage	Belgium	191.76	–	42.40	–	Douny et al. 2015
Mixed seeds oil	as purchased	Italy; grocery stores	0.05	0.00	–	–	Gabbanini et al. 2015
Mixed seeds oil	1h; 180 °C	Italy; grocery stores	1.41	0.09	–	–	Gabbanini et al. 2015
Mixed seeds oil	7h; 180 °C	Italy; grocery stores	3.47	0.11	–	–	Gabbanini et al. 2015
Mixed seeds oil	3 years, room temperature	Italy; grocery stores	–	–	–	–	Gabbanini et al. 2015
Corn oil	as purchased	Italy; grocery stores	nd	nd	–	–	Gabbanini et al. 2015
Corn oil	1h; 180 °C	Italy; grocery stores	2.02	0.10	–	–	Gabbanini et al. 2015
Corn oil	7h; 180 °C	Italy; grocery stores	2.59	0.23	–	–	Gabbanini et al. 2015
Corn oil	3 years, room temperature	Italy; grocery stores	2.68	0.04	–	–	Gabbanini et al. 2015
Soybean oil	as purchased	Italy; grocery stores	nd	–	–	–	Gabbanini et al. 2015

Soybean oil	1h; 180 °C	Italy; grocery stores	1.43	0.06	–	–	Gabbanini et al. 2015
Soybean oil	7h; 180 °C	Italy; grocery stores	2.87	0.08	–	–	Gabbanini et al. 2015
Soybean oil	3 years; room temperature	Italy; grocery stores	13.32	0.09	–	–	Gabbanini et al. 2015
Peanut oil	as purchased	Italy; grocery stores	nd	–	–	–	Gabbanini et al. 2015
Peanut oil	1h; 180 °C	Italy; grocery stores	1.11	0.02	–	–	Gabbanini et al. 2015
Peanut oil	7h; 180 °C	Italy; grocery stores	2.00	0.13	–	–	Gabbanini et al. 2015
Peanut oil	3 years, room temperature	Italy; grocery stores	–	–	–	–	Gabbanini et al. 2015
Sunflower oil	as purchased	Italy; grocery stores	nd	–	–	–	Gabbanini et al. 2015
Sunflower oil	1h; 180 °C	Italy; grocery stores	1.35	0.04	–	–	Gabbanini et al. 2015
Sunflower oil	7h; 180 °C	Italy; grocery stores	3.30	0.08	–	–	Gabbanini et al. 2015
Sunflower oil	3 years, room temperature	Italy; grocery stores	19.05	0.18	–	–	Gabbanini et al. 2015
Extra-virgin olive oil	as purchased	Italy; grocery stores	nd	–	–	–	Gabbanini et al. 2015
Extra-virgin olive oil	1h; 180 °C	Italy; grocery stores	–	–	–	–	Gabbanini et al. 2015
Extra-virgin olive oil	7h; 180 °C	Italy; grocery stores	–	–	–	–	Gabbanini et al. 2015
Extra-virgin olive oil	3 years, room temperature	Italy; grocery stores	–	–	–	–	Gabbanini et al. 2015
Avocado oil	as purchased	Italy; grocery stores	0.14	0.00	–	–	Gabbanini et al. 2015
Avocado oil	90min; 75 °C	Italy; grocery stores	0.37	0.02	–	–	Gabbanini et al. 2015
Blackcurrant oil	as purchased	Italy; grocery stores	0.55	0.02	–	–	Gabbanini et al. 2015
Blackcurrant oil	90min; 75 °C	Italy; grocery stores	0.80	0.03	–	–	Gabbanini et al. 2015
Apricot kernel oil	as purchased	Italy; grocery stores	0.05	0.00	–	–	Gabbanini et al. 2015
Apricot kernel oil	90min; 75 °C	Italy; grocery stores	0.11	0.00	–	–	Gabbanini et al. 2015
Echium oil	as purchased	Italy; grocery stores	0.18	0.01	–	–	Gabbanini et al. 2015
Echium oil	90min; 75 °C	Italy; grocery stores	0.18	0.01	–	–	Gabbanini et al. 2015
Sesame oil	as purchased	Italy; grocery stores	0.06	0.00	–	–	Gabbanini et al. 2015
Sesame oil	90min; 75 °C	Italy; grocery stores	0.09	0.00	–	–	Gabbanini et al. 2015
Wheat germ oil	as purchased	Italy; grocery stores	nd	–	–	–	Gabbanini et al. 2015
Wheat germ oil	90min; 75 °C	Italy; grocery stores	nd	–	–	–	Gabbanini et al. 2015
Kiwiseed oil	as purchased	France	0.52	0.02	0.17	0.02	Viau et al. 2016
Tuna oil	as purchased	France	0.20	0.06	0.16	0.02	Viau et al. 2016
Sunflower oil	as purchased	France; local supermarket	0.02	0.00	0.16	0.00	Viau et al. 2016
Rapeseed oil	as purchased	France; local supermarket	0.08	0.02	0.19	0.06	Viau et al. 2016
Soybean oil	Crude	China; local industry	644.2	–	16.3	–	Hua et al. 2016
Rapeseed oil	Crude	China; local industry	264.4	–	21.5	–	Hua et al. 2016
Soybean oil	After refining	China; local industry	260.0	–	5.65	–	Hua et al. 2016
Rapeseed oil	After refining	China; local industry	37.35	–	nd	–	Hua et al. 2016
Palm oil	as purchased	China; local industry	nd	–	nd	–	Ma and Liu 2017
Palm oil	5h; 180 °C	China; local industry	1.51E-03	1.50E-04	nd	–	Ma and Liu 2017
Corn oil	as purchased	China; local industry	nd	–	nd	–	Ma and Liu 2017
Corn oil	5h; 180 °C	China; local industry	4.78E-03	1.80E-04	nd	–	Ma and Liu 2017
Rapeseed oil	as purchased	China; local industry	nd	–	nd	–	Ma and Liu 2017
Rapeseed oil	5h; 180 °C	China; local industry	2.71E-03	1.30E-04	2.23E-03	1.50E-04	Ma and Liu 2017
Camellia oil	as purchased	China; local industry	nd	–	nd	–	Ma and Liu 2017
Camellia oil	5h; 180 °C	China; local industry	1.40E-03	1.10E-04	nd	–	Ma and Liu 2017
Linseed oil	as purchased	China; local industry	nd	–	nd	–	Ma and Liu 2017
Linseed oil	5h; 180 °C	China; local industry	2.80E-03	2.30E-04	5.96E-03	2.70E-04	Ma and Liu 2017

nd, not detected

Table 4. Occurrence of 4-hydroxy-2-alkenals in infant formulas, milk and dairy products.

