DEPARTMENT OF AGRICULTURAL, FOOD AND ENVIRONMENTAL SCIENCES

DEGREE COURSE: FOOD AND BEVERAGE INNOVATION AND MANAGEMENT

COLD PLASMA:

IMPACT ON THE LIPID OXIDATION OF FISH PRODUCTS

TYPE OF DISSERTATION: Research

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ACRONYMS AND ABBREVIATIONS

CP	cold plasma
CDPJ	corona discharge plasma jet
CO_2	carbon dioxide
$CO_3^{\bullet-}$	carbonate anion radical
COPs	cholesterol oxidized products
D	distilled
DBD	dielectric barrier discharge
DHA	docosahexaenoic acid
DNA	deoxyribonucleic acid
EPA	eicosapentaenoic acid
FFAs	free fatty acids
GC	gas chromatography
GC-MS	gas chromatography–mass spectrometry
H_2O_2	hydrogen peroxide
HNO_2	nitrous acid
HNO_3	nitric acid
$HO_2^{\bullet}$	hydroperoxyl
MUFA	monounsaturated fatty acid
N_2	nitrogen
NLs	neutral lipids
$NO^{\bullet}$	nitric oxide

NO_2	nitrogen dioxide
$NO_2^{\bullet}$	nitrogen dioxide radical
$NO_2\bullet$	nitrogen dioxide radical
NO_x	nitrogen oxide
O_2	singlet oxygen
$O_2^{\bullet-}$	superoxide anion
O_3	ozone
$OH^{\bullet}$	hydroxyl radical
$ONOO^-$	peroxynitrite
ONOO−	peroxynitrite
ONOOH	peroxynitrous acid
OONOH	peroxynitrous acid
ORP	oxidation-reduction potential
PAW	plasma activated water
PET	polyethylene terephthalate
PLs	polar lipids
PUFA	polyunsaturated fatty acids
PV	peroxide value
RNS	reactive nitrogen species
$RO^{\bullet}$	alkoxyl
$ROO^{\bullet}$	peroxyl
ROONO	alkylperoxynitrite
ROS	reactive oxygen species
RPM	revolution per minute
RS	reactive species
S.aureus	*Staphylococcus aureus*

SD	sterilised distilled
SFA	saturated fatty acid
SPE	solid phase extraction
SPE	solid phase extraction
TBARS	2-thiobarbituric acid reactive substances
TMS-ether	trimethylsilylether
UV	ultraviolet

1. INTRODUCTION AND AIM OF THE THESIS

The importance of food preservation and food safety concerns is significantly increasing among food producers and consumers. Nowadays people's request of microbial safety products with premium appearance, flavour, great nutritional value, and extended shelf-life has promoted the development of emerging food processing technologies as alternative or contributed to the conventional ones. The use of emerging technologies not only enhances the food quality preservation but also maintains microbial food safety.

Even though the use of heat-based technologies have many positive effects on food safety like: the elimination/reduction and inactivation of microbial contamination and microbial toxins and the inactivation of enzyme activities, but there is a big concern regarding the nutritional quality of the processed food. For example, in some food rich in water-soluble vitamins, which are heat-sensitive, such as vitamins C, B_1, B_2, B_6, and folic acid, the application of high temperatures can cause their disappearance and so the reduction in the nutritional quality of those products *Rechkemmer, (2007).* Thus, to overcome these problems alternative processing technologies have been studied and developed.

1.1. Plasma technology

Plasma is considered as the fourth state of matter. It is a neutral system which includes gaseous mixture of ions, free electrons, neutral particles (atoms, molecules), activated and metastable species (NO_x -), free radicals like reactive oxygen species (ROS), reactive nitrogen species (RNS) and photons.The required energy to produce these exited species and ions can be provided by thermal force or carried by either electric current or electromagnetic radiations. Plasma can be applied in a direct or indirect mode. The first consists in treating the sample directly by the plasma source, the second is about treating water through the plasma instrument first and then treat the sample with water after the plasma generation (PAW). The choice of the treatment mode has to be in line with the processing process to which the sample is subjected to.

This emerging technology to treat food is classified in two main categories: thermal or hot plasma and non-thermal or cold plasma. In the thermal one, molecules are dissociated into the

atoms at high temperature around 2000 °C. When the temperature goes up to more than 3000 °C, gas molecules will be ionized by losing electrons. In this state, gas has a liquid-like viscosity and free electric charges confer relatively high electrical conductivities (Talebizadeh et al., 2014). Since all particles are in thermal equilibrium, thermal plasma is known as equilibrium plasma.

Differently, in the non-thermal plasma or so-called cold plasma (CP), neutral ions and the overall gas are at lower temperature than electrons, which is why it is also referred as non-equilibrium plasma (Zhao et al., 2019). This technology is classified as a novel technology for food decontamination, which has raised tremendous attention during last years. Non-thermal plasma can be generated by applying high-energy electric field which disrupts and breaks-down the equilibrium state of the gas by formation of ions and electrons (Kulawik & Kumar Tiwari, 2019).

There are different ways to get cold plasma: plasma jet, dielectric barrier discharge (DBD), corona or spark discharge, pin/pins in water discharge or plasma discharge in water with gas bubbles. The commonly used gases are air, O_2, N_2, CO_2 and noble gases, including helium, argon, or even a mixture of them has been used to drive the discharge. According to the type of gas used, different reactive species are generated. ROS typically associated with antimicrobial activity and inactivation cascades are: hydrogen peroxide (H_2O_2), ozone (O_3), superoxide anion ($O_2^{\bullet-}$), hydroperoxyl ($HO_2^{\bullet}$), alkoxyl ($RO^{\bullet}$), peroxyl ($ROO^{\bullet}$), singlet oxygen (O_2), hydroxyl radical ($^{\bullet}OH$), and carbonate anion radical ($CO_3^{\bullet-}$). Likewise, examples of RNS include nitric oxide ($^{\bullet}NO$), nitrogen dioxide radical ($^{\bullet}NO_2$), peroxynitrite ($ONOO^-$), peroxynitrous acid (OONOH), and alkylperoxynitrite (ROONO) (Arjunan et al., 2015). In addition to the type of gas in which plasma is induced, many other factors affect the variation of the reactive species and their concentration, including: the configuration of the plasma source, power input to the gas, duration of treatment, and the humidity levels.

For the non-thermal plasma benefits, it is worthy to mention that this technology is an environmentally friendly and cost-efficient technology. Thus, it is gaining a lot of interest among various industries, other than food ones. It has been successfully applied in medicine, agriculture, water waste treatment, environmental protection and textile industries (Kulawik & Kumar Tiwari, 2019). For example, in the field of environmental protection, this technology has been used for different applications. Cold plasma was efficient in the degradation of pharmaceutical compounds in water (even in tap one), in the treatment of NO_x coming from diesel engines powered by diesel or biodiesel, in the treatment wastewater derived from manufacturing process of textile materials which uses high concentrations of dyes. In medicine

this technology was applied for the treatment of wounds, as well for cancer and skin diseases, where microbes are involved and as a surgical treatment of endometriosis. In agriculture field, non-thermal plasma found its application as stress reducer at germination and seedling stages. Moreover, this technology has been applied in nanotechnology field, for metal nanoparticles formation (Borra et al., 2015; Ghodbane et al., 2015; Haertel et al., 2014; Ishida et al., 2016; Misra et al., 2016; Randeniya & de Groot, 2015; Talebizadeh et al., 2014).

An important feature of non-equilibrium cold plasma, is its ability to generate biologically active agents, such as reactive oxygen species (ROS) and reactive nitrogen species (RNS), while remaining close to ambient temperature, which enables its safely application in food (Misra & Jo, 2017a).

In this field, cold plasma is mainly used as minimal processing preservation method of food, which reduces the risk of microbiological contamination and spoilage. However, this technology is applied in food field with many different aims (**Figure 1**). For example, as sanitation of surfaces, decontamination of fresh and packaged food, reduction of food allergens, inactivation of microorganisms and partially inactivation of enzymes.

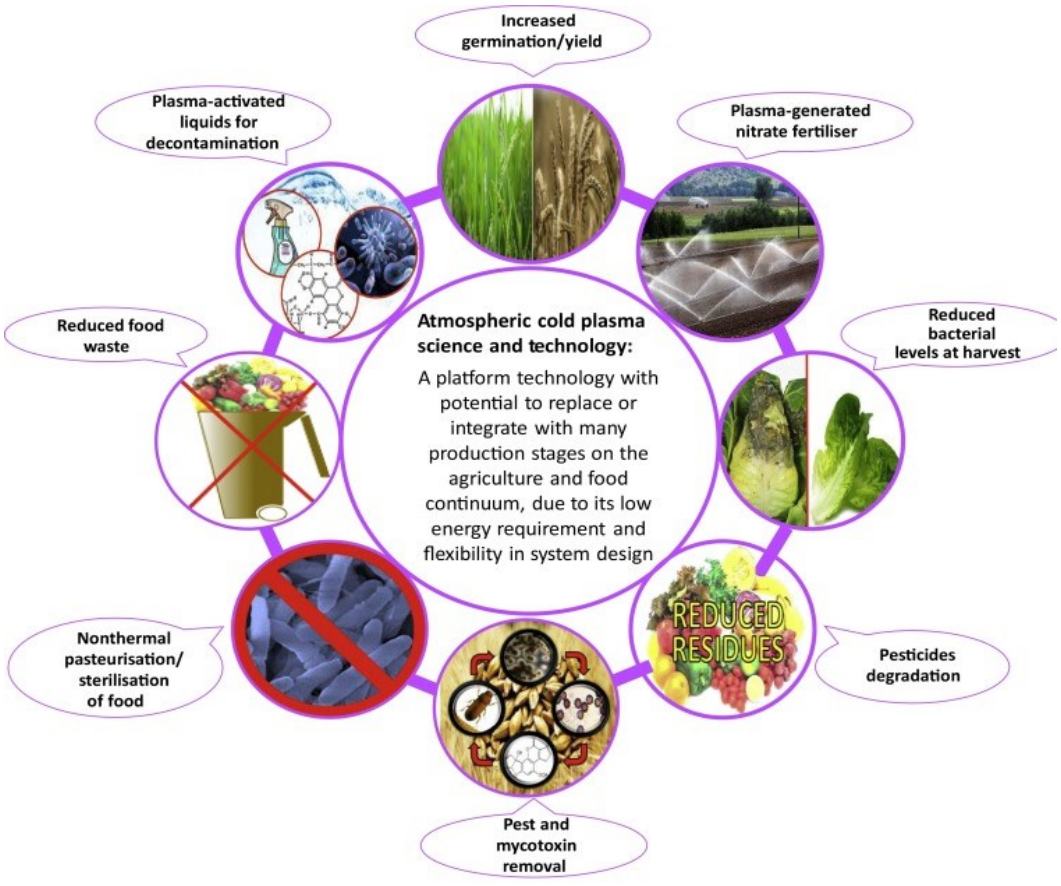

Figure 1. *Atmospheric cold plasma applications to agriculture and foods (Bourke et al., 2018)*

As mentioned above, cold plasma technology can be used as a tool to decontaminate biological materials, including fresh food. *Misra et al., (2014)* used cold plasma as a decontamination of starwberries. They concluded that, plasma technology is an effective decontamination method, because treating strawberries for 5 minutes with atmospheric cold plasma, allowed them to have a 2 $\log_{10}$ reduction of strawberries' background microflora (aerobic mesophilic bacteria, yeast and mould). Later in 2016, *Misra et al., (2016)* also studied the cold plasma's effect on enzymes activity in food. The latter, are often used as markers for processing efficiency and they have an important role in preserving food quality. Enzymes were inactivated by cold plasma due to the huge quantity of chemically active species present on it that cause the breaksdown of specific enzyme's bonds or the chemical modifications of their side chains. The mechanism of enzymes' inactivation depends on the power input of the discharge, the degree of exposure to reactive species, the mass transfer between the plasma-liquid phases, the structural complexity and the stability of the enzymes in their local environment. Moreover*, Moutiq et al., (2020)* conducted a study regarding the in-package decontamination of chicken breast using cold plasma technology. They observed a reduction in natural microflora of chicken, which was attributed to cold plasma's reactive oxygen and nitrogen species. Furthermore, they carried out shelf-life studies, analysing the population of mesophiles, psychrotrophs and *Enterobacteriaceae,* as well as samples colour and pH over a storage time of 24 days. At the end of this period, the studied populations were lower than the control. The authors concluded that, in-package cold plasma treatment is a valid way to prolong poultry products' shelf-life.

The effect of cold plasma on microbial cells can be attributed to the generated ROS from plasma, which can interact with vital cellular biomolecules, like DNA, proteins and enzymes in the cell. The action of cold plasma on bacterial cell structures results in a loss of functionality and thus ensuring product sterilization (

Figure 2). Depending on the type of bacteria, different inactivation mechanisms have been observed in plasma process. The different physical damages caused by plasma in gram-positive and gram-negative bacteria, are due to differences in the cell structure and composition. In gram-negative bacteria, plasma's antimicrobial particles, react directly with bacteria's outer membrane (Ucar et al., 2021). In particular, they cause the peroxidation of lipopolysaccharides (essential elements of gram- negative cell wall) (Bartis et al., 2013). Thus, biological membranes' function is compromised, because of the interaction of ROS with lipids, which leads to the formation of unsaturated fatty acids peroxides and to the oxidation of the amino acids in proteins (Misra et al., 2016). Differently, in gram-positive bacteria, ROS

produced from plasma generation, can either penetrate the cell wall and the membrane of the bacteria and then react with the internal cellular components (DNA and intracellular proteins), or they can break structurally important bonds in the cell wall (peptidoglycan), including C-O, C-N and C-C (Huang et al., 2020; M Yusupov et al., 2012; Maksudbek Yusupov et al., 2013).

The effect of all these actions of plasma chemical species, causes bacterial cell leakage and the loss of cell functionality (Misra & Jo, 2017b).

