2017-11-15

Characterisation of Cold Plasma Treated Beef and Dairy Lipids Using Spectroscopic and Chromatographic Methods

Chaitanya Sarangapani
Technological University Dublin, chaitanyakrishna.sarangapani@tudublin.ie

David Dorran
Technological University Dublin, david.dorran@tudublin.ie

Julie Dunne
Technological University Dublin, julie.dunne@tudublin.ie

See next page for additional authors

Recommended Citation

Sarangapani, C., Keogh, D., Dunne, J., Bourke, P. &Cullen, P.J. (2017). Characterisation of cold plasma treated beef and dairy lipids using spectroscopic and chromatographic methods. *Food Chemistry*, vol.235, pp.324.-333. doi:10.1016/j.foodchem.2017.05.016

Funder: Department of Agriculture, Fisheries and Food, Ireland.

Elsevier Editorial System(tm) for Food Chemistry

Manuscript Draft

Manuscript Number:

Title: Characterization of cold plasma treated beef and dairy lipids using spectroscopic and chromatographic methods

Article Type: Research Article (max 7,500 words)

Keywords: Cold plasma, dairy and beef fat, FTIR, 1H NMR, chromatography, lipid oxidation

Corresponding Author: Mr. chaitanya sarangapani, Masters

Corresponding Author's Institution: Institute of chemcial technology

First Author: chaitanya sarangapani, Masters

Order of Authors: chaitanya sarangapani, Masters

Abstract: The efficacy of cold plasma for inactivation of food-borne pathogens in foods is established. However, insights on cold plasma-food interactions in terms of quality effects, particularly for oils and fats are sparse. This study evaluated plasma induced lipid oxidation of model matrices namely dairy and meat fats. Product characterization was performed using FTIR, 1H NMR and chromatographic techniques. The oxidation of lipids by cold plasma followed the Criegee mechanism and typical oxidation products identified included ozonides, aldehydes (hexanal or pentenal, nonanal, nonenal) and carboxylic acids (9-oxononanoic acid, octanoic acid, nonanoic acid along with hydroperoxides (9-and13-hydroperoxy- octadecadienoylglycerol species). However, these oxidation products were only identified following extended treatment times of 30min and were also a function of applied voltage level. Understanding cold plasma interactions with food lipids and the critical parameters governing lipid oxidation is required to design appropriate industrial adoption of the technology for food products with high fat contents.

Characterization of cold plasma treated beef and dairy lipids using spectroscopic and chromatographic methods

Chaitanya Sarangapani[1], David Ryan Keogh[1], Julie Dunne[1], Paula Bourke[1]*, P.J. Cullen[1,2]

[1]Food and Health Research Centre, School of Food Science and Environmental Health, Dublin Institute of Technology, Dublin 1, Ireland

[2]Department of Chemical and Environmental Engineering, University of Nottingham, Nottingham, NG7 2RD, United Kingdom

Running Title: Characterization of cold plasma effects on food lipids

Highlights

- This study evaluated cold plasma induced lipid oxidation of model matrices namely dairy and meat fats.
- The oxidation of lipids by cold plasma followed the Criegee mechanism.
- Oxidation products and pathways were determined using FTIR, NMR and GC-MS analysis.
- A detailed mechanism of formation of oxidation products has been proposed.

Characterization of cold plasma treated beef and dairy lipids using spectroscopic and chromatographic methods

Chaitanya Sarangapani[1], David Ryan Keogh[1], Julie Dunne[1], Paula Bourke[1]*, P.J. Cullen[1,2]

[1]Food and Health Research Centre, School of Food Science and Environmental Health, Dublin Institute of Technology, Dublin 1, Ireland

[2]Department of Chemical and Environmental Engineering, University of Nottingham, Nottingham, NG7 2RD, United Kingdom

Running Title: Characterization of cold plasma effects on food lipids

Abbreviation list

DBD	Dielectric barrier discharge plasma
ACP	Atmospheric cold plasma
FTIR	Fourier transform Infrared radiation spectroscopy
NMR	Nuclear magnetic resonance spectroscopy
GC	Gas chromatography
GC-FID	Gas chromatography-flame ionization detector
GC-MS	Gas chromatography-Mass spectrometry
HC-PC	Hierarchal cluster-Principal Component
SFA	Saturated fatty acid
USFA	Unsaturated fatty acid

Abstract

The efficacy of cold plasma for inactivation of food-borne pathogens in foods is established. However, insights on cold plasma-food interactions in terms of quality effects, particularly for oils and fats are sparse. This study evaluated plasma induced lipid oxidation of model matrices namely dairy and meat fats. Product characterization was performed using FTIR, ^{1}H NMR and chromatographic techniques. The oxidation of lipids by cold plasma followed the Criegee mechanism and typical oxidation products identified included ozonides, aldehydes (hexanal or pentenal, nonanal, nonenal) and carboxylic acids (9-oxononanoic acid, octanoic acid, nonanoic acid along with hydroperoxides (9-and13-hydroperoxy- octadecadienoylglycerol species). However, these oxidation products were only identified following extended treatment times of 30min and were also a function of applied voltage level. Understanding cold plasma interactions with food lipids and the critical parameters governing lipid oxidation is required to design appropriate industrial adoption of the technology for food products with high fat contents.

Key words: Cold plasma, dairy and beef fat, FTIR, ^{1}H NMR, chromatography, lipid oxidation

1. Introduction

Consumer demands for high quality food and the detrimental effects associated with existing thermal technologies drive the development of alternative non thermal process technologies. Cold plasma technology has been widely used in etching and deposition of electronics, bonding of plastics, dying in textiles (Korner, Beck, Dommann, Onda, & Ramm, 1995; Naebe, Cookson, Rippon, Brady, Wang, Brack, et al., 2010). It has also demonstrated efficacy in bio-decontamination (Ziuzina, Patil, Cullen, Keener, & Bourke, 2014), treatment of food packaging materials (Pankaj, Bueno-Ferrer, Misra, Milosavljević, O'Donnell, Bourke, et al., 2014) and processing of foods (Sarangapani, Devi, Thirundas, Annapure, & Deshmukh, 2015). Plasma is a partially or wholly ionized state which consists of positively and negatively charged ions, free electrons, free radicals and intermediate highly reactive species, atoms, molecules and UV photons (Thirumdas, Sarangapani, & Annapure, 2015). Cold plasma can be generated under both atmospheric and low pressure conditions. However, for food processing, given the need for economical and continuous processing, atmospheric conditions are likely to be more suitable. The effects of the plasma reactive species in any biological or chemical system are likely to continue and diversify over a longer time frame than the initial plasma discharge resulting from formation of more stable secondary reactive species and subsequent chemical pathways. Cold plasma has been used for treatment of complex dyes, wastewater (Jiang, Zheng, Qiu, Wu, Zhang, Yan, et al., 2014), degradation of mycotoxins (Park, Takatori, Sugita-Konishi, Kim, Lee, Han, et al., 2007) .

Oils and fats form an important component of the human diet containing essential fatty acids. Polyunsaturated fatty acids cannot be made by our bodies, however, these are nutritionally important (Wang, Zhu, Lyu, Panigrahy, Ferrara, Hammock, et al., 2014). Unsaturated fatty acids

start decomposing upon isolation from their natural environment, resulting in rancidity (W¹sowicz, Gramza, Hes, Jeleñ, Korczak, & Malecka, 2004). This lipid oxidation occurs in pure fats and oils, but also in fat dense foods including peanuts, pork scratching's, oatmeal, muesli, milk and meat products (Jensen, Danielsen, Bertelsen, Skibsted, & Andersen, 2005). Therefore, oxidation is a concern for dairy and meat products owing to changes in the structure of proteins, fatty acid composition, reduced nutrient value, and degradation of sensory quality. These foods undergo changes in chemical composition as a function of time, process related conditions (heat, UV treatment, photolysis) or other interactions with environmental conditions. The consequent thermal degradation and autoxidation of fats leads to the formation of primary, secondary and tertiary oxidation products such as aldehydes, ketones, carboxylic acids. Ozone processing can lead to the formation of ozonides and carbonyl oxides. Nevertheless, these technologies are used in processing of oils and fats (Soriano Jr, Migo, & Matsumura, 2003; Soriano, Migo, & Matsumura, 2003). Several authors have reported on the efficacy of cold plasma for biocontrol of hams and cheeses (Song, Kim, Choe, Jung, Moon, Choe, et al., 2009), and raw meat (Han et al, 2016). However, an understanding of the impact on chemical quality parameters is also required. A limited number of studies have described how cold plasmas have controlled and accelerated oxidation using complex matrices such as fish oil (Vandamme, Nikiforov, De Roose, Leys, De Cooman, & Van Durme, 2016). However, the mechanisms of lipid oxidation due to plasma processing have not been fully established. The aim of this work is to investigate atmospheric air plasma induced lipid oxidation in relation to dairy and meat products by assessing fatty acid composition and to identify any primary and secondary products formed. Insights into cold plasma induced reaction pathways are provided by identifying changes in the functional groups

using FTIR spectroscopy along with identification of lipid oxidation products using ^{1}H NMR spectroscopy and chromatographic techniques.