Food matrices	Conditions	Country of origin	HNE mg/kg)		HHE mg/kg)		Reference
			Mean	SD	Mean	SD	
Infant formula A	as purchased	Korea; local markets	0.05	0.01	0.04	0.00	Surh, Lee, and Kwon 2007
Infant formula A	10 days storage, room temperature	Korea; local markets	0.17	0.02	0.07	0.01	Surh, Lee, and Kwon 2007
Infant formula B	as purchased	Korea; local markets	0.05	0.02	0.04	0.00	Surh, Lee, and Kwon 2007
Infant formula B	10 days storage, room temperature	Korea; local markets	0.21	0.01	0.11	0.02	Surh, Lee, and Kwon 2007
Infant formula C	as purchased	Korea; local markets	0.07	0.03	0.05	0.00	Surh, Lee, and Kwon 2007
Infant formula C	10 days storage, room temperature	Korea; local markets	0.29	0.03	0.11	0.00	Surh, Lee, and Kwon 2007
Infant formula D	as purchased	Korea; local markets	0.07	0.01	0.08	0.00	Surh, Lee, and Kwon 2007
Infant formula D	10 days storage, room temperature	Korea; local markets	0.28	0.09	0.12	0.06	Surh, Lee, and Kwon 2007
Infant formula E	as purchased	Korea; local markets	0.06	0.01	0.07	0.02	Surh, Lee, and Kwon 2007
Infant formula E	10 days storage, room temperature	Korea; local markets	0.42	0.11	0.12	0.02	Surh, Lee, and Kwon 2007
Infant formula F	as purchased	Korea; local markets	0.13	0.05	0.06	0.01	Surh, Lee, and Kwon 2007
Infant formula F	10 days storage, room temperature	Korea; local markets	0.50	0.04	0.19	0.03	Surh, Lee, and Kwon 2007
Infant formula G	as purchased	Korea; local markets	0.06	0.01	0.04	0.01	Surh, Lee, and Kwon 2007
Infant formula G	10 days storage, room temperature	Korea; local markets	0.24	0.06	0.09	0.01	Surh, Lee, and Kwon 2007
Infant formula H	as purchased	Korea; local markets	0.09	0.03	0.05	0.01	Surh, Lee, and Kwon 2007
Infant formula H	10 days storage, room temperature	Korea; local markets	0.49	0.05	0.12	0.01	Surh, Lee, and Kwon 2007
Infant formula I	as purchased	Korea; local markets	0.12	0.01	0.08	0.01	Surh, Lee, and Kwon 2007
Infant formula I	10 days storage, room temperature	Korea; local markets	0.79	0.03	0.22	0.03	Surh, Lee, and Kwon 2007
Infant formula J	as purchased	Korea; local markets	0.10	0.03	0.06	0.01	Surh, Lee, and Kwon 2007
Infant formula J	10 days storage, room temperature	Korea; local markets	0.65	0.23	0.16	0.03	Surh, Lee, and Kwon 2007
Infant formula K	as purchased	Korea; local markets	0.05	0.04	nd	–	Surh, Lee, and Kwon 2007
Infant formula K	10 days storage, room temperature	Korea; local markets	0.08	0.07	nd	–	Surh, Lee, and Kwon 2007
Infant formula L	as purchased	Korea; local markets	0.04	0.01	nd	–	Surh, Lee, and Kwon 2007
Infant formula L	11 days storage, room temperature	Korea; local markets	0.29	0.13	0.05	0.01	Surh, Lee, and Kwon 2007
Yoghurt	as purchased	Korea; local markets	1.10E-03	4.00E-04	nd	–	Surh, Lee, and Kwon 2007
Yoghurt PUFA fortified A	as purchased	Korea; local markets	1.70E-03	6.00E-04	nd	–	Surh, Lee, and Kwon 2007
Yoghurt PUFA fortified B	as purchased	Korea; local markets	1.00E-03	2.00E-04	nd	–	Surh, Lee, and Kwon 2007
Yoghurt PUFA fortified C	as purchased	Korea; local markets	1.33E-02	8.00E-03	nd	–	Surh, Lee, and Kwon 2007
Yoghurt PUFA fortified D	as purchased	Korea; local markets	6.10E-03	1.40E-03	nd	–	Surh, Lee, and Kwon 2007
Soymilk A	as purchased	Korea; local markets	1.19E-02	7.00E-04	1.20E-03	1.10E-03	Surh, Lee, and Kwon 2007
Soymilk B	as purchased	Korea; local markets	1.42E-02	6.30E-03	2.30E-03	2.20E-03	Surh, Lee, and Kwon 2007
Soymilk C	as purchased	Korea; local markets	1.81E-02	3.10E-03	3.90E-03	6.00E-04	Surh, Lee, and Kwon 2007
Soymilk D	as purchased	Korea; local markets	3.00E-02	4.80E-03	7.40E-03	1.70E-03	Surh, Lee, and Kwon 2007
Soymilk E	as purchased	Korea; local markets	1.42E-02	4.00E-03	3.30E-03	3.00E-03	Surh, Lee, and Kwon 2007
Milk A	as purchased	Korea; local markets	4.90E-03	4.00E-03	nd	–	Surh, Lee, and Kwon 2007
Milk B	as purchased	Korea; local markets	9.00E-03	6.70E-03	nd	–	Surh, Lee, and Kwon 2007
Milk C	as purchased	Korea; local markets	1.01E-02	7.70E-03	1.10E-03	9.00E-04	Surh, Lee, and Kwon 2007
Milk D	as purchased	Korea; local markets	1.00E-02	6.00E-03	5.00E-04	5.00E-04	Surh, Lee, and Kwon 2007
Milk E	as purchased	Korea; local markets	5.70E-03	1.20E-03	nd	–	Surh, Lee, and Kwon 2007
Milk F	as purchased	Korea; local markets	1.76E-02	5.40E-03	nd	–	Surh, Lee, and Kwon 2007
Milk G	as purchased	Korea; local markets	4.40E-03	1.10E-03	nd	–	Surh, Lee, and Kwon 2007
Natural mozzarella cheese A	30 min, 204 °C	United States; local store	0.01	–	–	–	Han and Csallany 2012
Natural mozzarella cheese B	30 min, 204 °C	United States; local store	0.01	–	–	–	Han and Csallany 2012
Natural mozzarella cheeses	30 min, 204 °C	United States; local store	0.01	–	–	–	Han and Csallany 2012
Natural mozzarella cheeses	30 min, 232 °C	United States; local store	0.18	–	–	–	Han and Csallany 2012
Imitation mozzarella cheese A	30 min, 204 °C	United States	0.09	–	–	–	Han and Csallany 2012
Imitation mozzarella cheese B	30 min, 204 °C	United States	0.13	–	–	–	Han and Csallany 2012
Imitation mozzarella cheese A	60 min, 204 °C	United States	1.12	–	–	–	Han and Csallany 2012
Imitation mozzarella cheese B	60 min, 204 °C	United States	0.78	–	–	–	Han and Csallany 2012
Imitation mozzarella cheeses	30 min, 204 °C	United States	0.11	–	–	–	Han and Csallany 2012
Imitation mozzarella cheeses	30 min, 232 °C	United States	0.88	–	–	–	Han and Csallany 2012
Cheese	as purchased	Belgium; supermarket chains	0.03	–	0.01	–	Papastergiadis et al. 2014a
Infant formula	as purchased	Belgium	0.21	0.01	0.02	0.003	Papastergiadis et al. 2014a
Full fat milk	as purchased	Belgium; supermarket chains	0.03	–	0.005	–	Papastergiadis et al. 2014a