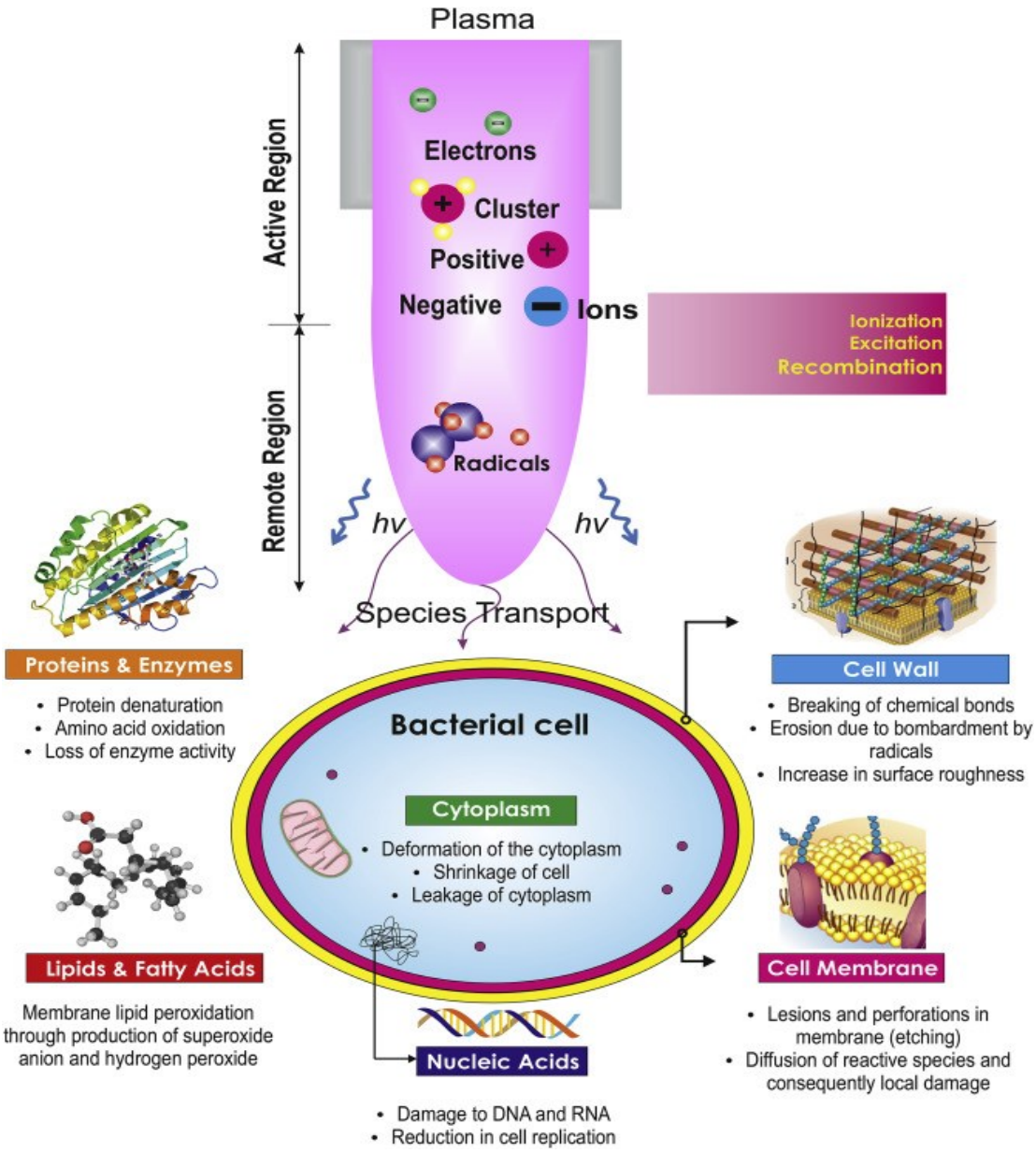

Figure 2. *A schematic of the action of cold plasma on bacterial cell structures resulting in loss of functionality and sterilization.*(*Misra & Jo, 2017b*)

Additionally, non-thermal plasma can be considered as a multimodal action technology, capable of lowering allergens in food. With this purpose, *Venkataratnam et al., (2020)* utilized this technology to reduce peanut allergenicity. Ara h 1 and Ara h 2 are considered the two main peanut's allergens. The authors subjected dry, whole peanut and defatted peanut flour to cold plasma, this, caused a reduction in antigenicity 65% for Ara h 1 and 66% Ara h 2 when purified from defatted peanut flour. Cold plasma was able to decrease peanut protein solubility and to cause changes in allergen structure, leading to a reduction in antigenicity. That was due to modifications in allergens' secondary structure caused by plasma reactive species. *Scholtz et al., (2019)* applied cold plasma on both wheat grains and flour. They discovered that this technology is efficient in the decontamination of wheat grain and flour, against bacteria and fungi, together with insect pests. Moreover, it inactivated enzymes, extended the shelf-life, as well as enhanced the germination and the initial state of growth leading to an increase of the yield.

This type of non-thermal technology used as mentioned before for the sanitation of unhygienic surfaces. The presence of food pathogens in processing areas' surfaces, such as *Salmonella*, can be a risk factor for food cross-contaminations. *Niemira et al., (2014)* tested cold plasma for the decontamination of food contact surfaces, contaminated with *Salmonella* biofilms. The authors grown three strains of *Salmonella* culture, in order to create an adherent biofilm on glass slides test surfaces. Those were exposed to plasma treatment at different time. A reduction of 2.13 log CFU/mL (99.3%) of mature *Salmonella*, was obtained with a 15 s of treatment. This, demonstrates that cold plasma is a valuable technology for rapid disinfection of food processing materials.

Thus, cold plasma is considered as an emerging disinfection, able to act on the product surface by causing a structural modifications, which is cost efficient and eco-friendly (Thirumdas et al., 2018).

1.2. Plasma activated water (PAW)

As mentioned before, plasma is composed by different species like singlet oxygen, superoxides, excited ions, electrons, atoms and ozone (Liao et al., 2017; Misra et al., 2011). Cold plasma can use water as a medium to transfer the generated species to the target, which is so-called plasma activated water (PAW). It is generated by water treatment with the plasma plume under or over the water surface. This treatment allows plasma-particles to interact with water molecules and trigger several chemical reactions, which in turn produce a unique mixture of biochemical reactive molecules (Zhao et al., 2020). During this process, plasma-

energetic particles are blocked in the aqueous liquids and several reactions at the gas-liquid interface is initiated, such as the transfer of gaseous species into the liquid and chemical reactions between gaseous species and liquid molecules (Wende et al., 2019).

Extremely dynamic and transient discharges are generated within the liquid and rapid breakdown processes occur. This is due to strong electric fields, or bubble implosions or laser pulses. Water plasma treatment causes non-equilibrium dissociation of water molecules, which leads to the formation of short living species, like hydroxyl ions (OH-) and hydrated (solvated) electrons. Fast reactions between hydroxyl ions and hydrated electrons occur and reactive oxygen species such as: superoxides (O_2-), ozone (O_3) and H_2O_2 are formed. (**Figure 3**) shows how the reaction proceeds:

$$H_2O + e^- \rightarrow OH^\bullet + H^\bullet + e^-$$

$$H_2O + e^- \rightarrow OH^\bullet + H^+ + 2e^-$$

$$OH^\bullet + OH^\bullet \rightarrow H_2O_2$$

$$H^\bullet + O_2 \rightarrow HO_2$$

$$OH^\bullet + H_2O_2 \rightarrow OOH + H_2O$$

$$O_2^- + H^+ \leftrightarrow OOH$$

$$O_2 + e^- \rightarrow O^+ + O^\bullet + 2e^-$$

$$O_2 + O^\bullet \rightarrow O_3$$

Figure 3. *ROS generation reactions (Zhao et al., 2020)*

Hydroxyl radicals (OH•) are highly reactive short-living species with very high redox potential. When organic content is present in the liquid, these species react with it, forming new radicals and triggering other reactions (Zhao et al., 2019). Ozone, when dissolved in a liquid is not very stable and its stability depends on the pH of the liquid. In presence of water vapor ozone disappears, since it reacts with OH• (Laurita et al., 2015). Differently from ozone, H_2O_2 is considered relatively stable and it could be converted to hydroperoxy radicals or superoxides in the form of hydroxyl radicals depending on the liquid environment. *Niquet et al., (2018*) demonstrated that H_2O_2 concentrations in PAW increased by increasing the

treatment time, and its concentration remained stable during storage. H_2O_2 is known as an important antimicrobial and cytotoxic agent in plasma activated liquid (Julák et al., 2018).

Besides the ROS described above, plasma treatment of water can generate other reactive species like nitric (HNO_3) and nitrous acid (HNO_2) and transient RNS compounds like peroxynitrous acid (ONOOH)/peroxynitrite ($ONOO^-$) and nitrogen dioxide (NO_2•) radicals. When air is present nitrogen and oxygen from the gaseous phase dissociate, forming nitrogen oxide (NO), this reacts with water, forming acids. Subsequently, nitric ions are formed from electron capture by NO_2 or by oxidation of NO and they cause the reduction of the pH thus the creation of an acidic environment. The drop of pH favours the dismutation of nitrite or the degradation of nitrite to nitrates. H_2O_2 reacts with nitrites, forming $ONOO^-$. The latter, lasts less than 1s in acidic solutions and it forms OH• and NO_2• or it transforms to nitrate ion (Tarabová et al., 2019). Given their oxidizing properties, these two forms of $ONOO^-$ are considered to have the greatest contribution to the germicidal effect in PAW. They are able to diffuse though the cell membrane and oxidize cellular components, or they can act as nitrating agents and mediate cytotoxic effects (Naïtali et al., 2012). Furthermore, by the reaction between $ONOO^-$ and carbon dioxide, carbonate radicals are formed (Bonini et al., 1999) and they can act as oxidizing agents (Pacher et al., 2007).

PAW's reactive species and ions lead to an acidic pH and an increase in electrical conductivity together with an oxidation-reduction potential (ORP). RONS formed in the liquid or liquid-gas phase have acid-base properties, because they are capable of releasing hydrogen into aqueous solutions. Moreover, water treated with plasma lead to an acidification of the solution, because it absorbs NO_3^- and NO_2^- generated by plasma; and also because in the liquid phase, NO_3^- is produced from NO_2^- (Anderson et al., 2016).

The formation of acidified compounds and the decrease in pH, depend on the plasma source (Lu et al., 2017), on the production rates and concentration ratios of RONS, which is different according to the type of water used: tap water or deionized water (Tachibana & Nakamura, 2019) and the feeding gases used (Takamatsu et al., 2012). Furthermore, the change in pH, also depends on the polarity of the applied voltage. Negative discharges lead to a greater decrease in the pH of the solutions, compared to positive ones (Zhao et al., 2020). In addition to the pH decrease, treating water with plasma causes changes in water's electrical conductivity. Such changes are due to the presence of ions and charged species in the liquid phase. Electrical conductivity and pH are directly related. In fact, once pH drops, H+ ions increase their mobility and the electrical conductivity of the solution increases.

ORP determines plasma effect against microorganisms. In case of high ORP, microbes' membrane is affected, because it can destroy the inner and outer cell membranes (McPherson, 1993).

Among all the ROS generated in PAW, H_2O_2 is the one with the highest influence in ORP (Lukes et al., 2012). Moreover, *Tian et al., (2015)* saw that plasma generation mode also affects ORP values. They observed that plasma jet beneath water surface have higher ORP values, compared to plasma jet above water surface and this was in line with the inactivation efficiency.

As demonstrated by an increasing number of reports, PAW is an effective method to inactivate a wide range of microorganisms. The main inactivation mechanisms are: oxidative stress and physical effects (**Figure 4**).

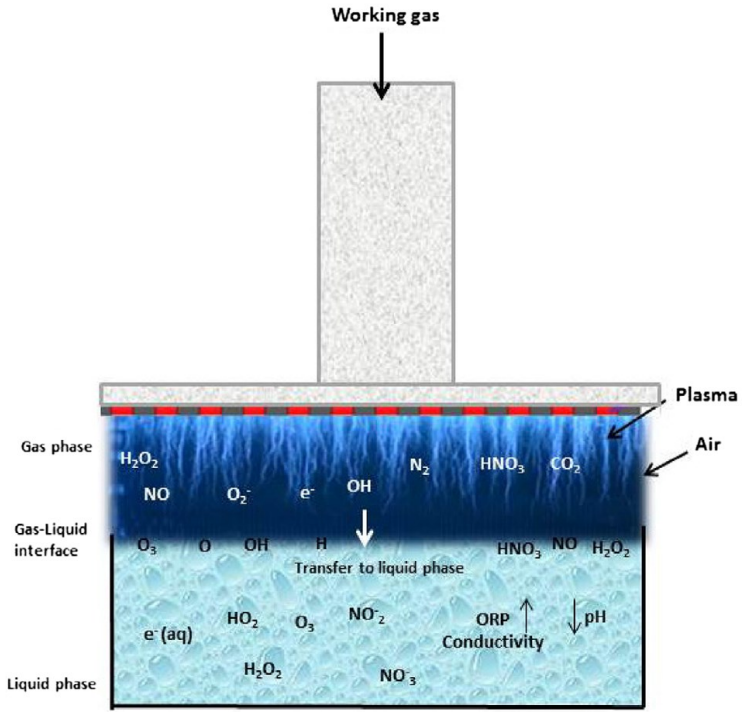

Figure 4. *The interactions of plasma in the gaseous phase with water in the liquid phase during PAW generation. (Zhao et al., 2020)*

Moreover, (**Figure 5**) shows the bacterial inactivation mechanisms of PAW in the oxidative and physical aspect.

Primary and secondary RONS generated in PAW, cause an increase in ORP and in electrical conductivity and a decrease in pH, which in turn cause physical stress on microbial cells. Besides physical stress, RONS are also responsible for the oxidative stress, which destroys peptidoglycan and initiates lipid and protein peroxidation on cell membrane. As a

result, membrane integrity is compromised, shrinkage, pores on cells, cracks, distortion and depolarization of cell membrane potential occur. RONS in the surrounding environment can also be transported into the internal environment, leading to the accumulation of intracellular RONS. Protons generated in PAW can go inside the cell, reducing the intracellular pH. Additionally, intracellular RONS can cause the oxidation of intracellular components, such as: DNA, proteins, lipids and carbohydrates, these can go into PAW, passing through the pores formed on cell membrane (Zhao et al., 2020).