2. Materials and Methods

2.1. Materials

Dairy fat (butter oil) and beef fat (99% pure) were purchased from a local super market (Dublin, Ireland) and samples were kept under refrigerated conditions (-20 ˚C) until used for analysis. Hexane, methanol (≥99.9% capillary GC-grade), sodium hydroxide, 50% boron trifluoride in methanol, Chloroform-d ($CDCl_3$) with 0.03% (v/v) TMS (Tetramethylsilane) were purchased from Sigma-Aldrich, Ireland.

2.2. Atmospheric air cold plasma treatment

The high voltage in package atmospheric cold plasma-dielectric barrier discharge (ACP-DBD) system employed for this work is described in Sarangapani et al., (2016). Fat samples (15 ± 1g) were placed in petri dishes and the samples were subjected to different doses of direct plasma treatment. The atmospheric air condition at the time of treatment was 40 ± 1% relative humidity (RH) and 16 ± 2°C, measured using a humidity-temperature probe connected to a data logger (Testo 176T2, Testo Ltd., UK). Atmospheric air was used as the working gas. Plasma treatment was performed at variable voltage (60-80 kV) and treatment duration (3-30 min). After processing, containers were stored at room temperature of 16-18 ℃ for 24 h in line with our previous findings that a sealed retention time is useful for biocontrol. This allows contact time of the generated and contained chemical reactive species with the samples. Control samples were not plasma treated.

2.3. FTIR spectroscopy

The IR spectra were recorded in absorbance mode at 4 cm^{-1} resolutions, using a Spectrum GX FT-IR (Perkin Elmer, Dublin, Ireland) equipped with an attenuated total reflectance (ATR) over the frequency range 4000–400 cm^{-1}. The sample measurements were replicated for all the individual samples of each treatment class. Analyses were carried out at room temperature of 25°C. The background was collected before every sample was measured.

2.4. Fatty acid composition

Samples were evaluated for fatty acid composition using the GC-FID. Individual fatty acid methyl esters (FAME) were identified using FAME standards (Sigma Chemicals, Ireland) were used to calculate the percentage of fatty acids based on its peak area. A BRUKER SCION 456 GC equipped with a flame ionization detector and Zebron ZB-5MS capillary column with dimension 30 m x 0.25 mm I.D and 0.25 µm thickness is employed. Helium was used as the carrier gas and the flow rate was set at 1mL min^{-1}. Samples were injected with a split ratio (1:10). The GC oven temperature was programmed as: 50°C held for 1 min, 50°C ramp to 200°C and held for 1min, ramp to 230°C at a rate of 8°C min^{-1} held for 20 min. The injector and detector temperatures were set at 250 and 280°C, respectively. Data was collected and integrated with a personal computer using MS workstation GC Software. The GC–MS analysis of the target compounds was performed using a Varian 3800 GC (JVA analytical Ltd. Ireland) with a 2200 Varian ion trap MS was used to analyze the samples. Chromatography was conducted on the same chromatographic column and under the chromatographic conditions described above. The mass detector was operated in the electron impact (EI) mode at 70 eV and electron multiplier voltage of 1.25 kV. The mass fragments of the derivatives were obtained in the full scan mode in the scan range from *m*/*z* 35 to 350. Data were collected using Varian software. The compounds present were tentatively identified based on computer matching

against commercial National Institute of Standards and Technology (NIST) libraries and spectral library (Dalton, Dragoset, & Wiersma) of pure substances and literature available.

2.5. NMR analysis

For ^{1}H and ^{13}C NMR spectroscopy, about 50 mg of the sample was accurately weighed and dissolved in $CDCl_3$ containing TMS as standard. Both spectra were obtained using a Bruker Avance 400MHz Spectrometer. Trace amount of $CHCl_3$ in the solvent used exhibits signals at 7.26 and 77.0 ppm in ^{1}H and ^{13}C NMR, respectively.

2.6. Determination of peroxide value

The peroxide value is determined by the procedure of Kirk & Sawyer (1991).

2.7. Statistical analysis

The results were analyzed by one-way ANOVA using SPSS (IBM statistical analysis Version 19), and the significance amongst the samples was compared at $p<0.05$ by the least significant difference post-hoc comparison, SPSS 19 version. Results represent the means of at least two separate experiments.

3. Results and discussion

3.1. FTIR analysis

The changes in the functional groups for both dairy and beef fat were revealed using FT-IR analysis. The representative spectra of the control and plasma treated dairy and beef fats are presented in **Fig.1** The analytical evaluation of the dairy and beef fat spectra were previously reported (M. a. D. Guillén & Ruiz, 2001). The IR spectra demonstrate that triglycerides were dominant. The major triglyceride peaks observed were around 2937 cm^{-1} corresponding to C–H stretching (asymmetry), 2856 cm^{-1} assigned to C–H stretching (symmetry), and a carbonyl peak 1746 cm^{-1} (C=O stretching) (Adeyemi, Mohiuddin, Mirghani, & Jameel, 2012; Yang, Irudayaraj, & Paradkar, 2005). Moreover, a weak signal was observed at 3000 cm^{-1} associated

183 with –C=*C–H* stretching groups of cis-unsaturation (Ahmad Fadzlillah, Che Man, Rohman,
184 Ismail, Mustafa, & Khatib, 2013). A weak peak around 1650 cm^{-1} was observed and corresponds
185 to C=C stretching (cis) and a major peak at 1454 cm^{-1} assigned to C–H bending (scissoring). The
186 stretching vibrations of the C–O bond of esters and bending vibrations of the methylene group
187 were present around 1300–1000 cm^{-1}. Other major peaks observed were at 1166 cm^{-1} which
188 corresponds to C–O (stretching) and C–H (bending), and 723 cm^{-1} which corresponds to C–H
189 bending (rocking) (M. D. Guillén & Cabo, 1997). The peak at 1117 cm^{-1} corresponds to C-H
190 (deformation) and 1097 cm^{-1} (C–H bending) vibration of fatty acids, respectively. The peak at
191 966 cm^{-1} has been previously reported as a marker band of trans fatty acids in fats and oils and
192 the peak is associated with –HC=CH out-of-plane deformation vibrations (Ahmad Fadzlillah,
193 Che Man, Rohman, Ismail, Mustafa, & Khatib, 2013). These frequencies observed in the FTIR
194 spectra were used as the basis for investigating the changes in the functional groups of plasma
195 treated fat samples.
196 Several changes were observed in the IR spectra of the plasma treated samples. ACP-DBD
197 plasmas are a source of a wide range of active species and reactive species such as O_3, H_2O_2,
198 OH. These plasma species can cleave double bonds of unsaturated fatty acids. The IR spectra of
199 the intensities corresponding to C=C decreased with increases in applied voltages and plasma
200 treatment times. These include the relatively weak band at 1650 cm^{-1} see **Fig.1. (I and II)** and
201 the =C–H stretch and bend at 3008 cm^{-1} and broadened peak at 723 cm^{-1}, respectively. The
202 dairy samples were more susceptible to plasma treatment than the beef samples, with changes
203 evident within shorter treatment times of 3 and 6 min for all applied voltages. This is attributed
204 to the higher concentration of unsaturated fatty acids (45%) in beef fat than dairy (31%). Similar
205 trends were reported for ozonation of sunflower methyl esters by Soriano *et al.,* (2003). Several

206 authors have reported the formation of carbonylic compounds as by-products of plasma
207 treatment . Dairy and meat triglycerides have a strong absorption band at 1743 cm^{-1} attributed
208 to stretching vibration of the carbonyl group (Moreno, Olivares, López, Martínez, & Reig, 1999).
209 As observed in **Fig.1 (c and d),** increasing plasma treatment time and applied voltage widens the
210 carbonyl band, which suggests the formation of new carbonyl compounds (Gao, Sun, Wan, Yu,
211 & Li, 2013). This is attributed to the production of saturated aldehyde, carboxylic acids or other
212 secondary oxidation products. The new aldehyde or ketone compounds formed come from the
213 oxidation of C=C bonds in existing unsaturated triglycerides. The emergent band around 1725
214 cm^{-1} suggests the formation of aldehyde in the plasma treated dairy fat samples, where a
215 shoulder was observed at 1700 cm^{-1} in plasma treated beef fat samples. However, the weak band
216 at 1725 cm^{-1} in the beef samples, overlaps with the stretching vibration at 1746 cm^{-1} of the ester
217 carbonyl functional group. Moreover, the presence of an aldehydic C-H stretch around 2900-
218 2700 cm^{-1} confirms the formation of aldehyde and other oxidation products. The presence of
219 new oxidation products in both the dairy and beef samples especially the carbonyl group (C=O)
220 can be evidenced by broadened bands around 725 cm^{-1} . This peak could be ascribed to
221 complicate oligomerization of olefins (Soriano Jr, Migo, & Matsumura, 2003). Interestingly, a
222 new band around 1105 cm^{-1} (**Fig.1. a and b)** was observed across all the plasma treated dairy
223 and beef samples, which was not observed for control or the blank. The intensity of this new
224 band increased with plasma treatment time and applied voltage. This peak likely corresponds to
225 C-O stretching of ozonide (Díaz, Hernández, Ledea, Sazatornil, & Moleiro, 2003). This suggests
226 that the majority of oxidation of fats using ACP-DBD occurs by direct attack of ozone to
227 produce ozonide, as described by the Criegee mechanism (Díaz, Hernández, Ledea, Sazatornil,
228 & Moleiro, 2003; Ledea, Díaz, Molerio, Jardines, Rosado, & Correa, 2003; Sega, Zanardi,