nd, not detected; PUFA, polyunsaturated fatty acids

Table 5. Occurrence of 4-hydroxy-2-alkenals in fish and fish products.

			HNE (mg/kg)		HHE (mg/kg)		
Food matrices	Conditions	Country of origin	Mean	SD	Mean	SD	Reference
Horse mackerel	as purchased	Japan; commercial markets	nd	–	–	–	Sakai et al. 1995
Sardine	as purchased	Japan; commercial markets	1,22E-07 – 7,72E-07	–	–	–	Sakai et al. 1995
Yellowfin tuna	as purchased	Japan; commercial markets	nd	–	–	–	Sakai et al. 1995
Skipjack	as purchased	Japan; commercial markets	nd	–	–	–	Sakai et al. 1995
Flying fish	as purchased	Japan; commercial markets	nd	–	–	–	Sakai et al. 1995
Yellowtail	as purchased	Japan; commercial markets	5,00E-08 – 2,95E-06	–	–	–	Sakai et al. 1995
Coho salmon	as purchased	Japan; commercial markets	3,41E-07 – 1,47E-06	–	–	–	Sakai et al. 1995
Yellowtail	as purchased	Japan; commercial markets	–	–	4.79E-07	–	Sakai et al. 1997
Horse mackerel	as purchased	Japan; commercial markets	–	–	1.94E-07	–	Sakai et al. 1997
Mackerel	as purchased	Japan; commercial markets	–	–	4.34E-07	–	Sakai et al. 1997
Carp	as purchased	Japan; commercial markets	–	–	1.05E-06	–	Sakai et al. 1997
Yellowtail sample 1	as purchased	Japan; commercial markets	5.47E-08	–	8.48E-07	–	Sakai et al. 1997
Yellowtail sample 2	as purchased	Japan; commercial markets	nd	–	7.98E-07	–	Sakai et al. 1997
Yellowtail sample 3	as purchased	Japan; commercial markets	nd	–	6.36E-07	–	Sakai et al. 1997
Yellowtail	as purchased	Japan; commercial markets	–	–	0.37	0.22	Sakai, Kazuhiro, and Eto 2000
Yellowtail	after 28 weeks of storage; −20 °C	Japan; commercial markets	–	–	6.94	0.50	Sakai, Kazuhiro, and Eto 2000
Smoked salmon I	as purchased	Japan; commercial markets	–	–	1.24	0.76	Munasinghe et al. 2003
Smoked salmon II	as purchased	Japan; commercial markets	–	–	nd	–	Munasinghe et al. 2003
Smoked salmon III	as purchased	Japan; commercial markets	–	–	0.01	0.00	Munasinghe et al. 2003
Fish meat sausage	as purchased	Japan; commercial markets	–	–	0.02	0.00	Munasinghe et al. 2003
Yellow croaker	as purchased	Korea; local markets	0.01	0.00	0.02	0.00	Surh and Kwon 2005
Mackerel raw	as purchased	Korea; local markets	0.01	0.00	0.01	0.01	Surh and Kwon 2005
Mackerel salted	as purchased	Korea; local markets	0.02	0.00	0.08	0.00	Surh and Kwon 2005
Tuna canned	as purchased	Korea; local markets	0.01	0.00	0.03	0.01	Surh and Kwon 2005
Spanish mackerel	as purchased	Korea; local markets	0.01	0.00	0.02	0.00	Surh and Kwon 2005
Hair tail	as purchased	Korea; local markets	nd	–	nd	–	Surh and Kwon 2005
Pacific saury raw	as purchased	Korea; local markets	0.02	0.01	0.06	0.02	Surh and Kwon 2005
Pacific saury canned	as purchased	Korea; local markets	0.01	0.00	0.04	0.00	Surh and Kwon 2005
Flat fish	as purchased	Korea; local markets	nd	–	nd	–	Surh and Kwon 2005
Alaska pollak	as purchased	Korea; local markets	0.01	0.00	0.02	0.00	Surh and Kwon 2005
Alabesque greenling	as purchased	Korea; local markets	0.01	0.00	0.05	0.01	Surh and Kwon 2005
Murex shell	as purchased	Korea; local markets	0.01	0.00	0.04	0.01	Surh and Kwon 2005
Granulated ark shell	as purchased	Korea; local markets	nd	–	nd	–	Surh and Kwon 2005
Hard-shelled mussel	as purchased	Korea; local markets	nd	–	nd	–	Surh and Kwon 2005
Pen shell	as purchased	Korea; local markets	nd	–	nd	–	Surh and Kwon 2005
Oyster	as purchased	Korea; local markets	0.04	0.01	0.14	0.00	Surh and Kwon 2005
Surf clam	as purchased	Korea; local markets	0.01	0.00	0.04	0.01	Surh and Kwon 2005
Jack-knife clam	as purchased	Korea; local markets	0.01	0.00	0.03	0.00	Surh and Kwon 2005
Venus clam	as purchased	Korea; local markets	0.02	0.01	0.07	0.02	Surh and Kwon 2005
Orient hard clam	as purchased	Korea; local markets	0.05	0.01	0.16	0.05	Surh and Kwon 2005
Little neck clam	as purchased	Korea; local markets	0.06	0.00	0.18	0.01	Surh and Kwon 2005
Fish sticks A	as purchased	Korea; local markets	0.01	0.01	nd	–	Surh, Lee, and Kwon 2007
Fish sticks B	as purchased	Korea; local markets	0.03	0.01	nd	–	Surh, Lee, and Kwon 2007
Fish sticks C	as purchased	Korea; local markets	0.04	0.02	0.01	0.01	Surh, Lee, and Kwon 2007
Fish sticks D	as purchased	Korea; local markets	0.04	0.02	0.01	0.01	Surh, Lee, and Kwon 2007
Tuna canned A	as purchased	Korea; local markets	0.02	0.02	nd	nd	Surh, Lee, and Kwon 2007
Tuna canned B	as purchased	Korea; local markets	0.03	0.01	nd	–	Surh, Lee, and Kwon 2007
Tuna canned C	as purchased	Korea; local markets	nd	–	nd	–	Surh, Lee, and Kwon 2007
Mackerel	26 weeks of storage; −10 °C	United Kingdom; Industry	0.45	0.02	–	–	Alghazeer and Howell 2008
Sardines canned	as purchased	Belgium	nd	–	0.03	0.00	Papastergiadis et al. 2014a
Frozen and fresh salmon	as purchased	Belgium; supermarket chains	0.01	–	0.03	–	Papastergiadis et al. 2014a
Smoked salmon	as purchased	Belgium; supermarket chains	0.02	–	0.00	–	Papastergiadis et al. 2014a

nd, not detected

reported results; and the presentation of the sum of HNE and HHE, instead of individual values.