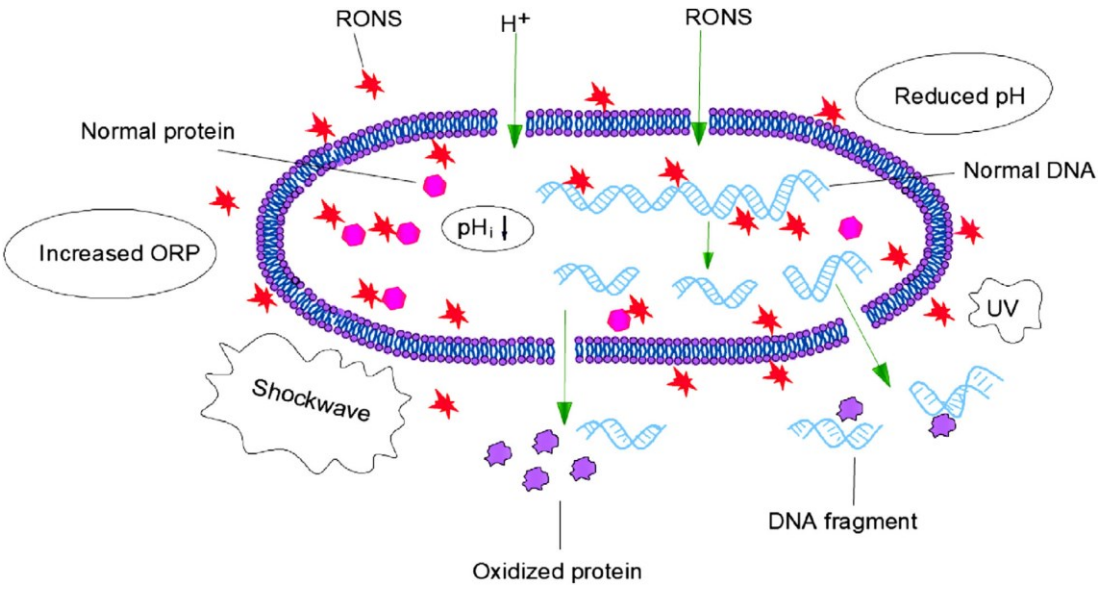

Figure 5. *Bacterial inactivation mechanisms of PAW in the oxidative and physical aspect. (Zhao et al., 2020)*

Non-thermal atmospheric pressure plasma has been proposed as a new tool for various biological and medical applications. In particular, PAW has been used as an anti-cancer, anti-metastatic, antimicrobial, regenerative medicine for blood coagulation and even as a dental treatment agent (Kaushik et al., 2018).

Additionally, PAW is a novel and promising alternative to traditional food sanitizers. Recently, the inactivation efficacy of PAW has been demonstrated on a wide range of food products against foodborne pathogens, spoilage microorganisms, and harmful chemicals.
Plasma generation mode affects PAW's antimicrobial efficiency. It can be generated above the water surface or underwater. *Tian et al., (2015)* saw a higher disinfection efficiency from plasma treatment beneath the water surface than in the one above it. The explanation to that, is given by the higher value of oxidative stress, which leads to a higher level of intracellular ROS. These, in turn compromise bacteria membrane integrity and eventually can also be lethal for bacteria.

Zhang et al., (2016) studied the effect of PAW treatment on *Staphylococcus aureus.* They noticed that the treatment caused an alteration in structure and in the chemical bonds of *S.aureus.* From the obtained results, the authors said that PAW can be a promising environmentally friendly electrochemical disinfectant for application in the medical and food industries.

Ikawa et al., (2016) tested the bactericidal effect of PAW against *Escherichia.coli.* Results demostrated that distilled water exposed to low-temperature atmospheric pressure helium plasma, has a strong bactericidal effect, even if suspeded for few minutes in the treated water. The main applications of this technology in food industry are: seed germination and plant growth, meat curing and surface decontamination.

Thirumdas et al., (2018) conducted a study on PAW's chemistry, physico-chemical properties and applications in food and agriculture. They concluded saying that PAW has a synergistic effect on the disinfection of seeds and on the promotion of seedling growth of seeds. So, PAW can be a treatment to fight against stressfull environmental conditions, as well to improve crop yield.

Lin et al., (2019) applied PAW on eggs' shell as decontamination treatment against *Salmonella* enterica serovar *Enteritidis*. They subjected eggs inoculated with *Salmonella.enteritidis,* to different treatment time obtaining 4 log reduction with 60s and 120s treatments. Furthermore, a better freshness index was detected in PAW eggs, compared to eggs treated with the commercial process (chlorinated water washing), as well a reduced surface damage of the cuticle. Results from this study show that PAW is not only effective as antibacterial agent, but it also allows to better preserve the quality of food products, compared to traditional preservation methods.

It should be noticed that different variables must be considered in PAW treatment. In addition to the plasma instrument employed, other variables like the power supply, the working gases and the treatment mode must be taken into account. The input energy of plasma is determined by voltage, power and frequency; these parameters affect the inactivation efficacy of PAW. The latter is also affected by the type of working gas used. Different gases and mixture of them can be used for PAW generation like air, oxygen, nitrogen, carbon dioxide and noble gases of helium, argon or a mixture of them. These, influence plasma composition, water interactions and as a result PAW's physico-chemical properties. Using air as working gas usually leads to a greater inactivation efficacy compared to noble gases. This is due to higher concentration of ROS like hydroxyl radicals, singlet oxygen, atomic oxygen and ozone which are generated in plasma, when oxygen is present. These species are considered as the

ones having the highest antimicrobial contribution in PAW technology (ROWAN et al., 2007; Vlad & Anghel, 2017; Zhang et al., 2012).

Furthermore, PAW's effectiveness is affected by gas flow rate (Machala et al., 2018; Royintarat et al., 2019) and by the treatment mode.

Moreover, plasma treatment time to activate water plays a key role in PAW inactivation efficiency. Many studies have demonstrated that longer treatment time gives greater physico-chemical changes of PAW, including more acidic pH and higher concentration of reactive species (Vlad & Anghel, 2017; Zhang et al., 2016). In addition to the treatment time to activate water, the exposure time of the sample with PAW, affects the antimicrobial effectiveness of the treatment (Zhao et al., 2020).

The use of PAW has many advantages over other types of treatments. It is better from the sustainability point of view. In fact, differently from traditional chemical sanitizers, it does not produce pollutant by-products. Then, it allows to save energy, since the only energy used is for PAW generation. Additionally, being a non-thermal treatment, it does not affect the quality of heat-sensitive samples. However, PAW technology has some drawbacks. Firstly, the process control and its validation are critical aspects, which are hard to define using PAW. Due to the intricacy of: plasma devices, experimental design and operations conditions, different reactions can be initiated, the latter produces a lot of reactive species, that are the responsible of PAW's inactivation properties; but these compounds are difficult to be controlled. Secondly, food constituents can significantly reduce the decontamination efficiency of PAW. So, treatment conditions should be optimized for each specific food product. Thirdly, the reactive species generated in PAW can cause chemical changes on food components, lipids' peroxidation is an example. This type of reaction causes the formation of aldehydes and keto acids, which in turn give unpleasant odours and flavours to food products (Zhao et al., 2020).

1.3. Lipid oxidation

Lipids are constituted of fatty acids; these, can be saturated, monounsaturated or polyunsaturated, according to the number of double bonds between the carbons. In presence of catalytic systems like light, heat and metals (e.g. Cu, Fe), lipid oxidation in food products is favoured. The reaction causes the formation of free radicals (they attack unsaturated fatty acids, causing a reduction in food quality) and other reactive species and goes ahead through auto-oxidation. The latter is the most common pathway of oxidation in food lipids.

The reaction proceeds in three distinct stages: initiation, propagation and termination (Lorenzo et al., 2018) (**Figure 6**).

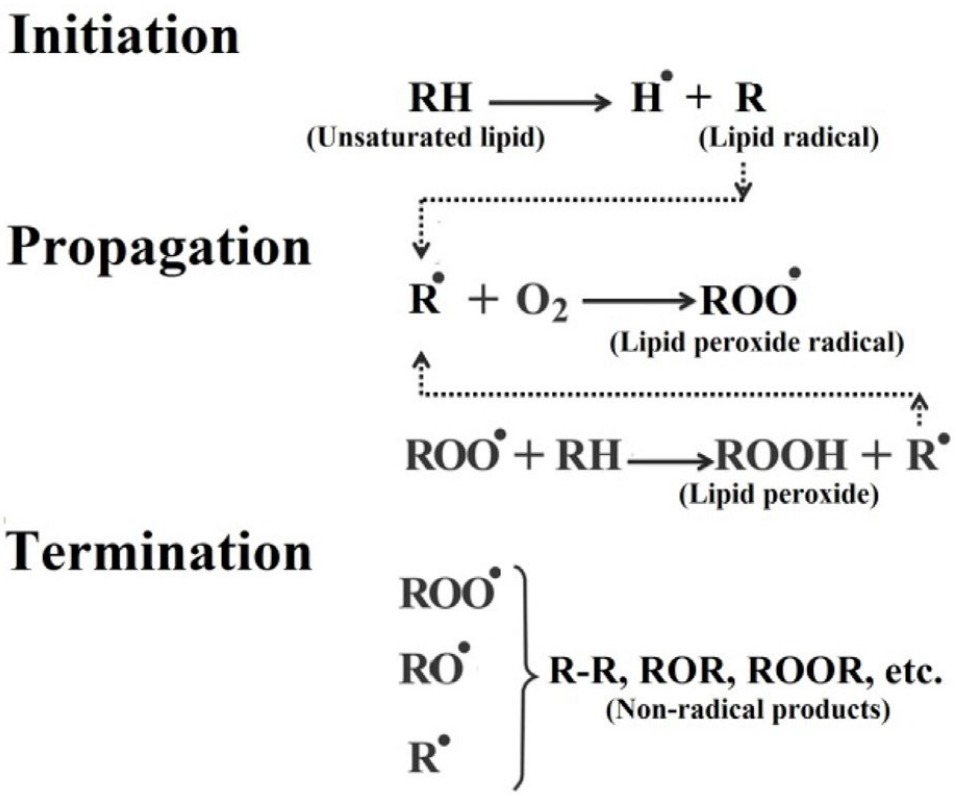

Figure 6. *The lipid autoxidation pathway. (Gavahian et al., 2018)*

The consequent degradation and autoxidation of fats leads to the formation of primary (are usually non-volatiles components), secondary (often comprise volatile components) and tertiary oxidation products (**Figure 7**).

Several analytical methods measuring primary and secondary oxidation products, can be used to detect the incidence of lipid oxidation in foods, as in peroxide value (PV) and 2-thiobarbituric acid reactive substances (TBARS) assays.

In cold plasma, electrical discharges are produced in feed gases containing N_2 and O_2, their collision with electrons result in a cascade of reactions forming reactive species. Ozone and singlet oxygen are two of the main responsible of plasma's antimicrobial action, however they are potential sources of oxidation of lipids in food (Gavahian et al., 2018). ROS obtained from plasma can initiate oxidation process, especially when treating food containing lipids (Van Durme & Vandamme, 2016). These reactive species (particularly OH• and O_2) have as primary target methyl groups in lipid moieties, with greater affinity for those linked to double

bonds. This because lower energy is needed for the abstraction of a hydrogen atom, compared to CH-bonds linked elsewhere. Hence, the highest is lipid unsaturation; the greatest is their susceptibility to ROS attacks. Linoleic acid (18:2) and α-Linolenic acid (18:3), containing two and three double bonds, respectively, are typical ROS sensitive fatty acids (Gavahian et al., 2018).

Polyunsaturated fatty acids are essential nutrients that must be introduced through the diet, since human body is not able to synthetize them. Seafood contains high amount of unsaturated fatty acids, in particular, fish and fish oil are rich in omega-3 fatty acids.

The oxidation of lipids by cold plasma leads to the formation of typical oxidation species (**Figure 7**) like ketones, aldehydes (hexanal, pentenal, nonanal and nonenal) and carboxylic acids (9-oxononanoic acid, octanoic acid, nonanoic acid), along with hydroperoxides (9- and 13-hydroperoxy-octadecadienoylglycerol species).

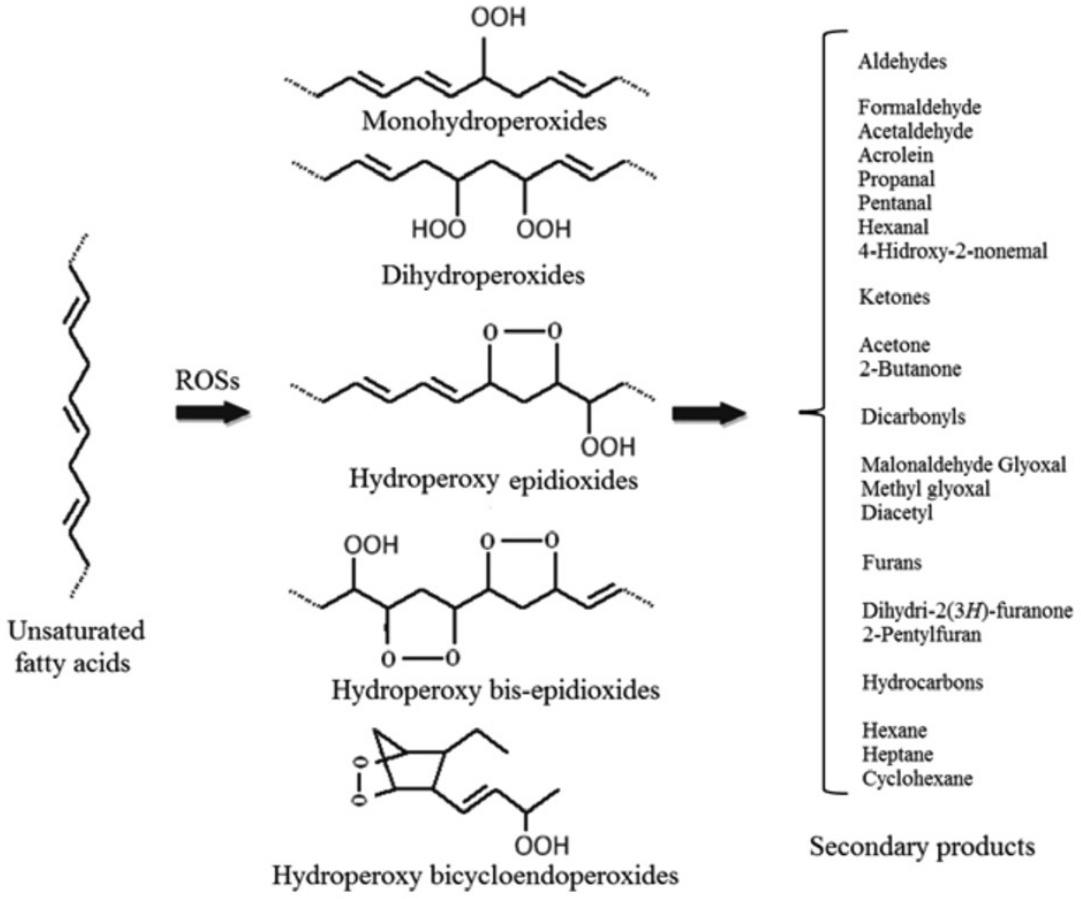

Figure 7. *Mechanism reaction for the formation of secondary products (Osório & Cardeal, 2013)*

Man has always benefited from fish consumption due its high content of ω-3 long chain polyunsaturated fatty acids (PUFA-n3). Omega-3 fatty acids in fish, may benefit heart health and reduce the risk of the insurgence of heart diseases. However, unsaturated fatty acids are more prone to lipid oxidation, which results in rancidity, off-flavours and odours.