229 Chiasserini, Gabbrielli, Bocci, & Travagli, 2010). The ozonide formed during plasma treatment
230 could be the 1,2,4-trioxolane ring which was reported by several authors (Díaz, Hernández,
231 Ledea, Sazatornil, & Moleiro, 2003; Soriano Jr, Migo, & Matsumura, 2003; Soriano, Migo, &
232 Matsumura, 2003) during ozonolysis of fatty acids methyl esters. Two other sharp bands appear
233 at 1175 cm^{-1} and 1195 cm^{-1} which also correspond to C-O stretching and O-C stretching of
234 ozonides (Soriano, Migo, & Matsumura, 2003). The other changes observed in the IR spectrum
235 associated with plasma treatment were peaks at 1379 cm^{-1} and 975 cm^{-1} , most likely associated
236 with C-O-C and stretching vibration of O-O, respectively (Georgiev, Anachkov, Batakliev, &
237 Rakovsky, 2013). The new bands formed at 969 and 829 cm^{-1} and small changes around 3470
238 cm^{-1} may be due to the formation of peroxide groups (Bellamy, 2013). The plasma treatment
239 also induced major changes in the region 1350-1475 cm^{-1}. These changes correspond to the
240 aldehydic group (C-H bend) at 1381 cm^{-1} and C-O-H bending at 1440 cm^{-1} to 1395 cm^{-1} ,which
241 is adjacent to the carbonyl group (Bailey, 2012). The scission of either initial or final ozonide
242 leads to the formation of aldehyde.

243 While trends can be observed in these IR spectra, chemometric analysis, specifically
244 hierarchical clustering, of the spectral data using principal component analysis (PCA) was
245 adopted to classify these plasma treated fats based on their FT-IR spectra. For this purpose, the
246 region between 700-1800 cm^{-1} was selected as representing the majority of the changes induced
247 in the functional groups such as C–H bending, C=O stretching, and C=C stretching and also the
248 unsaturated C=C bond. Principal component analysis of spectra within this region revealed two
249 principal components, explaining 87.3% of the data variance for the dairy sample. The cluster
250 analysis algorithm divided the dairy sample sets into six clusters (**see Fig. 2.(a)**), where a clear
251 separation between the control and the plasma treated samples was found. The replicates of the

dairy control sample are located at positive scores of PC1 and negative scores of PC2 and were grouped into cluster 1 and majority of the samples treated for 9 min at all the applied voltages (60, 70 and 80 kV) grouped into cluster 2 with 70 kV for 9 min (2^{nd} replicates as center). Moreover, the majority of samples treated for 3 min and 6 min were grouped into cluster 3. However, it may be noted that some of the replicates of treated samples are separately grouped into cluster 4, which might be due to overall variability in the plasma process (Misra, Pankaj, Frias, Keener, & Cullen, 2015). It can be observed that all replicates of samples treated for 60 kV for 30 min are cluster 5 similarly 70 kV for 30 min samples are cluster 6. Therefore, treatment time and applied voltage played an interactive role in the formation of oxidation products in dairy fat at higher treatment times.

It can be observed in **Fig.2.(b)** that two PCA scores for the beef samples explained 70.9% data variance and replicates of control sample are located in positive score of PC1 and negative score of PC2. There was a similar separation for the beef samples which are grouped into cluster 1. However, there was no clear separation obtained among the plasma treated beef samples. The majority of replicates of samples treated for 9 min or 30 min at all applied voltages were grouped into cluster 2 with the exception of the 70 kV 9 min samples. However, 70 kV 9 min samples were grouped into cluster 4 with two replicates of 60 kV 30 min, 80 kV 9 min and 80 kV 6 min. The majority of 3 min replicates of 60 kV and 70 kV were grouped into cluster 3 whereas 3 min replicates of 80 kV were grouped together with 6 min samples of 60 kV and 70 kV into cluster 5. The variability in the samples can be attributed to plasma induced changes in the functional groups such as formation of aldehydes around 1725 cm^{-1}, ozonide at 1105 cm^{-1} , peroxides at 969 cm^{-1} and 829 cm^{-1} in the beef fat samples. In addition, the natural variability in the composition of fatty acids among dairy and beef fats should be considered.

3.2. Fatty acid analysis

The fatty acid composition of control (untreated) and plasma treated dairy and beef fat are summarized in **Table 1.** The dairy and beef fats are mainly composed of oleic, palmitic and stearic acids. It can be observed that plasma treatment decreased the relative amount of unsaturated fatty acids from 34.06% to 28.47% for the dairy samples and from 44.05% to 38.60% for the meat samples. Ozone and hydroxyl radicals are considered the principal active species which cleave the double bonds of unsaturated fatty acids primarily oleic, palmtioleic and linoleic acid leading to oxidation. There was a decrease in the ratio of unsaturated to saturated fatty acids (UFA/SFA) with increasing applied voltage level, which may be attributed to the increased dissociation reactions to form free radicals, reactive species and unstable compounds at higher voltages. A similar observation with a reduction in the unsaturated fatty acids was also observed in ozonated vegetable oil and pork lard (Jurado-Alameda, García-Román, Altmajer-Vaz, & Jiménez-Pérez, 2012; Soriano, Migo, & Matsumura, 2003). This result supports the changes seen in the ^{1}H NMR and IR spectra of the plasma treated samples which is suggested to follow the Criegee mechanism (Soriano, Migo, & Matsumura, 2003). Several authors have reported that plasma treatment of organic chemicals results in the formation of new by-products (Gao, Sun, Wan, Yu, & Li, 2013; Sarangapani, Misra, Milosavljevic, Bourke, O'Regan, & Cullen, 2016). The GC-MS analysis of plasma treated fats was performed to identify the plasma degraded products. The unsaturated fatty acids were identified at retention times of; 16.49 min for palmitoleic, 19.19 for oleic, 20.09 for linoleic respectively. Interestingly, the analysis of chromatogram showed the formation of new peaks caused by the attack of reactive oxygen and nitrogen on the double bond of unsaturated fatty acids. Oleic acid was the primary fatty acid susceptible to oxidation by plasma species. The reaction of ozone to oleic acid forms primary ozonide (molozonide) which further decomposes into two Criegee intermediate radicals. These

299 results were supported by the ^{1}H NMR at the chemical shift δ 5.15 ppm and the IR spectra at
300 1105 cm^{-1} , 1195 cm^{-1} . It is known that decomposition of the primary ozonides leads to the
301 formation of aldehydes (or ketones) and carbonyl oxides (or Criegee intermediates) (Díaz,
302 Hernández, Ledea, Sazatornil, & Moleiro, 2003). The formation of nonanal and 9-oxonanonoic
303 acid in plasma treated samples support this hypothesis. Nonanal (aldehyde) compound (c) was
304 identified at a retention time of 9.69 min and exhibited $[M]^{+}$ ion at 142 and fragments ions at m/z
305 124 [M-18(loss of water)], m/z 114[M-28(loss of ethylene)], m/z 98[M-44(loss of CH_2=CH-
306 OH)] with other prominent peaks at m/z 95, 81, 71, 69, 43 and 41. Compound (b) was identified
307 as 9-oxonanonoic acid (Carbonyl oxide) with mass fragments at $[M]^{+}$ 172, and fragments ions at
308 m/z 154[M-18(loss of water)], m/z 144 [M-28 (loss of ethylene)], m/z 129 [M-44 (loss of
309 CH_2=CH-$OH^{\cdot}$)] (Supplementary matrial Fig.S7.) and other prominent peaks at m/z 111, 98, 87,
310 73, 59, 45, 41. Katrib *et al.,* (2004) reported that the Criegee intermediates undergo reactions
311 with oleic acid to form C27 molecules. Moreover, other products were also identified in the MS
312 spectra of plasma treated samples. The compound (a) nonanoic acid (more than 70% match) was
313 identified at 9.88 min in all the plasma treated samples and exhibited $[M]^{+}$ ion at 172, and other
314 major fragments at m/z 129, 5, 101, 87, 74, 55, 43, 41. Compound (d) was identified as azelaic
315 acid (96.6% match) exhibited $[M]^{+}$ ion at 185, and other fragments at m/z 152, 143, 124, 111, 97,
316 74, 55, 41. Interestingly, another compound identified at 9.206 min corresponds to octanoic acid
317 (more than 75% match) which exhibited $[M]^{+}$ ion at 158, 127[M-31(OCH_3)], m/z 101, 87, 74,
318 41.These compounds could be formed by the isomerization of Criegee intermediates (Hung,
319 Katrib, & Martin, 2005). A possible oxidation pathway **see Fig.2 (c)** for oleic is proposed based
320 on the intermediates formed. Further possible recombination of the carbonyl oxide and the
321 aldehyde (or ketone) yield secondary ozonides. The formation of 9-oxononononoic acid and

nonanal were also observed in the reaction of oleic acid droplets with nitrate radicals by Hung *et al.*,(2005). However, no nitrate radical reactions forming long chain carbon molecules and formation of peroxides including hydroperoxides were seen in the MS spectra. Extensive information on the formation of hydroperoxides can be found elsewhere (Bailey, 2012).