Oils and fats

Oils and fats processing usually aims to improve their quality, stability and safety (Albuquerque, Oliveira, and Costa 2018). Despite the removal of a large amount of impurities from the oil, processing can often originate new contaminants (Choe and Min 2006). In general, vegetable oils have several similar features, for example low moisture content and are mainly composed by triacylglycerides (Foster, Williamson, and Lunn 2009; Dubois et al. 2007). However, they also have differences which are linked with raw materials and factors such as climate, soil, plant varieties, geography and state of ripeness (Minihane and Harland 2007). These differences are most of the time responsible for their behavior and functionality, namely in what concerns fatty acids composition; and the presence of minor compounds, such as vitamin E, sterols and phenolics.

In Table 3, an overview of the occurrence data on 4-hydroxy-2-alkenals in vegetable oils is provided. It is

Table 6. Occurrence of 4-hydroxy-2-alkenals in meat and meat products.

			HNE (mg/kg)		HHE (mg/kg)		
Food matrices	Conditions	Country of origin	Mean	SD	Mean	SD	Reference
Beef meat	as purchased	Japan; commercial markets	2.19E-06 – 21.9E-06	–	–	–	Sakai et al. 1995
Pork meat	as purchased	Japan; commercial markets	1.56E-07 – 23.7E-06	–	–	–	Sakai et al. 1995
Pork meat	as purchased	Japan; commercial markets	9.42E-07	2.17E-07	–	–	Sakai et al. 1998
Pork meat	3 days of storage; 0 °C	Japan; commercial markets	1.10E-06	3.19E-07	–	–	Sakai et al. 1998
Pork meat	7 days of storage; 0 °C	Japan; commercial markets	1.16E-06	2.94E-07	–	–	Sakai et al. 1998
Pork meat	12 days of storage; 0 °C	Japan; commercial markets	4.37E-06	8.73E-07	–	–	Sakai et al. 1998
Pork meat	2 months of storage; −20 °C	Japan; commercial markets	2.97E-08	1.56E-08	–	–	Sakai et al. 1998
Pork meat	4 months of storage; −20 °C	Japan; commercial markets	7.98E-07	3.44E-07	–	–	Sakai et al. 1998
Pork meat	6 months of storage; −20 °C	Japan; commercial markets	7.97E-07	7.50E-07	–	–	Sakai et al. 1998
Pork meat	8 months of storage; −20 °C	Japan; commercial markets	2.34E-06	1.08E-06	–	–	Sakai et al. 1998
Pork meat	11 months of storage; −20 °C	Japan; commercial markets	2.19E-06	8.75E-07	–	–	Sakai et al. 1998
Pork meat	2 months of storage; −80 °C	Japan; commercial markets	1.33E-07	1.06E-07	–	–	Sakai et al. 1998
Pork meat	4 months of storage; −80 °C	Japan; commercial markets	4.64E-07	3.06E-07	–	–	Sakai et al. 1998
Pork meat	6 months of storage; −80 °C	Japan; commercial markets	1.37E-06	8.59E-07	–	–	Sakai et al. 1998
Pork meat	8 months of storage; −80 °C	Japan; commercial markets	8.59E-07	6.72E-07	–	–	Sakai et al. 1998
Pork meat	11 months of storage; −80 °C	Japan; commercial markets	1.72E-07	1.41E-07	–	–	Sakai et al. 1998
Milano sausage	as purchased	Italy; local supermarkets	nd				Zanardi et al. 2002
Cacciatore sausage	as purchased	Italy; local supermarkets	nd				Zanardi et al. 2002
Smoked sausage A	as purchased	Italy; local supermarkets	nd				Zanardi et al. 2002
Smoked sausage B	as purchased	Italy; local supermarkets	nd				Zanardi et al. 2002
Smoked sausage C	as purchased	Italy; local supermarkets	0.41	0.04			Zanardi et al. 2002
Smoked sausage D	as purchased	Italy; local supermarkets	0.28	0.14			Zanardi et al. 2002
Parma ham	as purchased	Italy; local supermarkets	nd				Zanardi et al. 2002
Cooked ham	as purchased	Italy; local supermarkets	nd				Zanardi et al. 2002
Zampone A	as purchased	Italy; local supermarkets	nd				Zanardi et al. 2002
Zampone B	as purchased	Italy; local supermarkets	0.34	0.09			Zanardi et al. 2002
Zampone C	as purchased	Italy; local supermarkets	0.13	0.09			Zanardi et al. 2002
Zampone D	as purchased	Italy; local supermarkets	0.15	0.06			Zanardi et al. 2002

(*continued*)

Table 6. Continued.