Several authors have reported the efficacy of cold plasma for biocontrol of different food products like fish, ham, cheese and raw meat, however an understanding of the impact of this type of treatment on chemical quality parameters is also required. Applying cold plasma as food treatment to ensure food safety is promising, nevertheless food quality preservation must be guaranteed.

1.4. Sardine (*Sardina pilchardus*)

Sardine (*Sardina pilchardus*) is an important Mediterranean commercial fish species. It is a gregarious plankton-eating fish that lives in the open sea, at different sea depth levels (Whitehead, 1985). It is one of the most important commercial fish species in the Mediterranean Sea and, in particular, in the Adriatic Sea (Santojanni et al., 2006). Sardine's lipid content changes seasonally in function of animal biology, food availability and sea water temperature. In general, Adriatic sardines in winter-spring seasons are lean (total lipids < 4%), while in summer-autumn they are fat (total lipids > 4%). In the Adriatic Sea, sardine fishing is usually practiced in winter-spring months, when also sardine reproduction is in process. Summer sardine fishing is highly reduced because of the suspension of fishing activities. The principal nutritional characteristics of sardine lipids are the presence of PUFA-n3, particularly EPA and DHA. In fact, fat sardines provide significantly higher quantities of EPA and DHA (De Leonardis & Macciola, 2004). *De Leonardis & Macciola* studied the lipid fraction of fresh Adriatic sardine (*Sardina pilchardus*) filets. Using SPE they divided filet sardine lipids into different categories: neutral lipids (NLs) (they are principally reverve lipids), glycerides (tri-, di- and mono-), hydrocarbons, cholesterol and others, free fatty acids (FFAs), polar lipids (PLs) (structural lipids, prevalently phospholipids). They saw that fat and lean sardine had different lipid composition. Generally, sardine filets showed an acidic profile equally distributed among saturated fatty acids (38.3%) , monounsaturated fatty acids (31.2%) and polyunsaturated ones (30.4%). PUFA-n3 were always higher than PUFA-n6. Polar lipds distinguished from the other for their higher amount of SFA (47.7%) and smaller quantity of PUFA-n6. Free fatty acids presented a higher quanity of SFA (63.4%) and (15.8%) of PUFA. The principal constituent of SFA was palmitic acid, followed by miristic and stearic acids. Values of about 1%, were detected for C15:0 and C17:0, while C20:0 and C24:0 were found

in variables quantities. Regarding MUFAs, they were mainly composed of oliec acid with the isomers C18:1n9, C18: 1n7, and C18: 1n5. Palmitic acid (C16:1n7) was also present in relevant amount. All PUFAs were PUFA-n3 or PUFA-n6, with the only exception of C16: 2n4. PUFA-n6 were mainly represented by linoleic acid (C18: 2n6), followed by C20:4n6. C20: 5n3 (EPA) and C22: 6n3 (DHA) were the most abundant of PUFA-n3. The authors also conducted gas chromatographic analyses on sardine sterols and found that cholesterol was the princial and pratically the unique sterol. Lean sardines owed higher amount of it, compared to fat ones, due to the fact that they are rich in structural lipids and cholesterol belongs to this category (De Leonardis & Macciola, 2004).

In cold plasma treatments, ozone and hydroxyl radicals are considered the principal active species, which cleave the double bonds of some unsaturated fatty acids that are present in sardine, such as oleic, palmitoleic and linoleic acids, leading to sardine's oxidation (Sarangapani et al., 2017).

1.5. Mussels (*Mytilus Galloprovincialis*)

Mediterranean mussels *(Mytilus Galloprovincialis)* farming has a great impact on the economy of many European countries. Every year, in Italy this mollusc is harvested in amount higher than 170,000 tons, which represents more than 75% of the total shellfish production (Sarangapani et al., 2017). This kind of mollusc is a filter-feeding organism which can accumulate bacteria in very high quantity. In fact, bivalve molluscs can accumulate microorganisms including pathogens from seawater. The type of microorganism accumulated and its amount, depend on seasonal, climatic and anthropogenic factors (Vernocchi et al., 2007). Water temperature, nutrient availability and reproductive cycle of mussels can affect meat yield, microbiological characteristics and the biochemical composition of these bivalve molluscs.

The use of PAW on mussels is a valuable method to reduce the bacterial load and increase their shelf-life. However, their lipid profile and the effect that plasma can have on it should be taken into account.

Vernocchi et al., (2007) characterized fresh Mediterranean mussels (*Mytilus Galloprovincialis*) harvested in Adriatic Sea. The authors noticed that in all the samples, seven fatty acids (myristic, stearic, palmitic, palmitoleic, oleic, eicosapentaenoic and docosahexaenoic acids) were the ones present in highest amount. Palmitic acid (C16:0) was the main saturated fatty acid in mussel, with a variable concentration from 20% to 28% during the different months. While, palmitoleic acid (C16:1) was the main monounsaturated fatty

acid. They also noticed an increase in the unsaturation level of fatty acids, particularly during the coldest months (C20:5 was the predominant). This, was mainly due to the need to maintain cell membranes' fluidity. *Orban et al., (2002)* demostrated that mussles are able to convert C20:5 to C22:5. However, the main source of some unsaturated fatty acids is phytoplancton. The latter is the most important food resource for these molluscs. So, unsaturation levels and fatty acid composition, are determined by the quantitative and qualitative availability of food (Freites et al., 2002).

AIM OF THE THESIS

The objective of the current study was to evaluate the lipid oxidation level through the quantitative and qualitative analysis of the target volatiles compounds, fatty acid composition and oxysterols in fillets of *Sardina pilchardus* and *Mytilus Galloprovincialis,* treated with plasma activated water (PAW).

2. MATERIAL AND METHODS

2.1. Sampling

Raw sardine filets (*Sardina pilchardus*) and mussels *(Mytilus Galloprovincialis)* were purchased from the local market. The fish specimens were kept refrigerated covered with ice inside polystyrene boxes and transported to the laboratory within 1 h of purchase. Once in the laboratory, sardine filets were decapitated and eviscerated. Subsequently they were cut in half, mixed and washed with tap water for 30 seconds. After washing the samples were dried using paper towel to remove the water excess.

Mussels were cleaned, removed from the shell, washed with tap water for 30 seconds and then dried using paper towel. After that, sardine and mussels were subjected tothe PAW treatment.

50 g of each sardine sample were dipped in two different types of PAW: demineralized (D) water bought from the supermarket and sterilised distilled water (SD), bought in the pharmacy, for 3 different time periods: 10, 20 and 30 minutes. Sardine filets treated with demineralized water were named: P10 D, P20 D, P30 D, while sardine filets treated with sterilised distilled were named: P10 SD, P20 SD, P30 SD. 1:3 solid:liquid ratio was used and samples were kept in agitation. As a control the samples were dipped for the same time period in D water (samples C10 D, C20 D, C30 D) and the others in SD water (samples C10 SD, C20 SD, C30 SD), maintaining them in agitation.

For mussels, 50 g of each sample were dipped in PAW using just one type of PAW: the sterilised distilled one, bought in the pharmacy. 3 different exposure times of the samples in PAW were used: 5, 10, 15 minutes. Mussels treated only with sterilised distilled water were named: P5 SD, P10 SD, P15 SD. 1:3 solid:liquid ratio was used and samples were kept in agitation. As a control the samples were dipped for the same time period in SD water (samples C5 SD, C10 SD, C15 SD), maintaining them in agitation.

Each sample was equally distributed, placed in plastic bags and stored at -20°C, before the lipid cold extraction was made.

Three replicates were performed respectively for sardine specimens treated with D and SD plasma activated water. Also for mussels three sampling were performed at each treatment time.

Moreover, one sample of raw sardine filet (sample TQ) was frozen as such. The same was done for raw mussels, frozen likewise (sample TQ).

2.2. PAW conditions

PAW was obtained with a "Microsecond pulsed corona" set up. The treatment was optimized in order to obtain the highest number of reactive species, always keeping the temperature at room temperature. The electrode used for plasma production is made of stainless steel. The volume of water subjected to activation was 500 mL. The total treatment time to activate water was set to 1 minute and the peak voltage was 18 kV with a pulse repetition frequency of 5 kHz. The resulted PAW contains 40-80 mg/l of nitrites and 3-10 mg/l of hydrogen peroxide. pH measurements were performed in SD and D waters prior and after the plasma treatment and different values were detected. Before plasma treatment SD water had a pH of 8.73, this shift to a more acidic pH around 4, after water activation through plasma. Once samples were dipped in activated SD water, the pH raised again and reached 6.6. D water showed different pH values. Before the treatment, the pH value of D water was 6.87, after water activation6.49 and then decreased to 6.3 after dipping the samples in it.

It was noticed that, dipping the samples for different time periods did not cause a change in pH, neither in PAW used to treat sardine, nor in the one used to treat mussels.

2.3. Total lipid extraction

Total lipids were extracted using cold extraction method, as described by *Bligh & Dyer, (1959)*. In brief, 50 g of each sample of sardine and mussel was homogenized in Erlenmeyer flask with choloroform:methanol (150 ml, 1:2, v/v), using IKA T 25 digital ULTRA-TURRAX®, 19.000 RPM for 80 seconds. The suspension was filtered through Whatman filter paper (Grade 4, 90 mm, Merck KGaA, Darmstandt, Germany). After filtration, the filtered cake was homogenized again in 50 ml of chloroform using IKA T 25 digital ULTRA-TURRAX®, 19.000 RPM, for 30 seconds. The solutions collected from the two filtrations were placed in the same separation funnel and they were washed one time with 100 ml of KCl aqueous solution (0.88% w/v). Subsequently, the separation funnel was covered with aluminium foils and leaved to stand for 24 hours. The day after the solvent with the oil (down part of the separation phase) were collected in a balloon flask and the solvent was evaporated with rotary Buchi™ Rotavapor™ (40° C). The oil was then recovered from the flask using diethyl ether and it was transferred in tubes. The latter were leaved in hot bath (42° C), in order to evaporate the solvent.

2.4. Volatile analysis by GC-MS

Volatile component analysis was conducted through Gas Chromatography–Mass Spectrometry (GC-MS), according to *Savini et al., (2017)*. Briefly, GC vials containing 0.5 g of oil of each sample, were prepared respectively for sardine filets and mussels.
The samples were placed in hot bath at 40°C and the fibre (SPME solid phase micro extraction) was exposed for 40 minutes.

The fibre thermal desorption in the GC-MS injector was done at 220°C for 3 minutes.

GC-MS analysis were performed using a gas Varian 3900 chromatograph with a Saturn 2100 T (Varian, Walnut Creek, CA,USA) ion trap mass spectrometer. The chromatographic separation was done through a ZB-5capillary column (30 m x 0.25 mm D.I. The thickness of the film was 0,25 µm, Phenomenex). In splitless modality (splitless time 0.3 min).

The oven program temperature started at 40°C for 5 minutes, at 140°C it was leaded to a 4°C/min increase and it reached 220°C with 10°C/min speed, the ion trap and the transfer line were set at 200 °C and 220 °C, respectively.

Volatiles compounds were classified and identified with Variation Software. The identification of the compounds was confirmed with Kovats retention Indices (RIs) and with mass spectral data collected in the NIST/EPA/NIH Mass Spectral Library (Version:2.0;2002).

2.5. Fatty acids analysis by GC-FID

Fatty acids analysis was performed as described by *Haddad et al., (2012)*. Before the analysis, fatty acids are commonly derivatized to form fatty acids methyl esters (FAMES) which are then detected by GC-FID. For the analysis, TRACE 1300 Gas Chromatograph (ThermoFisher Scientific, Waltham, Massachusetts, United States) was used. The column used is: TG-POLAR column. Its length is 60 m and the diameter is 0.25 mm. The thickness of the stationary phase is 0.2 µm. The maximum temperature is 260/275°C. The carrier gas is N_2.

The temperature of the oven was set at 150°C x 1 minute and increased 3°C/min, until a final temperature of 240°C was reached and this was kept for 5 minutes.

The temperature of the injector and the flame ionization detector (FID) were set at 250°C. Direct trans-esterification through acidic catalysation was performed for the transformation of the extracted lipids into their corresponded methyl esters (they have a higher volatility and lower polarity than the correspondent free acids).

The identification of the chromatographic peaks was done comparing the retention times of a blend of standards of 37 fatty acids methyl esters (FAMES).

Fatty acids composition (weight % of each fatty acid) was calculated by the areas'

normalization method.

2.6. Determination of cholesterol oxydized products (COPs)

Cholesterol oxidized products or oxysterols determination was done as described by *Larkeson et al., 2000* with slight modifications. It consists in three different steps: oil extraction by *Bligh & Dyer, 1959* method (as reported previously), cold saponification and solid phase extraction (SPE), as purification step in order to selectively extract those.