3.3.NMR analysis

Fig. 3 (a and b) shows the typical ^{1}H NMR spectra of the control and plasma treated samples of the dairy and beef fat respectively. ^{1}H NMR assignments of the main components of pure fats are δ(ppm); 0.90, $-CH_3$ (fatty acid terminal groups); 1.40-1.15, $-(CH_2)_n-$ (saturated fatty acid acyl groups); 1.71-1.50, –O-C(=O)–CH_2–CH_2– (acyl groups) ; 2.10-1.90, –CH_2–CH=CH– (acyl groups) ; 2.35-2.20, –OCO–CH_2– (acyl groups); 2.80-2.70, =CH-CH_2–CH=(acyl groups); 4.32-4.10, –CH_2OC(=O)R (C1 and C3 glyceryl CH_2 groups); 5.26-5.20, $(-CH_2)_2$C*H*OC(=O)R (C=2 glyceryl CH); 5.40-5.26, –C*H*=C*H*– (acyl groups) (M. a. D. Guillén & Ruiz, 2001). Thus, the chemical shifts are assigned to the saturated fatty acid component of triglycerides and free fatty acids between 0-4 ppm while 4-6 ppm are assigned to proton signals of glycerol backbone as well as olefin components of unsaturated fatty acids and triglycerides. It was evident from the NMR analysis that fats underwent structural changes upon plasma treatment. New signals at 9.76 ppm found in the ^{1}H NMR spectra of plasma treated dairy and beef correspond to aldehydic protons (Díaz, Hernández, Ledea, Sazatornil, & Moleiro, 2003). The formation of aldehydes were also observed at 1725 cm^{-1} and C-H stretch around 2900-2700 cm^{-1} in IR spectra. It was also observed that the area of these peaks increased with increases in plasma treatment time and applied voltage. The 9.3-10.5 ppm region of ^{1}H NMR spectra of plasma treated beef and dairy samples contained some doublet resonances at 9.49, 9.52, 9.67 ppm (see supplementary material Fig.S19 and Fig.S31). Claxson *et al.,* (1994) demonstrated that these signals arise from

unsaturated aldehydes and correspond to trans- 2-alkenals, alka-2,4-dienals and 4-hydroxy-trans-2-alkenals, respectively. The signal at 9.76 ppm corresponds to saturated aldehydes and it is attributed to hexanal or pentanal whereas, 9.49, 9.52 ppm signals are a mixture of trans-2-heptenal, -octenal or -nonenal (Haywood, Claxson, Hawkes, Richardson, Naughton, Coumbarides, et al., 1995). A relatively weak signal at 9.67 ppm was observed in the 30 min treated samples and showed coupling patterns at 6.15 ppm that could possibly be assigned to 4-hydroxy-trans-2-nonenal. The possible generation of these compounds is due to the oxidation of unsaturated fatty acids (particularly linoleic acid) by reactive oxygen and nitrogen species. Similar results were observed in ozonolysis of sunflower and sesame oil (Sega, Zanardi, Chiasserini, Gabbrielli, Bocci, & Travagli, 2010; Soriano Jr, Migo, & Matsumura, 2003; Soriano, Migo, & Matsumura, 2003). The distinct singlet resonances at 8.9, 8.8, 8.2, 8.0 and 6.2 ppm in the dairy samples and 9.20, 8.2, 8.0, 6.45 and 6.2 ppm detected in beef samples were assigned to hydroperoxide group (-OOH) protons. Similar hydroperoxide groups were detected in thermally stressed linoleate samples by Claxson *et al.,* (1994). There was an increase in the peak intensity with increases in plasma treatment time and applied voltage. The resonances at 8.2 and 8.0 ppm could be attributed to the reaction between oxidizing radicals (eg. •H, •O, •OH) and oleic acid in beef fat to form hydroperoxides. The reaction proceeds **(see Fig.4(a))** with a free radical attack at C-8 and C-11 of oleic acid and hydrogen abstraction which leads to allylic radicals which upon reaction with reactive oxygen species would result in formation of 9-hydroperoxy-*trans*-10-,11-hydroperoxy-*cis*-9,10-hydroperoxy-*trans*-8-, 8-hydroperoxy-*cis*-9-octadecenoates (Frankel, 1984). These results are in agreement with reports by Neff *et al.,* (1990) for autoxidation of olive oil and triolein. The chemical shift in values of 8.9 and 8.81 ppm observed in the dairy samples may correspond to hydroperoxide group protons of linoleic acid

producing a mixture of 9-hydroxy-*trans*-10, *cis*-12- and 13-hydroxy-*cis*-9, *trans*-1-octadecadienoate (Frankel, 1984). The evidence of these hydroperoxy species were confirmed by resonances centered at 4.05, 5.56, 6.35, 5.90 and 5.30 ppm which correspond to 13, 12, 11, 10, 9 position protons respectively, of conjugated diene *cis*, *trans*-13-hydroxydiene isomer (Haywood, et al., 1995). The steps involved **(see Fig.4(b))** include selective hydrogen abstraction to form a pentadienyl hybrid radical which upon reaction with plasma reactive species would result in 9- and 13-hydroperoxy- octadecadienoylglycerol species (Frankel, 1984). Similar hydroperoxy species were detected and a mechanism of formation explained by Chan *et al.,* (1980). Similar resonances were also detected in the oxidation of trilinolenin in the presence of oxygen with the formation of hydroperoxy epidioxy adducts identified as the main products (Chan, Matthew, & Coxon, 1980; Neff, Frankel, & Miyashita, 1990). It is reported that the resonances at 8.9 and 8.8 corresponds to 12- and 9-position methine proton multiplets of 13-hydroperoxy-9,12-epidioxy-10-octadecenoates and the 10- and 13-position methine proton multiplets of 9-hydroperoxy-l0,13- epidioxy-11-octadecenoate (Haywood, et al., 1995). These signals were also detected in thermally treated methyl linoleate, methyl oleate, soyabean oil and deteriorated oil (Claxson, et al., 1994; M. a. D. Guillén & Ruiz, 2001). The formation of these hydroperoxy epidioxide products were also reported in photosensitized methyl linoleate samples by Neff *et al.*,(1983). However, the absence of the signals at 4.66 ppm in both the dairy and beef ^{1}H NMR spectra rules out the formation of hydroperoxy epidioxide as plasma oxidized products. Further investigation was also carried out to elucidate the possible formation of other dihydroperoxides. As stated earlier, the resonances at 8.25, 8.12 ppm in both plasma treated samples corresponds to hydroperoxy group. Moreover, the multiplets centered at 6.45, 6.25, 4.87, 4.30-4.40, 3.67, 2.31, 1.50-1.80 and 0.92 ppm correspond to 8-13-dihydroperoxy-*trans*-9,*trans*-11- and 9,14-

392 dihydroperoxy-*trans*-10,*trans*-12-octadecadienoates (Neff, Frankel, Selke, & Weisleder, 1983).
393 The formation of hydroperoxides and diperoxide was further confirmed by the peroxide value
394 experiments. The plasma treatment increased the peroxide value of both dairy and beef fat. The
395 peroxide value of control samples was 7.5 mEq O_2/ kg for dairy and 10 mEq O_2/ kg for beef fat.
396 The 80KV 30 min of plasma treatment increased the peroxide value to 23 mEq O_2/ kg for dairy
397 and 17 mEq O_2/ kg for beef fat respectively. The formation of these peroxides is due to reaction
398 of plasma species with unsaturated fatty acid following the Criegee mechanism. However, the
399 mechanism of formation of these dihydroperoxides after plasma treatment should requires
400 further investigation.

401 A new signal at 5.15 ppm was seen in the ^{1}H NMR spectra of plasma treated dairy and
402 beef samples. This resonance was assigned to the ring proton of 1,2,4-trioxolane (Díaz,
403 Hernández, Ledea, Sazatornil, & Moleiro, 2003; Sega, Zanardi, Chiasserini, Gabbrielli, Bocci, &
404 Travagli, 2010) and the formation of this ozonide was also supported by bands at 1105 cm^{-1}
405 ,1170 cm^{-1}, 1195 cm^{-1} in the IR spectra. From **Fig.3 (a) and (b).**, this new signal resonance
406 increased with plasma treatment time and voltage. Moreover, there was also an increase in
407 resonances at 2.35–2.45 and 2.04–2.18 ppm upon plasma treatment. The signals at 2.35–2.45
408 ppm are attributed to methylene bridge protons connecting the 1,2,4-trioxolane ring to the double
409 bond. However, the chemical shifts at 2.04–2.18 ppm correspond to methylene bridge protons
410 now connecting two carbon rings (Sega, Zanardi, Chiasserini, Gabbrielli, Bocci, & Travagli,
411 2010). The ozonolysis of methyl oleate (Ledea, Díaz, Molerio, Jardines, Rosado, & Correa,
412 2003), methyl linoleate (Díaz, Hernández, Ledea, Sazatornil, & Moleiro, 2003), sunflower oil
413 (Soriano Jr, Migo, & Matsumura, 2003; Soriano, Migo, & Matsumura, 2003), sesame oil (Sega,
414 Zanardi, Chiasserini, Gabbrielli, Bocci, & Travagli, 2010) has been reported with the formation

