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# Chemical Modifications of Lipids and Proteins by Nonthermal Food Processing Technologies

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**ABSTRACT:** A range of nonthermal techniques have demonstrated process efficacy in ensuring product safety, extension of shelf life, and in general a retention of key quality attributes. However, various physical, chemical and biochemical effects of nonthermal techniques on macro and micro nutrients are evident, leading to both desirable and undesirable changes in food products. The objective of this review is to outline the effects of nonthermal techniques on food chemistry and the associated degradation mechanisms with the treatment of foods. Oxidation is one of the key mechanisms responsible for undesirable effects induced by nonthermal techniques. Degradation of key macromolecules largely depends on the processing conditions employed. Various extrinsic and intrinsic control parameters of high-pressure processing, pulsed electric field, ultrasound processing, and cold atmospheric plasma on chemistry of processed food are outlined. Proposed mechanisms and associated degradation of macromolecules, i.e., proteins, lipids, and bioactive molecules resulting in food quality changes are also discussed.

**KEYWORDS:** lipid oxidation, protein oxidation, high-pressure processing, pulsed electric field, ultrasound and cold plasma processing

## INTRODUCTION

Classical thermal technologies are based on the use of heat to extend food shelf life and ensure product safety by inactivating spoilage enzymes and microorganisms. Techniques such as thermal sterilization and pasteurization are a cornerstone of food processing. In these cases, heat is generated by electrical resistance or combustion which is transferred to the product. These technologies require relatively high energy which results in high costs and consequently are not environmental friendly. Use of novel thermal technologies are rapidly emerging, offering greater efficiency and process control, including, ohmic heating and dielectric heating, which includes radio frequency (rf) and microwave heating (MW). Such techniques have demonstrated process efficacy in ensuring product safety, extension of shelf life and good retention of critical quality attributes along with providing a more sustainable food processing sector.<sup>1,2</sup> The main difference from the traditional techniques is that the heat is generated directly inside the product, allowing a reduction of heat/energy loss, leading to lower costs and greener solutions.<sup>3</sup> However, when a product is heated, even to moderate temperatures, flavors, essential nutrients, and vitamins can be modified.<sup>4,5</sup> Alternatives to classical and novel thermal techniques are a range of technologies collectively called “nonthermal technologies”. These technologies are effective at ambient or sublethal temperatures, thereby minimizing negative thermal consequences. High-pressure processing, pulsed electric field, cold plasma, and ultrasound processing are the leading nonthermal technologies.<sup>6,7</sup> They can inactivate both pathogenic and spoilage microorganisms associated with food, resulting in extensions of shelf life with microbiological safety profiles. The

potential and adoption of such nonthermal treatments has been further expanded by regulatory agencies increasingly acknowledging their demonstrated efficacies.<sup>8</sup> Of note here is the expansion of the definition of pasteurization beyond solely a thermal treatment by the NACMCF (the U.S. National Advisory Committee on Microbiological Criteria for Foods Adopted August 27, 2004 Washington, DC) to include any treatments which can “reduce the most resistant microorganism(s) of public health significance to a level that is not likely to present a public health risk under normal conditions of distribution and storage”. Apart from their use as a single intervention technology, several studies have shown that such technologies used along with conventional techniques can ensure food safety with limited impacts on the food quality. For instance, ultrasound-assisted hot air drying can reduce the drying time of strawberries in the range of 13–44%, thus moderating the damage on food quality.<sup>9</sup> In the context of sterilization, using high pressure together with mild or high temperatures treatments to inactivate bacterial spores have also shown benefits.<sup>10</sup> Comparably, a combination of nonthermal technologies is also proposed (hurdle approach), to achieve effective microbial inactivation while mitigating negative effects on product quality. In order to meet growing consumer demand for high quality food, it is necessary to understand the mechanisms of action driving these potential technologies and the response of food chemistry to such processes. Applications

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of novel thermal and nonthermal technologies have been reviewed extensively covering various aspects of food quality and safety.<sup>11–13</sup> However, the effects of nonthermal techniques on food chemistry and the associated degradation mechanisms have not been reviewed to date. The objective of this work is to review the effects of four of the leading nonthermal technologies, namely, high-pressure processing, pulse electric fields, ultrasound, and cold plasma on biomolecules associated with food quality, focusing on lipids and proteins.