2.7. Cold saponifictaion for COPs analysis

Cold saponification was performed as described by *Larkeson et al., (2000*) with slight modifications. Briefly, 250 mg of sample was weighted in 50 ml volumetric flask and added of two internal stardards: 1 ml of 5alfa-cholestane (cholesterol standard) (solution 100 ppm in hexane/isopropanol, 3:2) and 25 µl of 19-hydroxycholesterol (COPs standard) (solution 500 ppm). The sample was taken to dryness and added of 10 ml KOH 1M, as base to facilitate the saponification step. The sample was left 18 h in agitation, in bath at 25°C, covered with aluminium foil, in order to complete the saponification process. The reaction was then stopped adding 10 ml of distilled water. The sample was then transferred to a 100 ml separation funnel added of diethylether, shaken vigorously and removed of the down part. This step was repeated three times. For the hydroscopic compounds washing, 20 ml of distilled water was added and gently shaken. The sample was washed two times and removed of the water fraction. The sample was then filtered with sodium sulphate and concentrated at rotavapor at 40°C. The total unsaponifiables prepared by cold saponification were dissolved in 1 ml of hexane/diethylether (75:25 v/v).

2.8. COPs determination after saponification

Silica solid phase (SPE) cartridges were used to concentrate the non-volatile polar fractions of the total unsaponifiable matter oxidized derivatives of cholesterol (hydroxy, epoxy and keto). The column was conditioned using 10 ml hexane. 900 µl of the unsaponified extract dissolved in 1 ml hexane/diethylether (75:25 v/v), was deposited in the column after the conditioning step. The tube was washed with an additional 2 ml of hexane:diethylether 75:25 (v/v) and the unsaponifiables were eluted through the column dropwise. Thereafter, the column was eluted with 6 ml of hexane diethylether 60:40 (v/v) and the eluates were discharged. COPs were eluted with 4 ml acetone. Acetone was dried under nitrogen and the residue derivatized to TMS (trimethylsilyl) derivatives for the subsequent analyses by GC-

MS. Silylation is advantageous in terms of thermostability and peak shape of TMS derivatives and allows also a better chromatographic resolution. Reproducible derivatization depends on the silylating reagent, the reaction conditions, and the ratio silylating reagent/hydroxyl groups. Sylon BTZ is the silylating reagent used in this study, it contains 10:2:1 pyridine (99.8%) : hexamethyldisilazane (≥99%) : cholorotrimethylsilane (≥99%), as solvent. In order to complete the silanization of COPs to TMSE, Sylon BTZ was leaved 60 minutes at room temperature. After 1hthe silanized sample was taken to dryness and then added of 100 µl hexane. After centrifuging, 1 µl was taken to be injected in the GC-MS.

Analysis of COPs by GC-MS. Quantification of COPs as TMS derivatives was carried out on a TRACE 1300 Gas Cromatograph, coupled to a ISQ 7000 mass spectrometer. The chromatographic separation was done through a Zebron ZB-5MS capillary column, (30 m x 0.25 mm D.I. The thickness of the film was 0,25 µm, Phenomenex). The samples were injected in splitless modality and the purge flow was 500 ml/min. Helium was used as carrier-gas at an inlet pressure of 15 ml/min. A temperature-programmed oven was set at 90°C for 50 sec and then raised to 300° C at a rate of 30° C/min and then held at this temperature for an additional 32 min.

COPs were classified and identified with Chromeleon Software.

2.9. Data analysis

On volatiles and fatty acids data, the Tukey–Kramer's honest significant difference (HSD) test was used to compare the experimental variables among the different sardine and mussel samples. All statistical analyses were carried out by the software JMP® Version 10 (SAS Institute Inc., Cary, NC, USA).

3. RESULTS AND DISCUSSION

3.1. Volatile analysis

In this study, the presence of primary and secondary lipid oxidation products has been determined in order to investigate the different degree of lipid oxidation of sardine and mussels, treated through PAW activated water for distinct times and using different types of water.

In the headspace of both sardine oil (treated with SD and D water) and mussels oil (treated with SD water), about fifty volatiles lipid oxidation products were fully identified (**Table 1**). From the total detected compounds, just twelve among the most relevant were selected and further analysed in sardine samples treated with the two type of water (**Figure 8** and **Figure 9**) and in mussel samples (**Figure 10**).

Table 1. *Major volatile compounds detected in sardine and mussel samples*

Category	Volatile Compounds	Category	Volatile Compounds
Aldehydes	pentenal isomer*	**Aliphatic hydrocarbons**	decane, x-methyl
	2-pentenal, (E)-*		n-undecane
	hexanal*		dodecene isomer
	2-hexenal, (E)-*		1-dodecene
	4-heptenal, (Z)-*		n-dodecane
	2,4-heptadienal, (E,Z)-*		dodecene isomer
	n-octanal*		dodecene isomer
	2,4-heptadienal, (E,E)-*		tridecane isomer
	n-nonanal*		1-tridecene
			n-tridecane
Ketones	3-penten-2-one, (E)-*		1-tetradecene
	3,5-octadien-2-one, (E,E)-*		n-tetradecane
			n-pentadecane
Alcohols	2-penten-1-ol, (Z)-*		1-hexadecene
			n-hexadecane
Ethers	cyclohexene, 1-methoxy-		heptadecene isomer

			heptadecene isomer
Aliphatic hydrocarbons	octadiene isomer		1-heptadecene
	octadiene isomer		n-heptadecane
	octadiene isomer		hexadecane, 4-methyl
	1-decene		
	n-decane	**Aromatic hydrocarbons**	ethylbenzene
	decane, x-methyl		m/p-xylene
	decane, x-methyl		o-xylene

*the most representative volatile lipid oxidation products

Aldehydes are most prominent volatiles produced during lipid oxidation and have been used to monitor the oxidation phenomena. The latter, when occurs in fish muscle is known to produce volatile aldehydic compounds including hexanal, heptanal, octanal, nonanal. *Iglesias & Medina, (2008)* conducted a study on lipid oxidation of fish products and they reported that hexanal is one of the main volatile compounds formed during the storage of Atlantic horse mackerel muscle at 4°C. Hexanal, and heptenal are generated from n6 PUFA oxidation, and they contribute to the strong intensities of fishy and rancid off-odours .

In sardine samples treated in D water. Among all secondary volatiles lipid oxidation products, n-nonanal was found as the most abundant, as well the major aldehyde formed, followed by 2,4-heptadienal, (E,E) and n-octanal respectively. In addition, alcohol and ketone were also formed. In particular 3,5-octadien-2-one, (E,E) was the most abundant among all ketones. While, low levels of the only alcohol considered: 2-penten-1-ol, (Z), were detected in all control and treated samples.

N-nonanal and 3,5-octadien-2-one, (E,E), were the two predominant secondary volatiles oxidized products found, with a higher concentration compared to the other volatiles. These two volatiles had a similar behaviour during the treatment. Both of them were already present in high quantity (12000-14000 %) in raw sardine samples (TQ) and after dipping the samples at 10 and 20 minutes in agitation in not-activated D water, an increase in the oxidation degree was detected. Whereas, after 30 minutes it decreased. These findings are in line with those obtained by *Park & Ha (2015).* They subjected dried filefish (*S. cirrhifer*) fillets to cold oxygen plasma, obtaining an increase in the levels of lipid oxidation by treatment time >10 minutes and a tremendous increase in lipid oxidation levels (measured as TBARS) for samples treated for 20 minutes in comparison to control. In PAW treated samples, both n-nonanal and 3,5-octadien-2-one, (E,E) increased by increasing the treatment time. 2,4-heptadienal, (E,E)- had a similar behaviour as the two above mentioned secondary volatiles lipid oxidation products, in the three control samples (C10, C20, C30). While, when dipped in PAW, after an increase

at 10 and 20 minutes in the aldehyde concentration, at 30 minutes those ones decreased. The two aldehydes: n-octanal and 4-heptenal, (Z) had the same behaviour in samples dipped in PAW water for 10, 20 and 30 minutes, but a different trend was noticed for the control samples since the two aldehydes decreased from TQ to C10 then increased from C10 to C20 and then decreased again from C20 to C30. 2-hexenal, (E) and hexenal followed the same trend of n-octanal and 4-heptenal, (Z), in all the samples, with the exception of TQ, C10 and C20. From TQ to C20 there was a linear increase in their concentration. The alcohol 2-penten-1-ol, (Z), raised from TQ to C10 and then decreased up to 30 minutes of dipping in agitation. Then increased by increasing the dipping time in activated water. The aldehyde 2-pentenal, (E), had the same behaviour, but it just increased from P20 to P30. The concentration of the aldehyde pentenal isomer and the ketone 3-penten-2-one, (E) showed not significant differences compared to other secondary volatiles lipid oxidation products and they did not vary significantly both in the control and in the treated ones, a slight increase was just noticed for the ketone in in the control sample dipped for 10 minutes in D water.

So, a general increase in secondary volatiles lipid oxidation products was noticed, especially by increasing the dipping time in PAW from 10 minutes to 20 minutes. However, *Pérez-Andrés et al., (2020)* obtained different results. They found no significant effect on lipid oxidation between the control samples and those treated at 80 kV, for extended treatment times of 5 minutes, employing an in-house dielectric barrier discharge atmospheric plasma system.

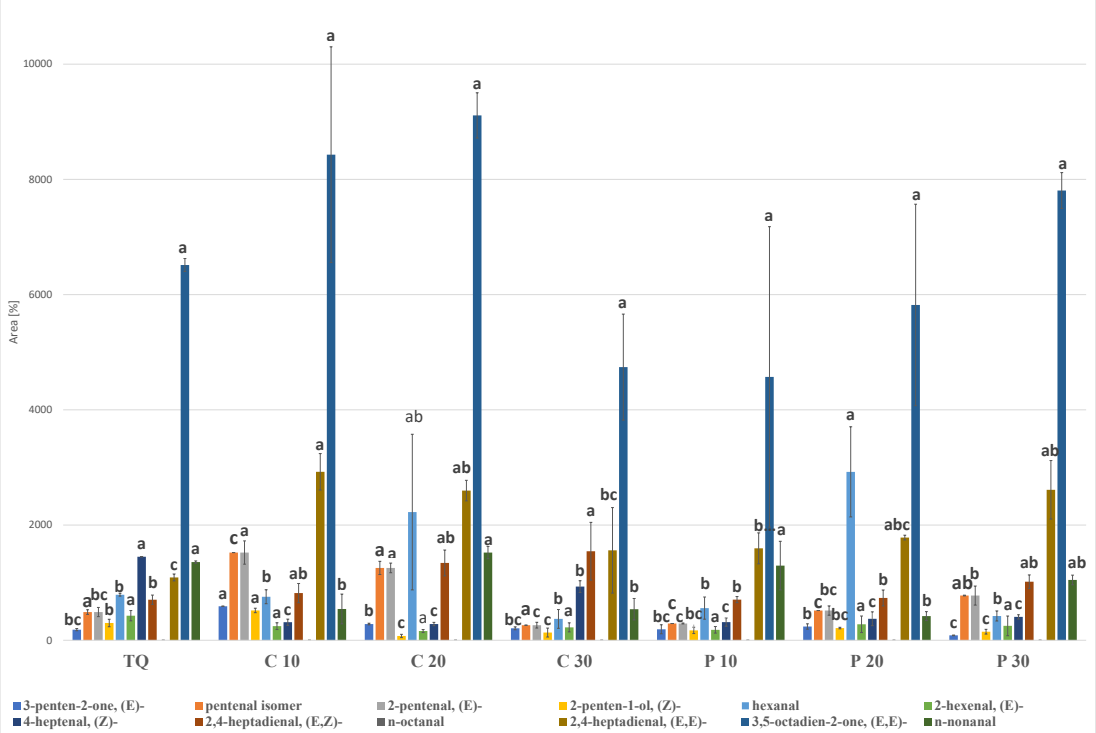

***Figure 8**. volatile compounds (% area) of fresh sardine (TQ), control (C10, C20, C30) and treated (P10, P20, P30) fillets in D water*

In sardine samples treated in SD water. a different trend in secondary volatiles lipid oxidation products, compared to D water, was noticed. When dipping the samples 10 minutes in SD water, these volatiles decreased. Also in this type of water, as in D one, the aldehyde n-nonanal, and the ketone 3,5 octadien-2-one (E,E) were the volatiles present in higher concentration either in TQ, control and plasma treated samples. These two compounds followed the same trend of other volatiles which were present in lower amount, such as: 2,4-heptadienal (E,E), n-octanal, 2,4-eptadienal (E,Z), 4-heptenal (Z), 2-hexenal (E), pentenal isomer and hexenal. In all control samples, these secondary volatiles lipid oxidation products, decreased when dipped for 10 minutes, then increased when dipped 20 minutes and later decreased again with 30 minutes dipping. These results agree with those reported by *Albertos et al., (2019),* who described an increase in secondary oxidation products, expressed as TBARS, when exposing Atlantic herring (*Clupea harengus*) to dielectric barrier discharge (DBD).

In our study, we observed a clear increase of the same volatiles when treating the samples in activated SD water, especially when sardine samples were dipped 10 minutes in it. Then, by lengthening the dipping time to 20 and 30 minutes almost a linear decrease in secondary volatile lipid oxidation products was noticed. These results are different from the ones obtained

in D water. In it, the higher increase in volatiles occurred in 20 minutes dipping of the samples in PAW water. The ketone 3-penten-2-one, (E) and the alcohol 2-penten-1-ol, (Z) followed the same trend, decreasing from TQ to C30 and then increasing in plasma treated samples. However, 3-penten-2-one, (E) was present in higher amount (400%) compared to the alcohol (200%) during 10 minutes treatment. Differently from the other volatile compounds, the aldehyde 2-pentenal (E) decreased in all the control samples, even lengthening the treatment time and raised in plasma treated ones. However, this was noticed after 20 minutes dipping in PAW and not after 10 minutes as for the other volatiles. *Choi et al., (2020)* obtained the same results. They measured the TBARS value, to determine the effects of DBD plasma treatment on dried blackmouth angler. This value increased with a gradual increase in the DBD plasma treatment time (1–30 min) on the surface of dried blackmouth angler *(Lophiomus setigerus),* that means an increase in the content of the secondary oxidation products.