of ozonides. The oxidation of fat by plasma treatment can be described by the Criegee mechanism. The electrophilic ozone molecule or other plasma active species attack the C=C double bonds of unsaturated fatty acid, leading to the formation of an unstable cyclic intermediate called an unstable initial ozonide (1-2-3-trioxolane). The unstable intermediate can be further decomposed to a cabonylic compound (aldehyde or ketone) to form stable 1-2-4 trioxolane, diperoxides and hydroperoxides (Ledea, Díaz, Molerio, Jardines, Rosado, & Correa, 2003). It was found that degradation of linoleic acid by ozone was found to be 1.5 times higher than that of oleic acid. The presence of greater amounts of unsaturated fatty acids (45%) in beef fat compared to dairy would result in relatively small increases in the reactivity of the C=C double bond due to a high steric effect. The reaction of plasma species such as ozone with fats to form products depends upon the composition of fatty acids and type of medium (solvent). The reaction of pure oleic acid with carbonyl oxide, by inter and intra molecular reactions, readily forms acyloxyalkyl hydroperoxides whereas, under dilute conditions favours ozonide formation and hydroperoxide. It was reported that ozonation of sunflower oil in presence of water yielded alkyl hydroperoxide and prevented the formation of ozonide (Soriano Jr, Migo, & Matsumura, 2003). Ledea et al., (2003) studied the ozonation of methyl oleate and reported the formation of ethoxy hydroperoxides in the presence of ethanol and ozonides and aldehydes in the presence of water. Considering the surrounding milieu of dairy and beef fats in food matrices, the interactions within aqueous environments require further investigation.

An extensive explanation into the formation of new products and their kinetics as a result of plasma treatment is proposed. The integral ratios of the proton signals of aldehydes, peroxides, ozonides and methylene groups with reference to the integral of TMS that remains constant during the process of some key protons resonances was evaluated. The formation of secondary

oxidation products with plasma treatment time and applied voltage was modelled using a first-order kinetic regression model (see supplementary material Fig.S33 and Fig.S34)**.** It can be observed from **Table 1** that the model is in agreement with the experimental data with high correlation coefficients explaining the increase in formation of new products in tandem and relation to plasma process parameters.

4. Conclusion

This work provides insights into ACP-DBD air plasma induced lipid oxidation with FTIR spectra of treated samples showing plasma induced changes in the functional properties of dairy and beef fat. In this present study the formation of secondary oxidation products were only seen at extended plasma treatment times of 30min. A linear trend explains the extent of the changes induced by the process conditions. The increase in ozonide band at 1105 cm^{-1}, 1195 cm^{-1}, formation of aldehydes 1725, 2950 cm^{-1} and 829, 969, 3470 cm^{-1} is attributable to the formation of hydroperoxides. These changes were dependent on treatment time and applied voltage.

^{1}H NMR analysis also identified the formation of several lipid oxidation products, of which aldehydes, such as hexanal or pentenal, mixtures of trans-2-heptenal, -octenal or -nonenal and 4-hydroxy-trans-2-nonenal are the most important. For the first time the formation of hydroperoxides of oleic acid was identified as 9-hydroperoxy-*trans*-10, 11-hydroperoxy-*cis*-9,10-hydroperoxy-*trans*-8-, 8-hydroperoxy-*cis*-9 octadecenoates and linoleic acid as 9- and 13-hydroperoxy- octadecadienoylglycerol species. Therefore, it is suggested that atmospheric air plasma treated fatty acids follow the Criegee mechanism. With the reaction of ozone and active species almost exclusively with carbon double bonds present in unsaturated fatty chains. Fatty acid composition analysis identified the reduction in oleic, palmitoleic and linoleic acid along with oxidation products as nonenal, azelaic acid, 9-oxononanoic acid, nonanoic acid and

461 octanoic acid. Moreover, these products can be used as oxidation markers for the chosen dairy
462 and beef fats.

463 **Acknowledgment**

464 The funding for this research was provided by the Food Institutional Research Measure (FIRM)
465 project entitled 'Cold Plasma treatment of waste water' (project number: 13/F/442) under the
466 National Development Plan administered by the Department of Agriculture, Fisheries and Food,
467 Ireland.

468

469

470

471

472

473

474

475

476

477

478

References

Adeyemi, N. A., Mohiuddin, A., Mirghani, M. E. S., & Jameel, A. T. (2012). Unconventional Method for Monitoring of Waste Cooking Oil Transesterification. In *Advanced Materials Research*, vol. 576 (pp. 357-361): Trans Tech Publ.

Ahmad Fadzlillah, N., Che Man, Y., Rohman, A., Ismail, A., Mustafa, S., & Khatib, A. (2013). Authentication analysis of butter from beef fat using Fourier Transform Infrared (FTIR) spectroscopy coupled with chemometrics. *International Food Research Journal, 20*(3), 1383-1388.

Bailey, P. S. (2012). *Ozonation in Organic Chemistry V2: Nonolefinic Compounds*: Elsevier.

Bellamy, L. (2013). *The infra-red spectra of complex molecules*: Springer Science & Business Media.

Chan, H. W.-S., Matthew, J. A., & Coxon, D. T. (1980). A hydroperoxy-epidioxide from the autoxidation of a hydroperoxide of methyl linolenate. *Journal of the Chemical Society, Chemical Communications*(5), 235-236.

Claxson, A. W., Hawkes, G. E., Richardson, D. P., Naughton, D. P., Haywood, R. M., Chander, C. L., Atherton, M., Lynch, E. J., & Grootveld, M. C. (1994). Generation of lipid peroxidation products in culinary oils and fats during episodes of thermal stressing: a high field 1H NMR study. *FEBS letters, 355*(1), 81-90.

Dalton, G., Dragoset, R., & Wiersma, G. National Institute of Standards and Technology Atomic Spectrum Database. In).

Díaz, M. F., Hernández, F., Ledea, O., Sazatornil, J. A. G., & Moleiro, J. (2003). 1H NMR Study of Methyl Linoleate Ozonation. *Ozone Science & Engineering, 25*(2), 121-126.

Frankel, E. (1984). Chemistry of free radical and singlet oxidation of lipids. *Progress in lipid research, 23*(4), 197-221.

Gao, L., Sun, L., Wan, S., Yu, Z., & Li, M. (2013). Degradation kinetics and mechanism of emerging contaminants in water by dielectric barrier discharge non-thermal plasma: the case of 17β-Estradiol. *Chemical engineering journal, 228*, 790-798.

Georgiev, V., Anachkov, M., Batakliev, T., & Rakovsky, S. (2013). Study on the stoichiometry and reaction products of extra virgin olive oil ozonation. *BULGARIAN CHEMICAL COMMUNICATIONS, 45*, 203-207.

Guillén, M. a. D., & Ruiz, A. (2001). High resolution 1 H nuclear magnetic resonance in the study of edible oils and fats. *Trends in Food Science & Technology, 12*(9), 328-338.

Guillén, M. D., & Cabo, N. (1997). Infrared spectroscopy in the study of edible oils and fats. *Journal of the Science of Food and Agriculture, 75*(1), 1-11.

Haywood, R. M., Claxson, A. W., Hawkes, G. E., Richardson, D. P., Naughton, D. P., Coumbarides, G., Hawkes, J., Lynch, E. J., & Grootveld, M. C. (1995). Detection of Aldehydes and Their Conjugated Hydroperox Ydiene Precursors in Thermally-Stressed Culinary Oils and Fats: Investigations Using High Resolution Proton Nmr Spectroscopy. *Free radical research, 22*(5), 441-482.

Hung, H.-M., Katrib, Y., & Martin, S. T. (2005). Products and mechanisms of the reaction of oleic acid with ozone and nitrate radical. *The Journal of Physical Chemistry A, 109*(20), 4517-4530.

Jensen, P. N., Danielsen, B., Bertelsen, G., Skibsted, L. H., & Andersen, M. L. (2005). Storage stabilities of pork scratchings, peanuts, oatmeal and muesli: Comparison of ESR spectroscopy, headspace-GC and sensory evaluation for detection of oxidation in dry foods. *Food Chemistry, 91*(1), 25-38.

Jiang, B., Zheng, J., Qiu, S., Wu, M., Zhang, Q., Yan, Z., & Xue, Q. (2014). Review on electrical discharge plasma technology for wastewater remediation. *Chemical Engineering Journal, 236*, 348-368.

Jurado-Alameda, E., García-Román, M., Altmajer-Vaz, D., & Jiménez-Pérez, J. L. (2012). Assessment of the use of ozone for cleaning fatty soils in the food industry. *Journal of Food Engineering, 110*(1), 44-52.

Katrib, Y., Martin, S. T., Hung, H.-M., Rudich, Y., Zhang, H., Slowik, J. G., Davidovits, P., Jayne, J. T., & Worsnop, D. R. (2004). Products and mechanisms of ozone reactions with oleic acid for aerosol particles having core-shell morphologies. *The Journal of Physical Chemistry A, 108*(32), 6686-6695.

Kirk, S., & Sawyer, R. (1991). *Pearson's composition and analysis of foods*: Longman Group Ltd.

Korner, N., Beck, E., Dommann, A., Onda, N., & Ramm, J. (1995). Hydrogen plasma chemical cleaning of metallic substrates and silicon wafers. *Surface and Coatings Technology, 76*, 731-737.