			HNE (mg/kg)		HHE (mg/kg)		
Food matrices	Conditions	Country of origin	Mean	SD	Mean	SD	Reference
Frankfurter A	as purchased	Italy; local supermarkets	0.71	0.14			Zanardi et al. 2002
Frankfurter B	as purchased	Italy; local supermarkets	0.62	0.05			Zanardi et al. 2002
Frankfurter C	as purchased	Italy; local supermarkets	0.27	0.05			Zanardi et al. 2002
Frankfurter D	as purchased	Italy; local supermarkets	0.46	0.04			Zanardi et al. 2002
Mortadella A	as purchased	Italy; local supermarkets	0.06	0.01			Zanardi et al. 2002
Mortadella B	as purchased	Italy; local supermarkets	0.16	0.04			Zanardi et al. 2002
Mortadella C	as purchased	Italy; local supermarkets	0.32	0.05			Zanardi et al. 2002
Mortadella D	as purchased	Italy; local supermarkets	0.08	0.02			Zanardi et al. 2002
Ham A	as purchased	Japan; local market	14.9	2.35			Munasinghe et al. 2003
Ham B	as purchased	Japan; local market	0.59	0.53			Munasinghe et al. 2003
Ham C	as purchased	Japan; local market	3.19	2.86			Munasinghe et al. 2003
Bacon A	as purchased	Japan; local market	1.28	0.40			Munasinghe et al. 2003
Bacon B	as purchased	Japan; local market	1.39	0.81			Munasinghe et al. 2003
Wiener sausage A	as purchased	Japan; local market	6.98	1.32			Munasinghe et al. 2003
Wiener sausage B	as purchased	Japan; local market	0.59	0.59			Munasinghe et al. 2003
Frankfurter sausage	as purchased	Japan; local market	2.69	2.40			Munasinghe et al. 2003
Pork meat	as purchased	Japan; local market	nd	nd			Munasinghe et al. 2003
Pork meat	3 days storage in 1% Sugi wood vinegar, 0 °C	Japan; local market	0.03	0.03			Munasinghe et al. 2003
Pork meat	7 days storage in 1% Sugi wood vinegar, 0 °C	Japan; local market	10.6	1.89			Munasinghe et al. 2003
Pork meat	10 days storage, 0 °C	Japan; local market	nd	nd			Sakai et al. 2004
Pork meat	10 days storage with 1% NaCl, 0 °C	Japan; local market	3.44E-03	3.12E-04			Sakai et al. 2004
Pork meat	10 days storage with 2% NaCl, 0 °C	Japan; local market	4.31E-02	3.44E-03			Sakai et al. 2004
Beef meat	10 days storage, 0 °C	Japan; local market	0.04	0.01			Sakai et al. 2004
Beef meat	10 days storage with 1% NaCl, 0 °C	Japan; local market	0.15	0.03			Sakai et al. 2004
Beef meat	10 days storage with 2% NaCl, 0 °C	Japan; local market	0.11	0.04			Sakai et al. 2004
Pork meat	raw; after 3 days of storage	Japan; local market	1.56E-08	6.25E-09			Sakai, Shimizu, and Kawahara 2006
Pork meat	cooked 100 °C with 1% NaCl; after 3 days of storage	Japan; local market	1.56E-09	1.56E-09			Sakai, Shimizu, and Kawahara 2006
Pork meat	cooked 100 °C with 2% NaCl; after 3 days of storage	Japan; local market	1.56E-09	1.56E-09			Sakai, Shimizu, and Kawahara 2006
Pork meat	5 min; cooked 100 °C	Japan; local market	8.28E-08	4.22E-08			Sakai, Shimizu, and Kawahara 2006
Pork meat	10 min; cooked 100 °C	Japan; local market	1.11E-07	2.19E-08			Sakai, Shimizu, and Kawahara 2006
Pork meat	15 min; cooked 100 °C	Japan; local market	2.39E-07	4.53E-08			Sakai, Shimizu, and Kawahara 2006
Pork meat	5 min; cooked 100 °C; after 3 days storage	Japan; local market	2.44E-07	8.44E-08			Sakai, Shimizu, and Kawahara 2006
Pork meat	10 min; cooked 100 °C; after 3 days storage	Japan; local market	4.64E-07	1.48E-07			Sakai, Shimizu, and Kawahara 2006
Pork meat		Japan; local market	3.14E-07	1.09E-07			Sakai, Shimizu, and Kawahara 2006

(*continued*)

Table 6. Continued.

Food matrices	Conditions	Country of origin	HNE (mg/kg)		HHE (mg/kg)		Reference
			Mean	SD	Mean	SD	
	15 min; cooked 100 °C; after 3 days storage						
Chicken meat	as purchased	France; local supermarket	nd				Gasc et al. 2007
Beef meat	as purchased	France; local supermarket	nd				Gasc et al. 2007
Liver pâté	as purchased	France; local supermarket	0.11				Gasc et al. 2007
Blood sausage	as purchased	France; local supermarket	12.5				Gasc et al. 2007
Bacon	as purchased	Belgium; supermarket chains	0.01		0.00		Papastergiadis et al. 2014a
Cured ham	as purchased	Belgium; supermarket chains	0.08		0.00		Papastergiadis et al. 2014a
Cured and cooked meat products	as purchased	Belgium; supermarket chains	0.11		0.01		Papastergiadis et al. 2014a
Cured minced meat products	as purchased	Belgium; supermarket chains	0.55		0.05		Papastergiadis et al. 2014a
Chilled cooked meals	as purchased	Belgium; supermarket chains	0.10		0.01		Papastergiadis et al. 2014a

nd, not detected

possible to confirm that the most analyzed compound is undoubtedly HNE and the most studied is soybean oil (Liu, Miao, and Toyo'oka 1996; Seppanen and Csallany 2006; Seppanen and Csallany 2004; Han and Csallany 2008; Hua et al. 2016; Gabbanini et al. 2015). In what concerns the reported amounts, very different values were found for the different origins of soybean oil. For instance, the determined HNE amounts varied between not detected and 644.2 mg/kg, for sunflower oil acquired in Italy and crude sunflower oils from China, respectively (Gabbanini et al. 2015; Hua et al. 2016). Nevertheless, it should be highlighted that crude oils are not edible oils; therefore, this value could be considered less important. Although, after refining, the same sample still contained high amounts of HNE (260 mg/kg), which may be of concern (Hua et al. 2016).

Palm oil, which is the most consumed worldwide, is the less studied regarding the presence of these compounds. To our knowledge, only Ma and Liu (2017) have evaluated palm oil, and HNE was not detected in the sample as acquired, and a very low amount (1.51 µg/kg) was detected when the sample was subjected to 180 °C for 5 h. The lack of interest in this type of food by researchers is probably due to its fatty acids composition because it is mainly composed by saturated and monounsaturated fatty acids, which are not considered precursors of these compounds.

Regarding HHE, the highest amounts were reported for linseed oil (42.4 mg/kg), rapeseed oil (21.5 mg/kg) and soybean oil (16.3 mg/kg) (Douny et al. 2015; Hua et al. 2016). However, two of these samples were crude oils from China and the amounts of HHE significantly decreased after processing, being not detected for rapeseed oil and 5.65 mg/kg for soybean oil.

Another interesting aspect concerning the origin of samples is for example the values reported by Hua et al. (2016) and Ma and Liu (2017), for oils acquired from local industries in China. For instance, both authors analyzed rapeseed oil, but Hua et al. (2016) reported that after refining, it still had 37.4 mg/kg of HNE and HHE was not detected, while Ma and Liu (2017) have not detected both compounds. This is probably linked with different processing conditions applied or even with the initial composition of the matrix (fatty acids and antioxidants) and oxidation *status*, among others.