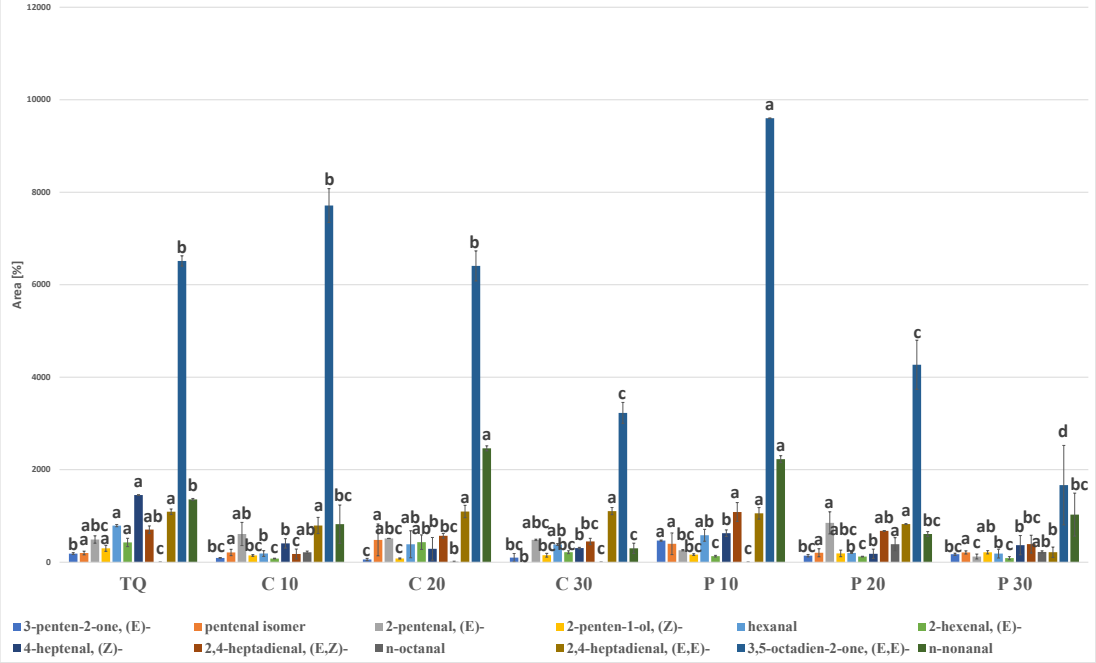

Figure 9. *volatile compounds (% area) of fresh sardine (TQ), control (C10, C20, C30) and treated (P10, P20, P30) fillets in SD water*

In mussel samples treated in SD water. The aldehyde n-nonanal, and the ketone 3,5 octadien-2-one (E,E) were the one of higher concentration either in TQ, control and plasma treated samples. As also noticed in sardine samples treated in D and SD water. N-nonanal, 2,4-heptadienal (E,E), 2,4-heptadienal (E,Z), 4-heptanal (Z), 2-hexenal (E), hexenal, 2-penten-1-ol, (Z), followed the same trend. They did not change significatively in control samples.

Conversely n- octanal showed a significant increase once lengthening the dipping time, on the contrary 3,5 octadien-2-one (E,E), decreased but not in a significant way. On the contrary, dipping the samples in PAW activated water for 10 minutes caused an increase in secondary volatiles lipid oxidation products. Similar results were obtained by *Puligundla et al.*, *(2018)*. They conducted a study on the application of corona discharge plasma jet (CDPJ) for the improvement of hygienic quality of *Gwamegi* (Semi-dried Pacific Saury). The CDPJ generated using 20 kV DC voltage, at 58 kHz frequency aimed at the decontamination treatment (1–10 min). 10 minutes treatment exert significant changes ($p < 0.05$) in color and thiobarbituric acid reactive substance levels. This means that an increase in secondary volatiles lipid oxidation products happened. On the contrary, in our study, once we left the samples in PAW water for 15 minutes a decrease in secondary volatiles lipid oxidation products, was noticed. Regarding 2-pentenal (E), pentenal isomer and 3-penten-2-one (E), they did not vary significantly among the control and the treated samples.

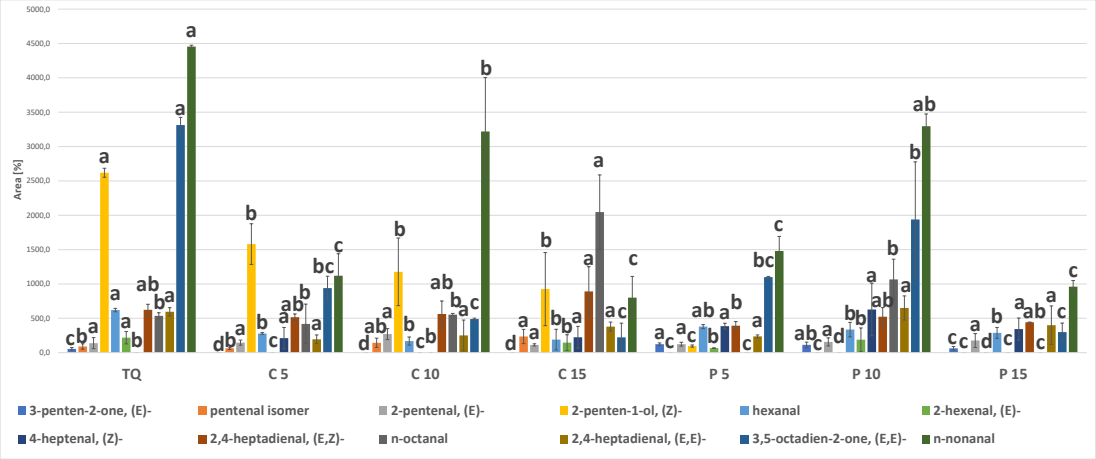

***Figure 10**. volatile compounds (% area) of mussels (TQ), control (C5, C10, C15) and treated (P5, P10, P15) samples in SD water*

It should be noticed that in both sardine and mussel samples treated using SD water the maximum increase in secondary volatiles lipid oxidation products occurred after 10 minutes dipping in PAW and by lenghtnening the holding time their presence sharply decreased. On the other hand, sardine samples treated in D water showed the highest amount in volatiles oxidized products once dipped 20 minutes in activated water and then their concentration slightly decrease. This results can be ascribed to the difference in the chemical composition of the two type of water used for the treatments. This discrepancy on the obtained results, could

be refered to the formation of different ROS and RNS, which in turn have a different effect in the product oxidation.

3.2. Fatty acids analysis

Sardine and mussels are important sources of valuable fatty acids like EPA and DHA. Therefore, studying the fatty acid profile, of PAW treated samples, is crucial to know the impact of the treatment on them. 37 fatty acids (**Table 2** and **Table 3**) were identified and quantified, in sardine samples treated using D and SD water. Among them, just seven: C16:0, C16:1, C17:0, C18:1Δ9c, C18:1Δ11 (vaccenic acid), C18:2cΔ9,12 and alpha 18:3 were selected (since they are the most important from a qualitative point of view), and their trend was analysed more in deep in sardine samples treated with the two type of water (**Figure 11** and **Figure 12**) and in mussel (**Figure 13**).

In sardine samples treated in D water and SD water. All the seven chosen fatty acids showed a lower concentration in TQ samples, compared to control and treated ones. In particular, C:16 was the one detected in the highest quantity in TQ (12,9%) compared to the fatty acids. Also *Albertos et al., (2017)* discovered palmitic acid as the main saturated fatty acid in fresh mackerel *(Scomber scombrus)* fillets, treated through dielectric barrier discharge (DBD) generated plasma.

It is valuable to notice that in our study, C:16:0, C17:0, C:18:1Δ9 and alfa 18:3 increased in their concentration in plasma treated samples, especially when they were dipped in PAW 20 and 30 minutes along. These results are in agreement with those obtained by *Albertos et al., (2017).* They reportedthat the levels of oleic acid (C:18:1Δ9), were lower for the control samples, compared to plasma treated ones. Especially when increasing the treatment from 1 to 5 minutes applying a 70kV to fresh mackerel *(Scomber scombrus)* fillets. This reduction could be attributed to the reactive oxygen and nitrogen species generated.

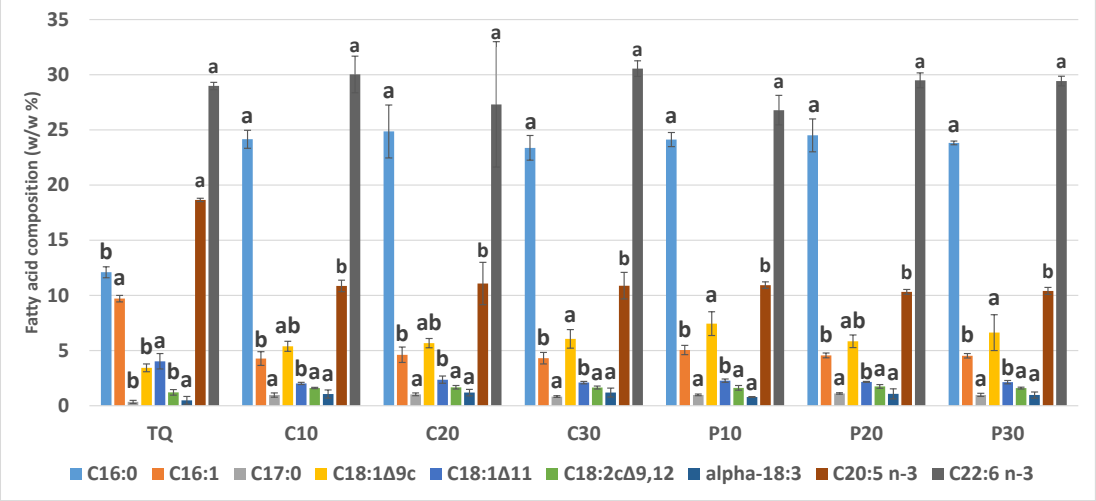

***Figure 11**. Fatty acid composition (w/w %) of sardine samples treated in D water*

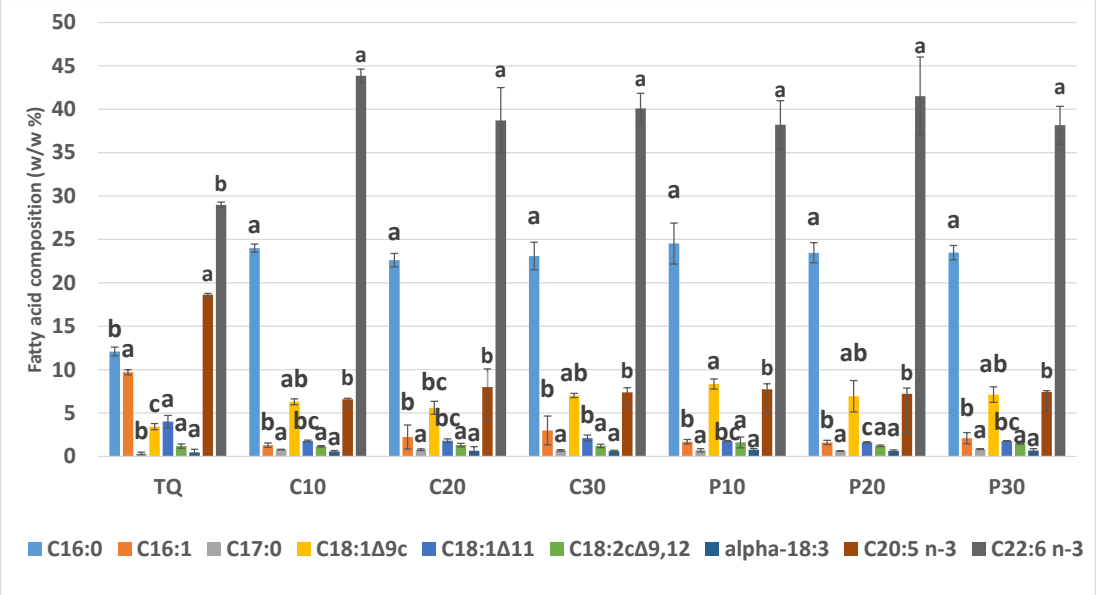

***Figure 12.** Fatty acid composition (w/w %) of sardine samples treated in SD water*

In mussel samples treated in SD water. 27 fatty acids were identified and quantified (**Table 4**). Among them, just seven: C16:0, C16:1, C17:0, C18:1Δ9c, C18:1Δ11 (vaccenic acid), C18:2 n-6 and alpha 18:3 were selected (since they are the most important from a qualitative point of view), and their trend was widely analysed. C16:0 and C16:1 were the predominant fatty acids in TQ samples. C16:0 decreased in the control when dipping the sample 5 minutes in not-treated water and then no significant difference were quantified in the treated ones. Regarding C16:1, C17:0, C:18:1Δ9c, C:18:1Δ11, C18:2 n-6 and alpha-18:3, not significant variations were detected among TQ, control and treated samples. Similar results were obtained

by *Kulawik et al., (2018),* who treated sushi samples with plasma generated using dielectric barrier discharge equipment.

The analysis of fatty acid composition showed that the most abundant ones in sushi were palmitic acid (C16:0); oleic acid (C18:1, n-9); linoleic acid (C18:2, n-6) eicosapentanoic acid (EPA, C20:5, n-3) and docosahexanoic acid (DHA, C22:6 n-3). However, no significant differences in the content of each fatty acid were found between the treated and untreated samples for both sushi products. Although, lipid oxidation occurred, since an increase in secondary oxidation products (expressed as TBARS) was noticed. Therefore, the non-thermal plasma treatment did not affect the fatty acids composition of sushi samples, even if an increase in oxidation (measured as TBARS) was detected.