Ledea, O., Díaz, M., Molerio, J., Jardines, D., Rosado, A., & Correa, T. (2003). 1H-NMR spectroscopy study of oleic acid and methyl oleate ozonation in different reaction conditions. *Revista CENIC Ciencias Químicas, 34*(1), 3-8.

Misra, N. N., Pankaj, S. K., Frias, J. M., Keener, K. M., & Cullen, P. J. (2015). The effects of nonthermal plasma on chemical quality of strawberries. *Postharvest Biology and Technology, 110*, 197-202.

Moreno, M. M., Olivares, D. M., López, F. A., Martínez, V. P., & Reig, F. B. (1999). Study of the formation of carbonyl compounds in edible oils and fats by 1 H-NMR and FTIR. *Journal of molecular structure, 482*, 557-561.

Naebe, M., Cookson, P. G., Rippon, J., Brady, R. P., Wang, X., Brack, N., & van Riessen, G. (2010). Effects of plasma treatment of wool on the uptake of sulfonated dyes with different hydrophobic properties. *Textile research journal, 80*(4), 312-324.

Neff, W., Frankel, E., & Miyashita, K. (1990). Autoxidation of polyunsaturated triacylglycerols. I. Trilinoleoylglycerol. *Lipids, 25*(1), 33-39.

Neff, W., Frankel, E., Selke, E., & Weisleder, D. (1983). Photosensitized oxidation of methyl linoleate monohydroperoxides: hydroperoxy cyclic peroxides, dihydroperoxides, keto esters and volatile thermal decomposition products. *Lipids, 18*(12), 868-876.

Pankaj, S. K., Bueno-Ferrer, C., Misra, N., Milosavljević, V., O'Donnell, C., Bourke, P., Keener, K., & Cullen, P. (2014). Applications of cold plasma technology in food packaging. *Trends in Food Science & Technology, 35*(1), 5-17.

Park, B. J., Takatori, K., Sugita-Konishi, Y., Kim, I.-H., Lee, M.-H., Han, D.-W., Chung, K.-H., Hyun, S. O., & Park, J.-C. (2007). Degradation of mycotoxins using microwave-induced argon plasma at atmospheric pressure. *Surface and Coatings Technology, 201*(9–11), 5733-5737.

Sarangapani, C., Devi, Y., Thirundas, R., Annapure, U. S., & Deshmukh, R. R. (2015). Effect of low-pressure plasma on physico-chemical properties of parboiled rice. *LWT-Food Science and Technology*.

Sarangapani, C., Misra, N., Milosavljevic, V., Bourke, P., O'Regan, F., & Cullen, P. (2016). Pesticide degradation in water using atmospheric air cold plasma. *Journal of Water Process Engineering, 9*, 225-232.

Sega, A., Zanardi, I., Chiasserini, L., Gabbrielli, A., Bocci, V., & Travagli, V. (2010). Properties of sesame oil by detailed 1 H and 13 C NMR assignments before and after ozonation and their correlation with iodine value, peroxide value, and viscosity measurements. *Chemistry and physics of lipids, 163*(2), 148-156.

Song, H. P., Kim, B., Choe, J. H., Jung, S., Moon, S. Y., Choe, W., & Jo, C. (2009). Evaluation of atmospheric pressure plasma to improve the safety of sliced cheese and ham inoculated by 3-strain cocktail Listeria monocytogenes. *Food Microbiology, 26*(4), 432-436.

Soriano Jr, N. U., Migo, V. P., & Matsumura, M. (2003). Ozonation of sunflower oil: spectroscopic monitoring of the degree of unsaturation. *Journal of the American Oil Chemists' Society, 80*(10), 997-1001.

Soriano, N. U., Migo, V. P., & Matsumura, M. (2003). Functional group analysis during ozonation of sunflower oil methyl esters by FT-IR and NMR. *Chemistry and physics of lipids, 126*(2), 133-140.
Thirumdas, R., Sarangapani, C., & Annapure, U. (2015). Cold Plasma: A novel Non-Thermal Technology for Food Processing. *Food Biophysics, 10*(1), 1-11.
Vandamme, J., Nikiforov, A., De Roose, M., Leys, C., De Cooman, L., & Van Durme, J. (2016). Controlled accelerated oxidation of oleic acid using a DBD plasma: Determination of volatile oxidation compounds. *Food Research International, 79*, 54-63.
W¹sowicz, E., Gramza, A., Hes, M., Jeleñ, H. H., Korczak, J., & Malecka, M. (2004). Oxidation of lipids in food. *Pol J Food Nutr Sci, 13*, 87-100.
Wang, W., Zhu, J., Lyu, F., Panigrahy, D., Ferrara, K. W., Hammock, B., & Zhang, G. (2014). ω-3 polyunsaturated fatty acids-derived lipid metabolites on angiogenesis, inflammation and cancer. *Prostaglandins & other lipid mediators, 113*, 13-20.
Yang, H., Irudayaraj, J., & Paradkar, M. M. (2005). Discriminant analysis of edible oils and fats by FTIR, FT-NIR and FT-Raman spectroscopy. *Food Chemistry, 93*(1), 25-32.

Figure legands

Fig.1. Overlaid raw FT-IR spectra of control and plasma treated of fat samples over region 600-3500 cm^{-1} (I) dairy (II) beef fat, where the spectra over region 950-1300 cm^{-1} was shown in (a) dairy and (b) beef and the spectra over region 1650- 1800 cm^{-1} was shown in (c)dairy and (d) beef. Arrow indicates increase in plasma treatment

Fig.2. HC-PC of the raw data (700-1800cm-1) of control and plasma treated (a) dairy (b) beef fat.In the data markers, the first digit indicates the voltage, the second time, and the last following underscore indicates the replicate Where (c) represents proposed oxidation mechanism of oleic acid from GC-MS.

Fig.3. 1H NMR spectra of control (untreated) a) plasma treated dairy fat and b) plasma treated beef fat samples. Note the bottom spectrum showing control and the expansion showing the oxidized products, the first digit indicates the applied voltage, the second digit indicates treatment

Fig.4. Proposed mechanism of hydroperoxides from a) oleic acid (Frankel, 1984) and b) linoleic acid (Frankel, 1984; Neff, Frankel, Selke, & Weisleder, 1983)

Table legends:

Table 1: Fatty acid profiles of the control (untreated) and plasma treated fats.

Table 2: Linear regression (y =ax+ b, R^2 parameter) of integral of selected proton chemical shifts

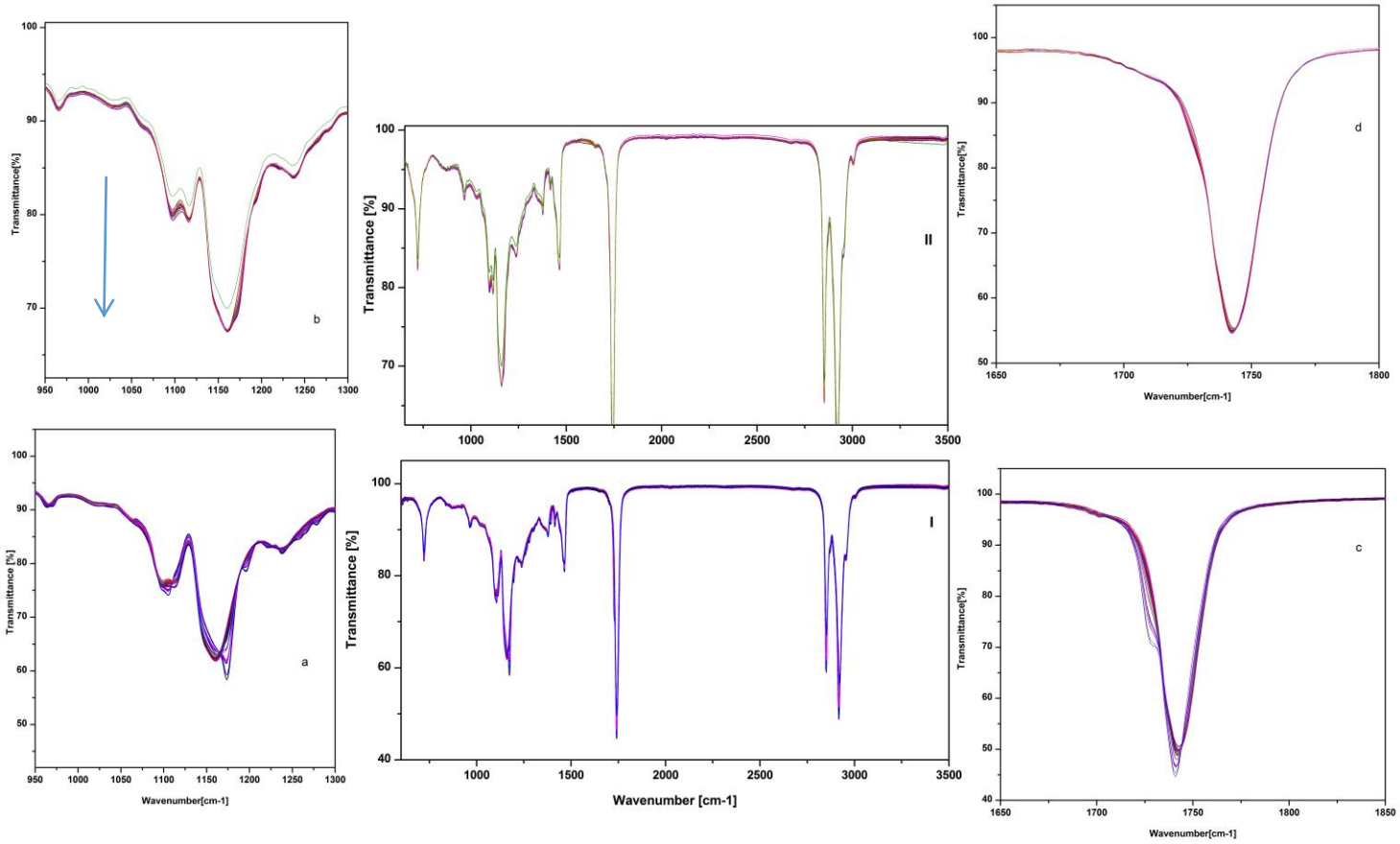
Transmittance[%]
b
Transmittance [%]
II
Trasmittance[%]
d
Wavenumber[cm-1]
Transmittance [%]
a
Transmittance [%]
I
Transmittance[%]
c
Wavenumber [cm-1]
Wavenumber[cm-1]

Fig.1.