In what concerns the samples analyzed as purchased, very low amounts of HNE were reported (Table 3). Furthermore, for almost 35% of the results reported for oils, HNE was not detected and the highest result (5.76 mg/kg) was reported for sunflower oil. This is a crucial information, because it allows us to clearly understand that most of the oils analyzed so far are safe when they are purchased by consumers, independently on the type of oil and the local where it is sold (Gabbanini et al. 2015; Liu, Miao, and Toyo'oka 1996; Ma and Liu 2017; Papastergiadis et al. 2014a; Surh and Kwon 2003; Viau et al. 2016).

Infant formulas, milk and dairy products

In Table 4, an overview of the reported results for HNE and HHE occurrence in infant formulas, milk and dairy products is provided. Infant formulas and milk are especially focused because children and young people, which have a lower body weight than adults, consume a high quantity of these products; therefore, their exposure risk is higher.

Table 7. Occurrence of 4-hydroxy-2-alkenals in other food products.

			HNE (mg/kg)		HHE (mg/kg)		
Food matrices	Conditions	Country of origin	Mean	SD	Mean	SD	Reference
Peanuts A	packed under modified atmosphere	Belgium	0.60	-	nd	-	Papastergiadis et al. 2014a
Peanuts B	packed under modified atmosphere	Belgium	0.12	-	nd	-	Papastergiadis et al. 2014a
Walnuts A	packed under modified atmosphere	Belgium	0.14	-	0.02	-	Papastergiadis et al. 2014a
Walnuts B	packed under modified atmosphere	Belgium	0.41	-	0.03	-	Papastergiadis et al. 2014a
Dry nuts	as purchased	Belgium; supermarket chains	0.31	-	0.01	-	Papastergiadis et al. 2014a
Peanuts	as purchased; raw	Germany; local market	1.56E-07[a)]	-		-	Globisch et al. 2015
Peanuts	20min; 170 °C	Germany; local market	6.25E-07[a)]	-		-	Globisch et al. 2015
Peanuts	40 min; 170 °C	Germany; local market	9.37E-07[a)]	-		-	Globisch et al. 2015
Cookies A	as purchased	Belgium	0.13	-	2.50E-02	-	Papastergiadis et al. 2014a
Cookies B	as purchased	Belgium	0.10	-	2.10E-02	-	Papastergiadis et al. 2014a
Cookies C	as purchased	Belgium	0.18	-	2.10E-02	-	Papastergiadis et al. 2014a
Potato crisps	as purchased	Belgium; supermarket chains	0.12	-	8.00E-03	-	Papastergiadis et al. 2014a
Cookies	as purchased	Belgium; supermarket chains	0.09	-	4.00E-03	-	Papastergiadis et al. 2014a
Soya based products	as purchased	Belgium; supermarket chains	0.06	-	2.00E-03	-	Papastergiadis et al. 2014a
Fried snacks	as purchased	Belgium; fast-food shops	0.62	-	2.00E-03	-	Papastergiadis et al. 2014a
Fruit juice A	as purchased	Korea; local markets	0.00	0.00	nd	nd	Surh, Lee, and Kwon 2007
Fruit juice B	as purchased	Korea; local markets	0.00	0.00	nd	nd	Surh, Lee, and Kwon 2007
Raw cereal/ vegetable powder A	as purchased	Korea; local markets	0.14	0.07	0.03	0.01	Surh, Lee, and Kwon 2007
Raw cereal/ vegetable powder B	as purchased	Korea; local markets	0.06	0.01	0.02	0.01	Surh, Lee, and Kwon 2007
Raw cereal/ vegetable powder C	as purchased	Korea; local markets	0.31	0.07	0.02	0.00	Surh, Lee, and Kwon 2007
Raw cereal/ vegetable powder D	as purchased	Korea; local markets	0.19	0.06	0.03	0.01	Surh, Lee, and Kwon 2007
Raw cereal/ vegetable powder E	as purchased	Korea; local markets	0.13	0.04	0.03	0.02	Surh, Lee, and Kwon 2007
Snacks A	as purchased	Korea; local markets	0.06	0.05	0.01	0.00	Surh, Lee, and Kwon 2007
Snacks B	as purchased	Korea; local markets	0.02	0.01	nd	nd	Surh, Lee, and Kwon 2007
Snacks C	as purchased	Korea; local markets	0.07	0.02	nd	nd	Surh, Lee, and Kwon 2007
Snacks D	as purchased	Korea; local markets	0.08	0.02	0.01	0.01	Surh, Lee, and Kwon 2007
Snacks E	as purchased	Korea; local markets	0.04	0.03	nd	nd	Surh, Lee, and Kwon 2007
Snacks F	as purchased	Korea; local markets	0.03	0.00	nd	nd	Surh, Lee, and Kwon 2007
French fries	5h; 185 °C	United States; local grocery store	0.49	0.05	-	-	Seppanen and Csallany 2004
French fries	as purchased		0.07	-	0.00		

(*continued*)

Table 7. Continued.

Food matrices	Conditions	Country of origin	HNE (mg/kg)		HHE (mg/kg)		Reference
			Mean	SD	Mean	SD	
		Belgium; fast-food shops					Papastergiadis et al. 2014a
French fries A	as purchased	United states; fast-food restaurants	0.00	0.00	-	-	Csallany et al. 2015
French fries B	as purchased	United states; fast-food restaurants	0.00	0.00	-	–	Csallany et al. 2015
French fries C	as purchased	United states; fast-food restaurants	0.00	0.00	-	–	Csallany et al. 2015
French fries D	as purchased	United states; fast-food restaurants	0.00	0.00	-	–	Csallany et al. 2015
French fries E	as purchased	United states; fast-food restaurants	0.00	0.00	-	–	Csallany et al. 2015
French fries F	as purchased	United states; fast-food restaurants	0.00	0.00	-	–	Csallany et al. 2015
Egg yolk powder	Enriched with n-3 fatty acids; pasteurized and dried	France	6,85E-05 – 1,48E-04	-	5.47E-05	-	Meynier et al. 2014
Porphyra spp.	Grade A; dried	Japan; Industry	0.04[b)]	0.07[b)]	1.83[b)]	1.66[b)]	Tanaka et al. 2016
Porphyra spp.	Grade B; dried	Japan; Industry	0.05[b)]	0.05[b)]	7.11[b)]	4.80[b)]	Tanaka et al. 2016
Porphyra spp.	Grade C; dried	Japan; Industry	0.04[b)]	0.04[b)]	6.71[b)]	4.03[b)]	Tanaka et al. 2016
Porphyra spp.	Grade D; dried	Japan; Industry	0.06[b)]	0.03[b)]	6.79[b)]	4.41[b)]	Tanaka et al. 2016

nd, not detected; [a)] mg/kg of oil; [b)] mg/kg of dry weight

It is recommended that breastfeeding should be exclusive until six months of age, but sometimes it is not possible to follow this recommendation and infant formulas are needed to assure a normal growth development and a good health status (Silva et al. 2018).