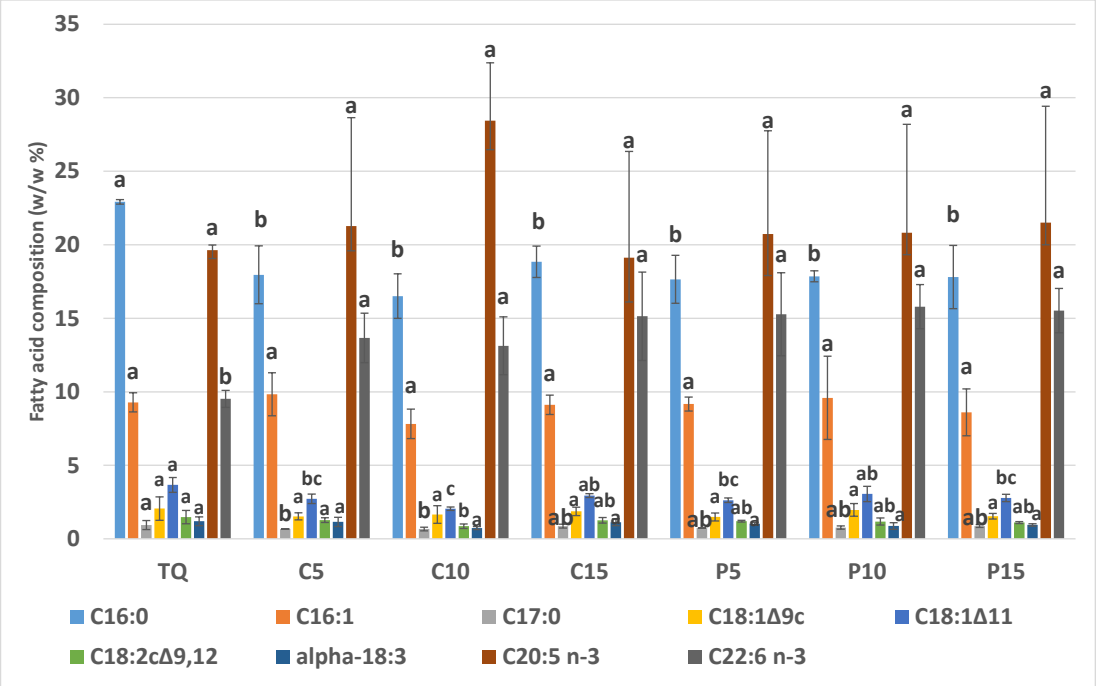

***Figure 13**. Fatty acid composition (w/w %) of mussel samples treated in SD water*

Table 2. *Major fatty acids detected in sardine samples treated in D water, calculated as percentage areas*

Fatty acid	TQ	C 10	C 20	C 30	P 10	P 20	P 30
C10:0	0,02 ± 0,20	0,05 ± 0,02	0,09 ± 0,10	0,04 ± 0,02	0,03 ± 0,01	0,04 ± 0,01	0,04 ± 0,02
C12:0	0,31 ± 0,40	0,14 ± 0,05	0,20 ± 0,18	0,13 ± 0,06	0,15 ± 0,03	0,10 ± 0,01	0,11 ± 0,02
C13:0	0,02 ± 0,15	0,07 ± 0,02	0,08 ± 0,07	0,05 ± 0,01	0,05 ± 0,01	0,06 ± 0,01	0,06 ± 0,01
C14:0	5,45 ± 0,39	5,98 ± 0,93	2,44 ± 3,08	5,55 ± 0,89	5,81 ± 0,31	5,67 ± 0,26	5,56 ± 0,44
iso-C15:0	0,01 ± 0,65	0,01 ± 0,01	0,22 ± 0,37	0,01 ± 0,01	0,01 ± 0,01	0,01 ± 0,01	0,01 ± 0,01
anteiso-C15:0	0,01 ± 0,30	0,03 ± 0,02	0,12 ± 0,16	0,02 ± 0,02	0,03 ± 0,02	0,03 ± 0,01	0,02 ± 0,01
C14:1Δ9	0,06 ± 0,25	0,16 ± 0,04	0,15 ± 0,11	0,15 ± 0,03	0,15 ± 0,03	0,16 ± 0,01	0,14 ± 0,01
C15:0	0,41 ± 0,75	1,03 ± 0,06	0,61 ± 0,50	0,94 ± 0,09	0,95 ± 0,08	1,05 ± 0,04	0,94 ± 0,04
C15:1Δ10	0,02 ± 0,80	0,00 ± 0,01	0,27 ± 0,46	0,01 ± 0,01	0,01 ± 0,01	0,00 ± 0,01	0,01 ± 0,01
C16:0*	12,09 ± 0,50	24,14 ± 0,82	8,49 ± 13,56	23,37 ± 1,12	24,12 ± 0,64	24,50 ± 1,49	23,81 ± 0,16
iso-C17:0	0,16 ± 0,45	0,79 ± 0,07	0,44 ± 0,36	0,63 ± 0,04	0,69 ± 0,14	0,78 ± 0,03	0,65 ± 0,02
anteiso-C17:0	0,09 ± 0,68	0,17 ± 0,04	0,30 ± 0,34	0,13 ± 0,04	0,14 ± 0,04	0,17 ± 0,02	0,14 ± 0,11
C16:1*	9,70 ± 0,30	4,27 ± 0,62	1,73 ± 2,21	4,31 ± 0,53	5,04 ± 0,42	4,55 ± 0,22	4,52 ± 0,20
C17:0*	0,34 ± 0,15	0,96 ± 0,19	0,43 ± 0,46	0,83 ± 0,08	0,98 ± 0,07	1,11 ± 0,07	0,97 ± 0,14
C17:1Δ10	1,23 ± 0,19	0,35 ± 0,19	0,24 ± 0,09	0,41 ± 0,20	0,43 ± 0,15	0,50 ± 0,07	0,30 ± 0,14
C18:0	2,27 ± 0,29	3,86 ± 0,46	1,54 ± 2,01	3,74 ± 0,36	3,86 ± 0,12	3,87 ± 0,23	3,96 ± 0,15
C18:1Δ9c*	3,43 ± 0,35	5,38 ± 0,46	2,06 ± 2,88	6,05 ± 0,85	7,44 ± 1,08	5,84 ± 0,57	6,62 ± 1,62
C 18:1Δ11*	4,02 ± 0,70	2,02 ± 0,10	0,94 ± 0,98	2,09 ± 0,11	2,26 ± 0,15	2,15 ± 0,04	2,13 ± 0,15
C18:2 n-6*	1,20 ± 0,25	1,60 ± 0,05	0,63 ± 0,84	1,65 ± 0,14	1,61 ± 0,21	1,74 ± 0,17	1,59 ± 0,08
gamma-18:3	0,40 ± 0,36	0,08 ± 0,01	0,15 ± 0,19	0,09 ± 0,03	0,09 ± 0,01	0,09 ± 0,01	0,09 ± 0,01
C20:0	0,34 ± 0,74	0,75 ± 0,42	0,64 ± 0,19	0,58 ± 0,46	1,00 ± 0,17	0,83 ± 0,49	0,78 ± 0,27
alpha-18:3*	0,48 ± 0,35	1,05 ± 0,37	0,59 ± 0,40	1,20 ± 0,40	0,79 ± 0,03	1,07 ± 0,46	0,97 ± 0,28
C20:1Δ11	0,54 ± 0,55	1,31 ± 0,45	0,77 ± 0,47	1,31 ± 0,19	1,38 ± 0,39	1,53 ± 0,25	1,56 ± 0,06
C21:0	0,03 ± 0,14	0,02 ± 0,02	0,06 ± 0,07	0,02 ± 0,03	0,02 ± 0,02	0,02 ± 0,01	0,03 ± 0,02
C20:2Δ11,14	0,35 ± 0,12	0,27 ± 0,06	0,15 ± 0,11	0,28 ± 0,01	0,27 ± 0,02	0,28 ± 0,00	0,28 ± 0,02
C20:3 n-6	0,47 ± 0,10	0,07 ± 0,02	0,06 ± 0,04	0,11 ± 0,07	0,08 ± 0,01	0,08 ± 0,01	0,07 ± 0,03
C22:0	1,39 ± 0,58	1,01 ± 0,08	0,56 ± 0,46	0,97 ± 0,09	1,05 ± 0,12	1,01 ± 0,04	0,98 ± 0,09
C20:3 n-3+C20:4n6	0,18 ± 0,41	0,10 ± 0,03	0,18 ± 0,20	0,09 ± 0,06	0,12 ± 0,04	0,10 ± 0,01	0,13 ± 0,05
C22:1Δ13	0,14 ± 0,33	0,19 ± 0,10	0,21 ± 0,12	0,15 ± 0,04	0,27 ± 0,15	0,17 ± 0,06	0,27 ± 0,03
C22:1Δ11	1,13 ± 0,21	0,63 ± 0,11	0,31 ± 0,28	0,68 ± 0,06	0,70 ± 0,10	0,69 ± 0,05	0,69 ± 0,04
C20:5 n-3	18,64 ± 0,15	10,85 ± 0,52	3,84 ± 6,07	10,87 ± 1,20	10,92 ± 0,29	10,31 ± 0,23	10,39 ± 0,32
C23:0	0,00 ± 0,46	0,01 ± 0,00	0,16 ± 0,26	0,01 ± 0,00	0,00 ± 0,01	0,01 ± 0,00	0,00 ± 0,00
C22:2Δ13,16	0,03 ± 0,78	0,01 ± 0,01	0,26 ± 0,45	0,02 ± 0,01	0,01 ± 0,01	0,01 ± 0,01	0,02 ± 0,01
C24:0	1,74 ± 0,14	0,37 ± 0,14	0,22 ± 0,13	0,39 ± 0,24	0,39 ± 0,01	0,25 ± 0,12	0,31 ± 0,09
C24:1Δ15	1,92 ± 0,19	1,40 ± 0,26	0,61 ± 0,68	1,63 ± 0,78	1,34 ± 0,77	0,86 ± 0,02	1,55 ± 0,69
C22:5n3	2,38 ± 0,22	0,86 ± 0,11	0,40 ± 0,40	0,94 ± 0,13	1,00 ± 0,18	0,89 ± 0,12	0,89 ± 0,17
C22:6 n-3	28,98 ± 0,33	30,02 ± 1,66	10,67 ± 16,77	30,55 ± 0,70	26,78 ± 1,34	29,48 ± 0,67	29,42 ± 0,44

**the most representative fatty acids*

***Table 3**. Major fatty acids detected in sardine samples treated in SD water, calculated as percentage areas*

Fatty acid	TQ	C 10	C 20	C 30	P 10	P 20	P 30
C10:0	0,02 ± 0,20	0,04 ± 0,03	0,09 ± 0,10	0,07 ± 0,04	0,07 ± 0,03	0,05 ± 0,02	0,03 ± 0,01
C12:0	0,31 ± 0,40	0,06 ± 0,03	0,16 ± 0,21	0,13 ± 0,09	0,14 ± 0,06	0,10 ± 0,04	0,07 ± 0,03
C13:0	0,02 ± 0,15	0,03 ± 0,01	0,06 ± 0,08	0,05 ± 0,03	0,05 ± 0,02	0,04 ± 0,02	0,02 ± 0,01
C14:0	5,45 ± 0,39	1,87 ± 0,53	0,93 ± 0,82	0,76 ± 0,20	0,59 ± 0,34	0,38 ± 0,20	0,30 ± 0,10
iso-C15:0	0,01 ± 0,65	0,02 ± 0,04	0,24 ± 0,36	0,21 ± 0,16	0,24 ± 0,10	0,17 ± 0,07	0,11 ± 0,05
anteiso-C15:0	0,01 ± 0,30	0,00 ± 0,01	0,10 ± 0,17	0,09 ± 0,08	0,12 ± 0,05	0,08 ± 0,03	0,05 ± 0,02
C14:1Δ9	0,06 ± 0,25	0,05 ± 0,01	0,10 ± 0,13	0,08 ± 0,06	0,09 ± 0,03	0,06 ± 0,03	0,04 ± 0,02
C15:0	0,41 ± 0,75	0,60 ± 0,08	0,48 ± 0,35	0,30 ± 0,20	0,29 ± 0,08	0,19 ± 0,11	0,12 ± 0,06
C15:1Δ10	0,02 ± 0,80	0,00 ± 0,01	0,27 ± 0,46	0,24 ± 0,23	0,31 ± 0,13	0,22 ± 0,09	0,15 ± 0,07
C16:0*	12,09 ± 0,50	24,00 ± 0,46	8,32 ± 13,58	7,45 ± 6,60	9,21 ± 3,81	6,54 ± 2,70	4,35 ± 1,98
iso-C17:0	0,16 ± 0,45	0,34 ± 0,07	0,28 ± 0,20	0,18 ± 0,11	0,16 ± 0,05	0,11 ± 0,06	0,07 ± 0,03
anteiso-C17:0	0,09 ± 0,68	0,32 ± 0,45	0,48 ± 0,18	0,37 ± 0,17	0,24 ± 0,11	0,17 ± 0,06	0,12 ± 0,06
C16:1*	9,70 ± 0,30	1,31 ± 0,25	0,62 ± 0,60	0,49 ± 0,21	0,43 ± 0,20	0,28 ± 0,13	0,20 ± 0,07
C17:0*	0,34 ± 0,15	0,79 ± 0,04	0,33 ± 0,40	0,26 ± 0,19	0,28 ± 0,11	0,19 ± 0,09	0,13 ± 0,06
C17:1Δ10	1,23 ± 0,19	0,65 ± 0,42	0,42 ± 0,23	0,36 ± 0,11	0,23 ± 0,12	0,16 ± 0,07	0,12 ± 0,04
C18:0	2,27 ± 0,29	4,06 ± 0,17	1,51 ± 2,21	1,30 ± 1,04	1,52 ± 0,62	1,06 ± 0,45	0,71 ± 0,31
C18:1Δ9c*	3,43 ± 0,35	6,30 ± 0,34	2,33 ± 3,44	2,04 ± 1,57	2,35 ± 0,97	1,63 ± 0,69	1,10 ± 0,48
C 18:1Δ11*	4,02 ± 0,70	1,78 ± 0,09	0,86 ± 0,86	0,60 ± 0,44	0,63 ± 0,21	0,43 ± 0,21	0,28 ± 0,13
C18:2 n-6*	1,20 ± 0,25	1,16 ± 0,09	0,50 ± 0,58	0,39 ± 0,27	0,41 ± 0,16	0,28 ± 0,13	0,19 ± 0,08
gamma-18:3	0,40 ± 0,36	0,04 ± 0,01	0,14 ± 0,19	0,11 ± 0,09	0,13 ± 0,05	0,09 ± 0,04	0,06 ± 0,03
C20:0	0,34 ± 0,74	0,14 ± 0,05	0,31 ± 0,38	0,24 ± 0,17	0,26 ± 0,10	0,18 ± 0,08	0,12 ± 0,05
alpha-18:3*	0,48 ± 0,35	0,58 ± 0,11	0,34 ± 0,24	0,23 ± 0,12	0,19 ± 0,07	0,13 ± 0,07	0,09 ± 0,04
C20:1Δ11	0,54 ± 0,55	0,29 ± 0,03	0,29 ± 0,26	0,19 ± 0,14	0,20 ± 0,06	0,13 ± 0,07	0,09 ± 0,04
C21:0	0,03 ± 0,14	0,01 ± 0,02	0,06 ± 0,07	0,05 ± 0,03	0,05 ± 0,02	0,03 ± 0,01	0,02 ± 0,01
C20:2Δ11,14	0,35 ± 0,12	0,17 ± 0,02	0,10 ± 0,08	0,07 ± 0,04	0,06 ± 0,02	0,04 ± 0,02	0,03 ± 0,01
C20:3 n-6	0,47 ± 0,10	0,11 ± 0,01	0,07 ± 0,05	0,05 ± 0,03	0,04 ± 0,01	0,03 ± 0,02	0,02 ± 0,01
C22:0	1,39 ± 0,58	1,53 ± 0,18	0,76 ± 0,70	0,54 ± 0,32	0,52 ± 0,19	0,34 ± 0,17	0,23 ± 0,10
C20:3 n-3+C20:4n6	0,18 ± 0,41	0,07 ± 0,02	0,16 ± 0,21	0,13 ± 0,10	0,15 ± 0,06	0,10 ± 0,05	0,07 ± 0,03
C22:1Δ13	0,14 ± 0,33	0,03 ± 0,02	0,13 ± 0,18	0,11 ± 0,08	0,12 ± 0,05	0,08 ± 0,04	0,06 ± 0,02
C22:1Δ11	1,13 ± 0,21	0,37 ± 0,02	0,20 ± 0,18	0,13 ± 0,10	0,14 ± 0,04	0,09 ± 0,05	0,06 ± 0,03
C20:5 n-3	18,64 ± 0,15	6,58 ± 0,12	2,28 ± 3,72	2,04 ± 1,81	2,52 ± 1,04	1,79 ± 0,74	1,19 ± 0,54
C23:0	0,00 ± 0,46	0,05 ± 0,08	0,20 ± 0,23	0,17 ± 0,08	0,16 ± 0,08	0,10 ± 0,05	0,08 ± 0,03
C22:2Δ13,16	0,03 ± 0,78	0,01 ± 0,01	0,27 ± 0,44	0,24 ± 0,22	0,30 ± 0,12	0,21 ± 0,09	0,14 ± 0,07
C24:0	1,74 ± 0,14	0,51 ± 0,06	0,24 ± 0,24	0,18 ± 0,11	0,17 ± 0,07	0,12 ± 0,05	0,08 ± 0,03
C24:1Δ15	1,92 ± 0,19	1,53 ± 0,43	0,72 ± 0,71	0,62 ± 0,16	0,50 ± 0,29	0,32 ± 0,17	0,26 ± 0,08
C22:5n3	2,38 ± 0,22	0,75 ± 0,08	0,35 ± 0,35	0,26 ± 0,16	0,26 ± 0,10	0,17 ± 0,08	0,12 ± 0,05
C22:6 n-3	28,98 ± 0,33	43,85 ± 0,79	14,99 ± 25,00	13,59 ± 12,16	16,92 ± 7,03	12,04 ± 4,94	8,00 ± 3,65