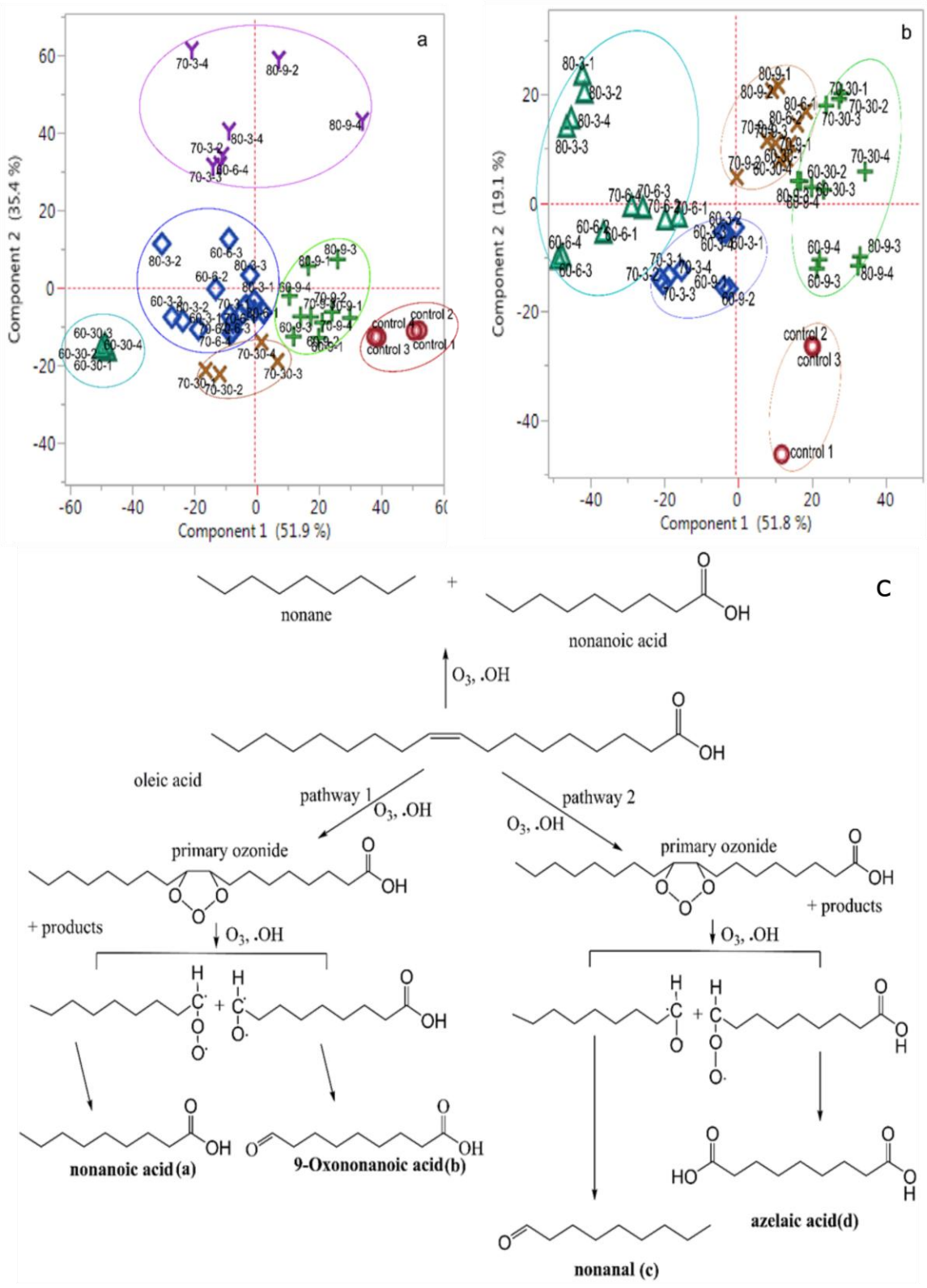
a
Component 2 (35.4 %)
Component 1 (51.9 %)
b
Component 2 (19.1 %)
Component 1 (51.8 %)
control 1
control 2
control 3
C
nonane
nonanoic acid
O_3, .OH
oleic acid
pathway 1
pathway 2
primary ozonide
+ products
nonanoic acid (a)
9-Oxononanoic acid(b)
azelaic acid(d)
nonanal (c)

Fig.2.

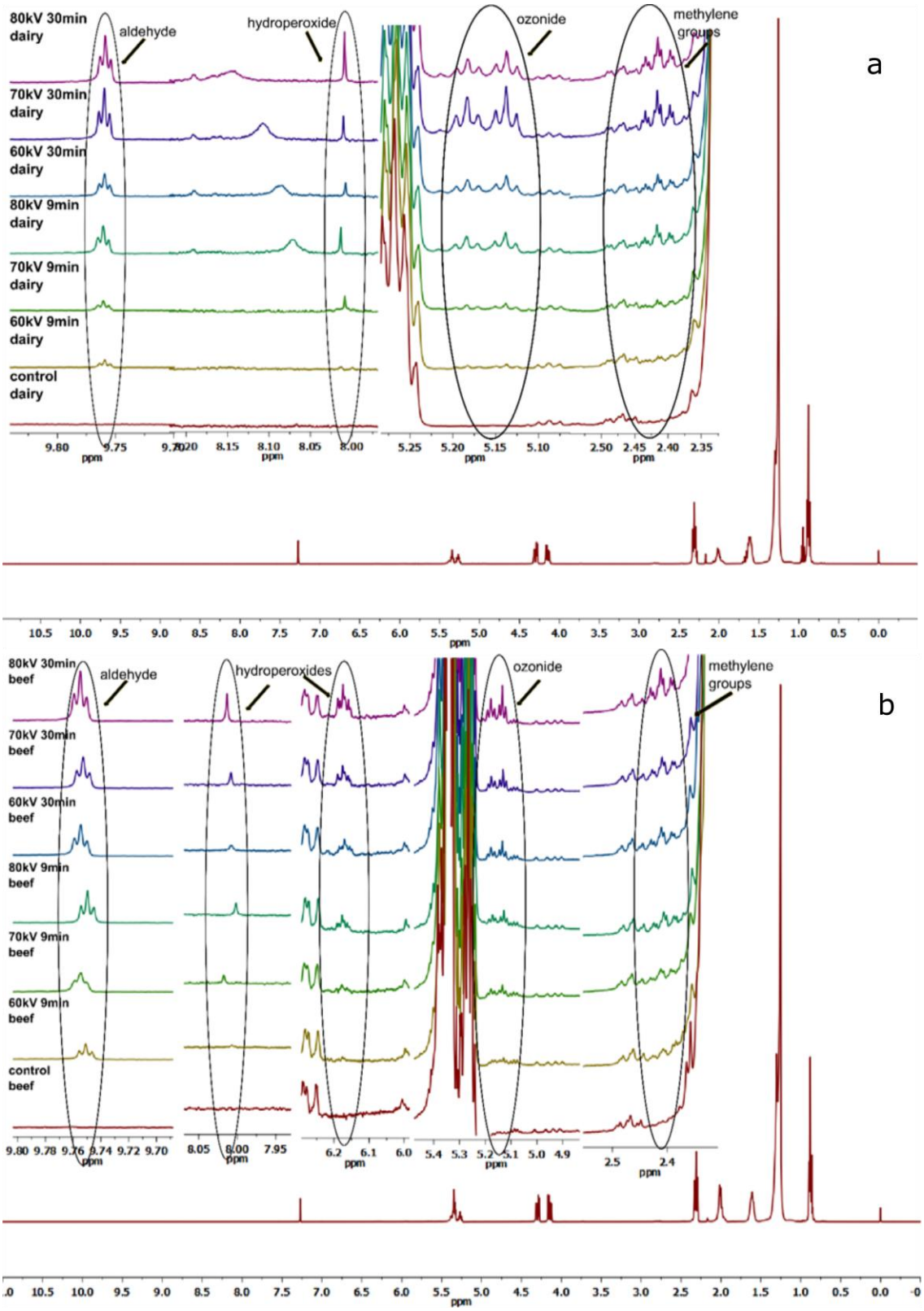
80kV 30min dairy
70kV 30min dairy
60kV 30min dairy
80kV 9min dairy
70kV 9min dairy
60kV 9min dairy
control dairy
aldehyde
hydroperoxide
ozonide
methylene groups
a
80kV 30min beef
70kV 30min beef
60kV 30min beef
80kV 9min beef
70kV 9min beef
60kV 9min beef
control beef
aldehyde
hydroperoxides
ozonide
methylene groups
b
ppm

Fig.3.