The amounts reported for HNE content in infant formulas varied between 0.041 and 0.291 mg/kg, while for HHE it ranged from not detected to 0.188 mg/kg (Papastergiadis et al. 2014a; Surh, Lee, and Kwon 2007). For instance, Surh, Lee, and Kwon (2007) have analyzed 12 samples of different infant formulas at the moment of purchase and a great variability of results for HNE was observed, being in some cases 3 times higher. Some of the possible reasons appointed for the results found are: different techniques for pulverizing fats and oils in the manufacture of infant formulas; differences in the drying methods applied during manufacture; and differences between their composition, namely fatty acids, antioxidants (ascorbic acid and lecithin) and pro-oxidant metals (iron, zinc, copper and manganese) (Surh, Lee, and Kwon 2007).

Comparing infant formulas, soymilk and milk, it is possible to observe that at the moment of purchase, in general, the values for HNE content are higher for infant formulas than for the other. HNE content varied between 0.004 and 0.032 mg/kg for milk and from 0.012 to 0.030 mg/kg in soymilk, while HHE ranged from not detected to 0.0011 and from 0.0012 to 0.0074 mg/kg, for milk and soymilk, respectively (Papastergiadis et al. 2014a; Surh, Lee, and Kwon 2007). Concerning the other evaluated dairy products, namely cheeses and yoghurt with and without fortification with PUFA, the amounts for HNE are low for both products, except for cheese when it is thermally treated (1.12 mg/kg, when it is exposed to 204 °C for 60 min) (Han and Csallany 2012).

Fish and fish products

Fish and fish products are a source of high-quality proteins, n-3 fatty acids, as well as minerals and vitamins, which are linked with many potential health benefits (Food and Agriculture Organization of the United Nations/World Health Organization 2010).

One of the most studied matrices regarding the presence of HNE and HHE is salmon. According to our literature review, HHE content reported for smoked salmon (Table 5) varied between not detected and 1.24 mg/kg, while HNE amounts were lower than 0.015 mg/kg (Munasinghe et al. 2003; Papastergiadis et al. 2014a; Sakai and Kuwazuru 1995).

The information on the abundance of HNE and HHE occurrence in fish and fish products is scarce (Table 5). The highest concentrations of HHE were reported for yellowtail (6.94 mg/kg) and smoked salmon (1.24 mg/kg). Although, it is worthy of mention that the result reported for yellowtail is after 28 weeks of storage at −20 °C, being the HHE content much lower (0.37 mg/kg) at the moment of purchase (Sakai, Kazuhiro, and Eto 2000). This fact confirms that HNE is a good marker of food decomposition, namely concerning oxidation reactions (Alghazeer and Howell 2008). For the other types of fish, the HHE amounts were lower than for example for the other food groups discussed previously.

In what concerns HNE occurrence in fish and fish products, the reported amounts varied between not detected and 0.06 mg/kg, except for mackerel after 26 weeks of storage at −10 °C (0.45 mg/kg) (Alghazeer and Howell 2008). More studies are needed about the presence of these hazardous compounds in this type of matrices, because it undoubtedly affects the safety of fish and fish products, as well as its quality and nutritional value.

Meat and meat products

Over the last decades, trends in the consumption of meat and meat products have significantly changed. Consumers

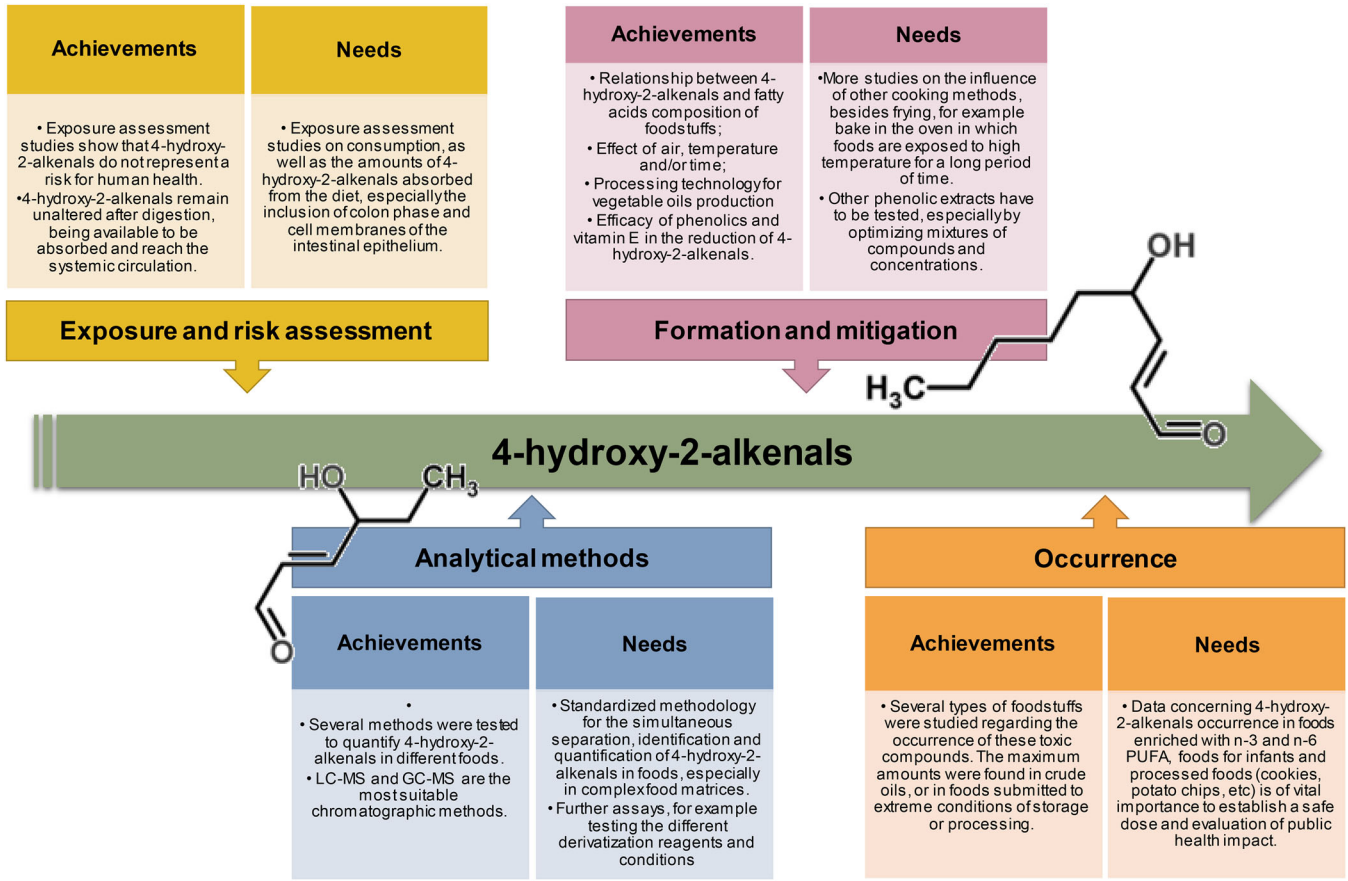


Figure 1. Summary concerning the achievements and the needs for 4-hydroxy-2-alkenals in foods, namely on exposure and risk assessment, analytical methods, formation and mitigation, and occurrence.