**the most representative fatty acids*

Table 4. *Major fatty acids detected in mussel samples treated in SD water, calculated as percentage areas*

Fatty acid	TQ	C 5	C 10	C 15	P 5	P 10	P 15
C14:0	2,73 ± 0,20	3,17 ± 0,65	2,30 ± 0,21	2,57 ± 0,23	2,79 ± 0,14	2,70 ± 0,58	2,43 ± 0,33
C15:0	0,68 ± 0,40	0,63 ± 0,08	0,48 ± 0,06	0,59 ± 0,07	0,56 ± 0,04	0,60 ± 0,14	0,58 ± 0,09
C16:0*	22,92 ± 0,15	17,96 ± 1,97	16,51 ± 1,52	18,84 ± 1,06	17,65 ± 1,63	17,86 ± 0,38	17,81 ± 2,15
Anteiso 17:0	0,26 ± 0,39	0,41 ± 0,06	0,36 ± 0,05	0,42 ± 0,01	0,45 ± 0,03	0,32 ± 0,14	0,44 ± 0,03
C16:1*	9,28 ± 0,65	9,83 ± 1,47	7,82 ± 1,00	9,12 ± 0,66	9,17 ± 0,48	9,59 ± 2,83	8,60 ± 1,60
C17:0*	0,94 ± 0,30	0,67 ± 0,01	0,67 ± 0,13	0,87 ± 0,14	0,74 ± 0,01	0,77 ± 0,11	0,87 ± 0,09
C17:1Δ10	7,44 ± 0,25	8,96 ± 0,71	7,10 ± 0,78	8,77 ± 0,37	9,20 ± 0,31	6,91 ± 1,43	8,53 ± 0,92
C18:0	3,97 ± 0,75	4,75 ± 0,32	4,98 ± 1,18	4,93 ± 0,80	4,84 ± 0,64	5,49 ± 0,72	5,09 ± 0,46
C18:1Δ9c*	2,06 ± 0,80	1,53 ± 0,24	1,66 ± 0,60	1,87 ± 0,28	1,49 ± 0,27	1,97 ± 0,43	1,54 ± 0,19
C 18:1Δ11*	3,67 ± 0,50	2,73 ± 0,32	2,05 ± 0,12	2,95 ± 0,14	2,63 ± 0,16	3,06 ± 0,52	2,79 ± 0,25
C18:2 n-6*	1,48 ± 0,45	1,28 ± 0,16	0,86 ± 0,15	1,26 ± 0,19	1,21 ± 0,06	1,18 ± 0,24	1,11 ± 0,07
gamma-18:3	0,08 ± 0,68	0,10 ± 0,01	0,05 ± 0,01	0,09 ± 0,04	0,10 ± 0,02	0,05 ± 0,03	0,09 ± 0,03
alpha-18:3*	1,20 ± 0,30	1,15 ± 0,32	0,73 ± 0,11	1,13 ± 0,23	1,03 ± 0,09	0,88 ± 0,23	0,95 ± 0,08
C20:1Δ11	2,29 ± 0,15	2,98 ± 0,40	2,07 ± 0,20	3,21 ± 0,56	2,82 ± 0,27	2,49 ± 0,99	2,95 ± 0,37
C21:0	0,09 ± 0,19	0,14 ± 0,01	0,12 ± 0,04	0,14 ± 0,02	0,11 ± 0,03	0,07 ± 0,04	0,14 ± 0,03
C20:2Δ11,14	0,82 ± 0,29	0,80 ± 0,12	0,49 ± 0,14	0,33 ± 0,17	0,84 ± 0,15	0,60 ± 0,18	0,48 ± 0,20
C20:3 n-6	0,26 ± 0,35	0,19 ± 0,06	0,20 ± 0,12	0,23 ± 0,03	0,18 ± 0,04	0,16 ± 0,07	0,18 ± 0,03
C22:0	4,73 ± 0,70	3,34 ± 0,41	2,44 ± 0,32	3,32 ± 0,66	3,24 ± 0,28	3,60 ± 0,63	3,43 ± 0,40
C20:3 n-3(20:4 n6)	0,03 ± 0,25	0,05 ± 0,05	0,00 ± 0,01	0,09 ± 0,03	0,03 ± 0,04	0,00 ± 0,01	0,05 ± 0,01
C22:1Δ13	0,01 ± 0,36	0,08 ± 0,03	0,09 ± 0,02	0,04 ± 0,04	0,10 ± 0,02	0,07 ± 0,04	0,09 ± 0,03
C22:1Δ11	0,33 ± 0,74	0,27 ± 0,06	0,18 ± 0,06	0,31 ± 0,04	0,26 ± 0,02	0,27 ± 0,11	0,27 ± 0,05
C20:5 n-3	19,64 ± 0,35	21,28 ± 7,38	28,44 ± 3,94	19,12 ± 7,24	20,73 ± 7,03	20,82 ± 7,37	21,50 ± 7,92
C22:2Δ13,16	0,02 ± 0,55	0,02 ± 0,01	0,09 ± 0,09	0,09 ± 0,09	0,03 ± 0,04	0,01 ± 0,01	0,03 ± 0,01
C24:0	1,53 ± 0,14	1,21 ± 0,18	0,78 ± 0,05	1,28 ± 0,20	1,25 ± 0,16	1,39 ± 0,16	1,28 ± 0,20
C24:1Δ15	0,63 ± 0,12	0,41 ± 0,01	0,59 ± 0,34	0,67 ± 0,37	0,99 ± 1,08	0,57 ± 0,02	0,66 ± 0,38
C22:5n3	3,39 ± 0,10	2,39 ± 0,48	1,79 ± 0,22	2,63 ± 0,61	2,31 ± 0,30	2,79 ± 0,53	2,58 ± 0,44
C22:6 n-3	9,52 ± 0,58	13,67 ± 1,68	13,13 ± 1,97	15,14 ± 3,00	15,28 ± 2,82	15,79 ± 1,50	15,52 ± 1,50

**the most representative fatty acids*

3.3. COPs analysis

In sardine samples treated using D and SD water twelve epoxy-, keto- and hydroxy-derivatives of cholesterol were identified (**Figure 14**). In both plasma treated and control samples, nine hydroxy-sterols, one cheto-sterol and two epoxy-sterols were detected. Three of them were fully separated among them and from the internal standard: the 19-hydroxycholesterol. The latter was used for COPs quantification. Three phytosterols and 2 types of cholesterol: dehydrocholesterol and dehydrocholesterol, were also found.

The total amount of oxysterols ranging from 79 to 298 μg/g total lipids were found in D samples and 94 to 211 μg/g total lipids in SD ones. Not-significant differences were identified among processed and control samples. However, a broad increase in cholesterol oxidized products was discovered in treated samples, compared to control ones; with an exception for samples treated for 30 minutes in SD water.

Mussels samples treated in SD water showed a different oxysterol profile compared to sardine samples. In both plasma and control samples, three hydroxy-sterols and one cheto-sterol were identified. One of them was fully separated from the internal standard: the 19-hydroxycholesterol. This was used for COPs quantification. Two phytosterols and 2 types of cholesterol: dehydrocholesterol and dehydrocholesterol, were also found. The total amount of oxysterols rage from 106 to 128 μg/g total lipids, with no significant differences among treated and control samples. In accordance with our results *Chudy & Teichert, (2021*) found 7α-hydroxycholesterol (7-KC) as the dominant oxysterol in milk and egg powder, after six months of storage.

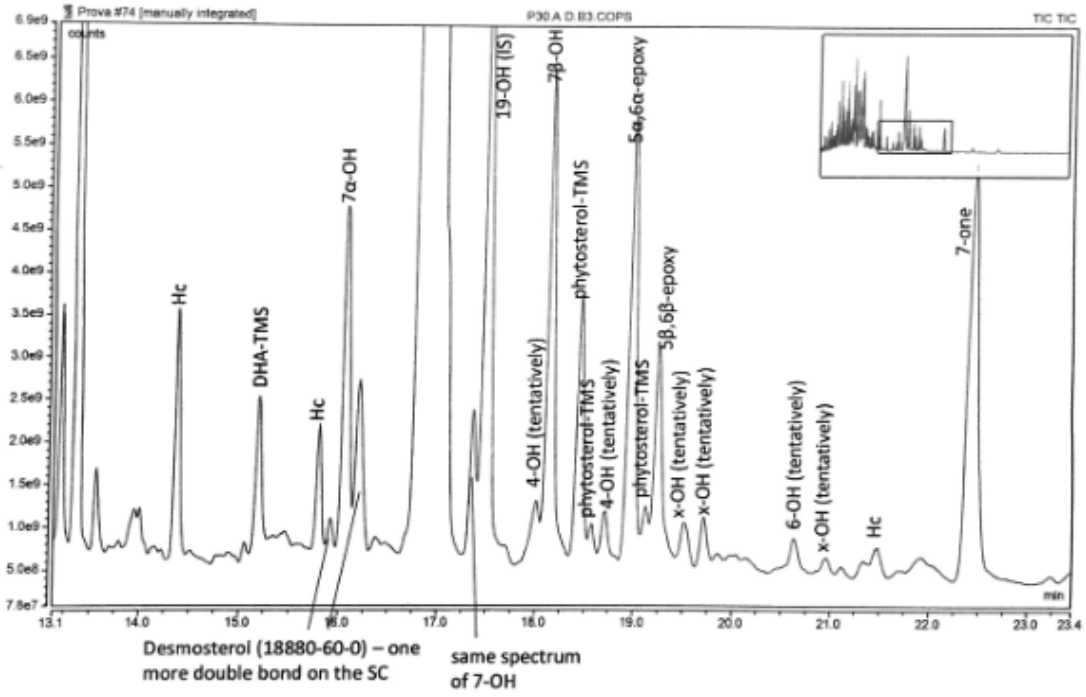

***Figure 14**. GC-MS TRACE of the identified oxysterols in a sample of sardine*

4. CONCLUSIONS

In conclusion the parameters used for plasma activated water generation, caused significant undesired changes in the lipid fraction. As a matter of fact, a general increase in secondary lipid oxidation products was detected, respectively in mussel samples and in sardine (by using two types of waters), at all different treatment times. Nevertheless, it should be mention that a good preservation of the fatty acid profile (especially for C:16:0, C17:0, C:18:1Δ9 and alfa 18:3) in plasma treated samples, once prolonging the dipping time.

As known by literature cold plasma as a valuable non-thermal treatment able to reduce the microbial load. Thus, the first step is trying to optimize the process parameters in order to reach microbial inactivation as well as minimize the negative effect on food quality features. Furthermore being sardine and mussels rich in highly unsaturated lipid fraction is important to understand the sensory impact of chemical changes caused by plasma process. It is worth to mention that the use of plasma activated water (D and SD), during this study, proved a different impact on the secondary oxidized products. Thus, further analysis, on chemical reactions, induced by plasma exposure, according to different operating conditions, must be performed for better understand the mechanism and the species produced by PAW generation, to achieve the desired safety and quality goals. Finally, those researches on PAW are necessary for regulatory approval preliminary to the further application and scale-up of this technology to industrial processing.

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