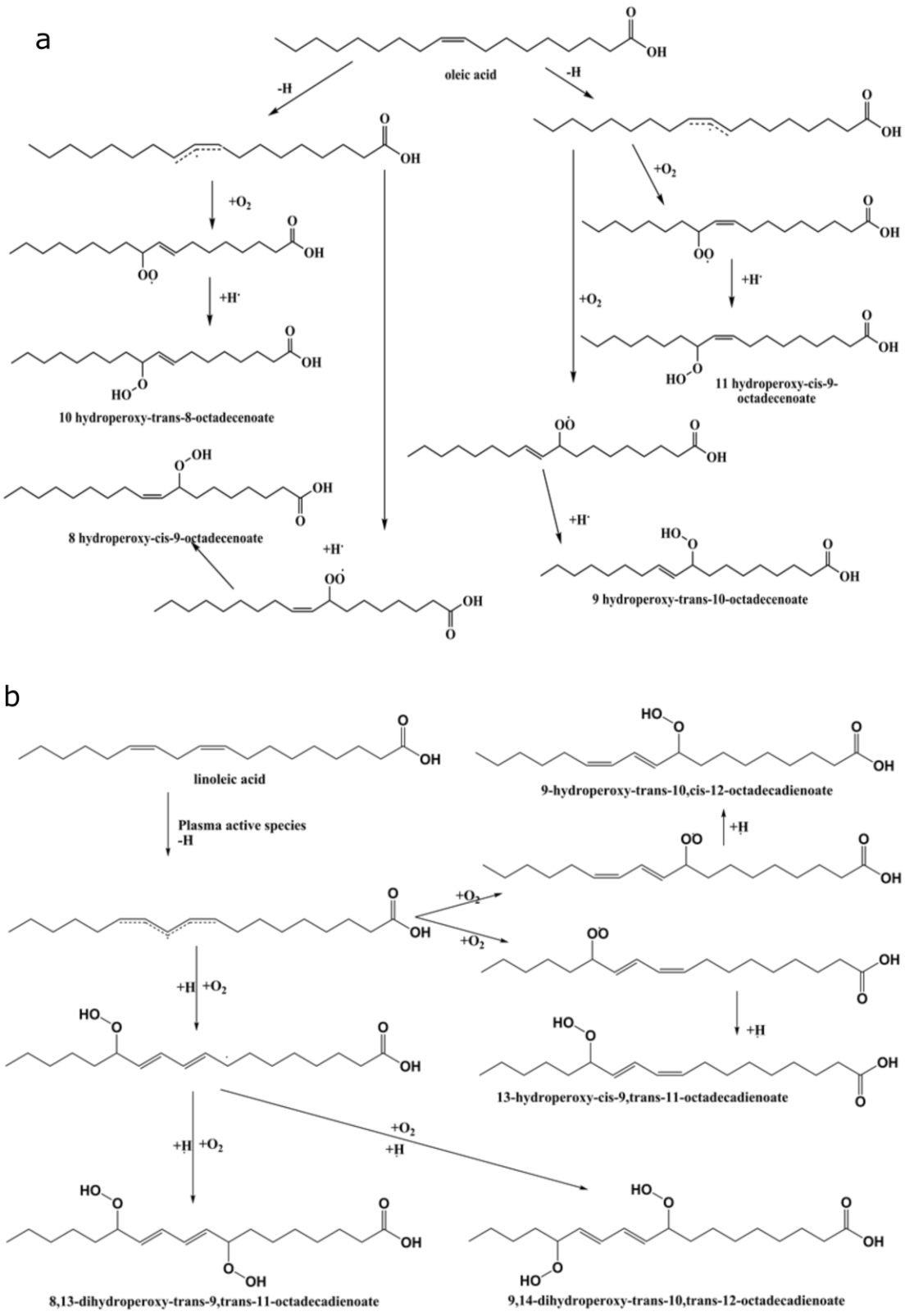
a
oleic acid
-H
-H
+O2
+O2
+H·
+O2
+H·
10 hydroperoxy-trans-8-octadecenoate
11 hydroperoxy-cis-9-octadecenoate
8 hydroperoxy-cis-9-octadecenoate
+H·
+H·
9 hydroperoxy-trans-10-octadecenoate
b
linoleic acid
Plasma active species
-H
9-hydroperoxy-trans-10,cis-12-octadecadienoate
+H
+O2
+O2
+H +O2
+H
13-hydroperoxy-cis-9,trans-11-octadecadienoate
+H +O2
+O2
+H
8,13-dihydroperoxy-trans-9,trans-11-octadecadienoate
9,14-dihydroperoxy-trans-10,trans-12-octadecadienoate

Fig.4.

Table 1

Fattyacid	Relative percentage[z]			
	Voltage (kV)			
	Control	60kV	70kV	80kV
	Dairy fat			
Butyric (C4:0)	3.22±0.21[a]	3.27±0.12[a]	3.36±0.88[b]	3.45±0.54[c]
Myristic (C14:0)	11.01±0.11[a]	12.12±0.31[c]	11.59±0.39 [b]	11.72±0.24[b]
Palmitic (C16:0)	31.86±2.11[a]	31.91±0.88 [a]	34.46±0.42[c]	32.99±1.11[a]
Palmitoleic(C16:1)	2.07±0.12[a]	2.40±0.11[b]	2.42±0.09[b]	2.25±0.22[a]
Stearic (C18:0)	11.31±1.53[d]	9.00±2.41[a]	10.18±0.81[b]	10.81±0.23[c]
Oliec(C18:1)	27.36±2.14[c]	27.30±0.85[c]	26.92±0.41[b]	24.14±0.56[a]
Linoleic(C18:2)	3.19±1.2[d]	1.83±0.11[c]	1.68±0.34[b]	1.21±0.19[a]
Linolenic(C18:3)	1.44±0.01[d]	1.11±0.08[b]	0.92±0.13[c]	0.85±0.12[a]
SFA	57.41±2.11[b]	55.41±1.21[a]	59.60±0.85[d]	58.97±1.02[c]
UFA	34.06±1.59[d]	32.65±1.45[c]	31.94±0.51[b]	28.47±0.56[a]
UFA/SFA ratio	0.59±0.08[d]	0.58±0.02[c]	0.53±0.07[b]	0.48±0.12[a]
	Beef fat			
Myristic (C14:0)	3.46±0.45[a]	3.48±0.12[b]	3.59±0.12[c]	3.62±0.24[d]
Palmitic (C16:0)	25.41±1.98[a]	25.81±0.22[b]	25.86±0.34[b]	25.99±0.56[c]
Palmitoleic(C16:1n-9)	4.00±0.18[b]	3.72±0.32[a]	3.71±0.10[a]	4.21±0.56[c]
Stearic (C18:0)	18.31±0.45[a]	18.32±0.12[a]	18.34±1.22[a]	18.51±0.10[b]
Oleic(C18:1n-9c)	36.86±1.11[d]	35.10±0.88[c]	32.92±0.98[b]	31.64±1.08[a]
Linoleic(C18:2n-6c)	3.19±0.18[d]	3.14±0.08[c]	3.09±0.13[b]	2.75±0.18[a]
SFA	47.19±2.12[a]	47.62±1.85[b]	47.80±0.65[c]	48.12±0.25[d]
UFA	44.05±1.18[a]	41.94±0.85[b]	39.71±1.58[c]	38.60±0.56[d]
UFA/SFA	0.93±0.05[a]	0.88±0.01[b]	0.83±0.01[c]	0.80±0.01[d]

[z] plasma treatment time of 30 min; Only fatty acids with a relative amount higher than 1% are shown where control is untreated sample, UFA: unsaturated fatty acid; SFA: saturated fatty acid. All the data are expressed as mean ± standard deviation. Means with the different superscript letters differ significantly ($P < 0.05$)

Table 2

Sample	Chemical shift (PPM)	Voltage(kV)	a	b	R^2(Adj)
Dairy	9.76	60	0.0040	0.0137	0.85
		70	0.0116	0.0022	0.99
		80	0.0122	0.0203	0.96
	5.12-5.20	60	0.0213	0.0203	0.86
		70	0.0630	0.0691	0.97
		80	0.0355	0.0832	0.78
	2.4	60	0.0410	0.0868	0.94
		70	0.0792	-0.0465	0.99
		80	0.0609	0.2312	0.82
Beef	9.76	60	0.0069	0.0194	0.89
		70	0.0085	0.0053	0.99
		80	0.0143	0.0004	0.99
	6.1-6.2	70	0.0012	0.0035	0.89
		80	0.0057	0.0011	0.94
	5.12-5.20	60	0.0189	-0.0124	0.99
		70	0.0276	0.0376	0.97
		80	0.0511	0.0615	0.98
	2.4	60	0.0482	0.529	0.65
		70	0.0441	-0.0234	0.99
		80	0.0092	-0.0793	0.99