are aware of the potential health risks associated with a high intake of red meat, therefore, the demand for white meat has increased (Lucarini et al. 2018).

Sakai et al. have extensively evaluated the HNE content in pork meat under different conditions of storage (Sakai et al. 1995, 2004, Sakai, Shimizu, and Kawahara 2006; Sakai et al. 1998). However, the reported values for HNE content are very low in comparison with other food matrices such as fish and edible oils (Table 6), which is probably linked with the low content of PUFA in pork meat. Although, Munasinghe et al. have also studied this type of food and HNE content ranged from not detected to 10.6 mg/kg (Munasinghe et al. 2003). However, this value was for pork meat stored at 0 °C for 10 days, which clearly exceeds the recommended period of storage at this temperature.

Regarding meat products, up to now different types of products were evaluated mainly concerning HNE, such as sausages, ham, bacon and mortadella (Munasinghe et al. 2003; Papastergiadis et al. 2014a; Zanardi et al. 2002). The HNE amounts were slightly higher in these type of processed products than for pork meat, being the highest amount (14.9 mg/kg) reported for ham (Munasinghe et al. 2003). Among the different types of sausage evaluated, the highest reported HNE contents were for blood sausage (12.5 mg/kg), followed by Wiener sausage (6.98 mg/kg) (Munasinghe et al. 2003; Gasc et al. 2007).

Undoubtedly, there is a lack of data on 4-hydroxy-2-alkenals occurrence in meat and meat products. Nevertheless, considering the reported results, if appropriate conditions of storage are applied, HNE amounts are probably not a risk for human health.

Other foods

In Table 7, data on the occurrence of 4-hydroxy-2-alkenals in other foods such as nuts, bakery products, snacks, potato products and algae are shown. In fact, this is a miscellaneous group of foods because there are few data to allow performing other types of comparisons.

Nuts consumption is associated with potential health benefits, mainly due to the presence of PUFA, but also of other bioactive compounds, such as tocopherols and phytosterols. Globisch et al. and Papastergiadis et al. have evaluated the presence of 4-hydroxy-2-alkenals in peanuts, although Globisch et al. reported the results per kg of oil, while Papastergiadis et al. reported the results per kg of sample, which are not comparable (Table 7). The HNE content ranged from 0.12 to 0.60 mg/kg for peanuts, while for walnuts it varied between 0.14 and 0.41 mg/kg (Papastergiadis et al. 2014a). Nevertheless, HHE was not detected in peanuts, and for walnuts low values were reported (0.02 and 0.03 mg/kg).

Potato crisps and French fries are palatable foods, highly appreciated by people of all ages. In addition, the supply of this type of products has increased a lot in the last years, existing nowadays a wide range of products. There has also

been a change in the type of oil used in frying, and there is a tendency to use oils rich in unsaturated fatty acids, making this an interesting group to be studied regarding the presence of 4-hydroxy-2-alkenals (Albuquerque et al. 2012). Concerning potato products, namely French fries and potato crisps, the highest HNE value (0.49 mg/kg) was reported by Seppanen and Csallany for French fries, but the frying procedure was conducted under extreme conditions since thermal oxidized soybean oil was used (Seppanen and Csallany 2004). On the other hand, Csallany et al. evaluated the HNE contents in six types of French fries, being the values for all the samples lower than 0.001 mg/kg (Csallany et al. 2015).

Algae are increasingly being consumed for functional benefits linked with their nutritional and bioactive composition (Wells et al. 2017). In what concerns lipids, algae have a low content of fat, but it is mainly composed by PUFA, namely EPA. As far as authors know, only Tanaka et al. have evaluated the occurrence of 4-hydroxy-2-alkenals in dried laver *Porphyra* spp. that is consumed not only in Japan but also in other regions, such as Europe and America (Tanaka et al. 2016). HHE content in dried laver *Porphyra* spp. varied between 1.83 and 6.79 mg/kg, while HNE content was much lower, ranging from 0.04 to 0.06 mg/kg (Table 7).

Final remarks

Avoiding lipid oxidation in foods is of utmost importance and crucial to assure the safety and quality of foods, because it is responsible for the formation of hazardous compounds such as 4-hydroxy-2-alkenals. Figure 1 summarizes the achievements and the needs in the near future concerning 4-hydroxy-alkenals in foods.

With this study, it was possible to understand that these compounds occur all over the food groups, but essentially in oils and fats and foods with a high content in n-3 and n-6 PUFA that are precursors of HHE and HNE, respectively. This is worthy of special attention, mainly because there is a trend to increase the consumption of these foods, but also to enrich other foods that do not naturally contain this type of fatty acids, which possibly will represent an increase in the intake of 4-hydroxy-2-alkenals. However, the literature also evidenced that there are few studies showing that it is possible to mitigate or retard the formation of these compounds in foods using antioxidants, like vitamin E and phenolics.

As far as authors know, up to now, there is not a standard analytical methodology to determine 4-hydroxy-2-alkenals in foods, which is a difficult task taking into account the complexity of the different food matrices. Furthermore, some of the existent methods lack sensitivity and do not have acceptable recoveries. Regarding exposure and risk assessment, up to now, a low number of studies have dedicated their research to this subject, which is critical because this data is needed to establish safe doses for these aldehydes. Nevertheless, the reported results already indicate that a considerable amount of these compounds remain unaltered after digestion, which increases its availability to reach the systemic circulation.

In summary, apart from what the future holds, plentiful research should be done soon, especially concerning the occurrence of these toxic compounds in foods enriched with n-3 and n-6 PUFA. Additionally, it will only be possible to realize the desired goals, if joint efforts, that must encompass food industry, governing bodies, research and academia, are taken. Nevertheless, we believe that it is possible to eliminate or effectively reduce 4-hydroxy-2-alkenals in foods using simple and economic practices.

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