## ■ HIGH-PRESSURE PROCESSING

High-pressure processing (HPP) is a method of food processing where food is subjected to elevated pressures (up to 900 MPa). HPP is the leading nonthermal technology in terms of research to date, consumer and regulatory acceptance, and industrial adoption with a wide range of food products on the global market. HPP technology has been reviewed extensively highlighting the range of applications it can offer in the food industry, assessed alone or in combination with conventional techniques.<sup>10,14–16</sup> HPP is an efficient nonthermal technology to inactivate a wide variety of pathogenic and spoilage vegetative cells, yeasts, mold, spores, and viruses associated with food products.<sup>17,18</sup> Intrinsic food parameters governing process efficacy include water activity, pH, and composition of food such as fats and oils.<sup>19</sup> It is known that compression increases the temperature of the food by approximately 3 °C/100 MPa<sup>4</sup> and potentially up to 8.7 °C/100 MPa if the samples have high levels of fats and oils.<sup>20</sup> The rapid increase in temperature during compression and subsequent cooling upon decompression is a unique benefit of high pressure-based technologies to reduce product thermal exposure during treatment.<sup>21</sup>

Pressure can affect the physical properties of the food matrix such as the superficial tension, density, viscosity, dipolar moment, dielectric constant, and thermal properties as well as equilibrium processes including ionization, dissociation of weak acids, and acid–base equilibrium.<sup>22</sup> Moreover, high pressures can impact the rate of these reactions by delaying or accelerating them. In addition, HPP can modify the pH of the environment as it enhances the formation of ions from ionizable substances. A change in pH can affect protein denaturation, growth of microorganisms and the kinetics of chemical reactions.<sup>22</sup> Even if the temperatures applied are considered as low, high pressure processing technology can affect various nutrients and bioactive molecules. For example, high-weight molecules such as proteins are formed by van der Waals forces and hydrogen and hydrostatic bonds which are weak, which can be affected by HPP. However, lower molecular weight molecules like vitamins are basically formed by covalent bonds and are typically sufficiently strong to withstand HPP conditions.

**Lipids.** One of the most common reactions associated with food is the oxidation of lipids. It results in a modification of color, flavors, functional properties, and nutritional values and may lead to the formation of toxic subproducts.<sup>23</sup> First, a free alkyl is formed by removing a hydrogen atom from the  $\alpha$ -methylene group of a fatty acid. This initiation step is strongly encouraged by heat, light, or by the presence of metal ions and enzymes initially present in the food. The second step is called propagation. The free radical formed being highly reactive reacts with molecular oxygen to form a lipid peroxy radical. This in turn can react with other fatty acids and generate hydroperoxide and further free radicals. Finally, this new free

radical can reinitiate this process with other fatty acids. This chain reaction mechanism stops when two free fatty acids radicals react and create a nonradical, which can happen after 10–100 cycles. The termination step can also occur under the presence of antioxidant molecules (vitamin E, vitamin C, catalase, etc.) which can neutralize free radicals.<sup>24,25</sup> Lipid oxidation is commonly measured using the TBARS method (Thiobarbituric Acid Reactive Substances), expressed in milligrams of malonaldehyde (MDA) per kilogram of sample.<sup>26</sup> According to Connell,<sup>27</sup> TBAR values of 1–2 mg MDA/kg sample is the range of acceptability of odor/taste in fish.

The effects of high pressure on lipid oxidation have been investigated.<sup>28</sup> High pressure should not initiate lipid oxidation, as the heterolytic cleavage to form the free radical is not favored by increases in pressure. However, the formation of covalent bonds during the propagation steps could be encouraged by pressure. Cheffel<sup>29</sup> observed that at values above 350 MPa, and sarcoplasmic and myofibrillar proteins were denatured and myoglobin and oxymyoglobin converted to the denatured ferric form. As a consequence of these transformations, lipid oxidation was catalyzed. Orlien et al.<sup>30</sup> found that lipid oxidation levels depend more on the applied pressure than on the processing time and suggested that lipid oxidation is due to damage of the cell membrane which could lead to the release of free radicals or their precursors. Bolumar et al.<sup>31</sup> applied a range of pressures for different treatment times and temperatures (5, 25, and 40 °C) and observed that increasing these parameters raised the production of free radicals, thus encouraging lipid oxidation which may be due to synergistic effects of high pressure and temperature. In addition, they established thresholds for radical formation of 400 MPa at 25 °C and at 500 MPa at 5 °C. Bolumar et al.<sup>32</sup> suggested that HPP induced the formation of free radicals either by an iron-catalyzed Fenton's reaction mechanism or by the formation of protein-derived radicals. Reddy et al.<sup>33</sup> applied 300 and 600 MPa over 5 and 10 min on raw chevon samples followed by storage at 4 °C for a month and reported a significant increase of lipid oxidation at 600 MPa during the storage period. In a similar study, Wang et al.<sup>34</sup> stored yak fat at 4 and 15 °C during 20 days after being HHP treated at 0.1, 100, 200, 400, and 600 MPa. Lipid oxidation was observed to increase with a rise in pressure, storage temperature, and treatment time. Indeed, the TBARS values obtained were much higher at 400 and 600 MPa compared with 200 MPa, revealing higher rates of lipid oxidation. These results match with the findings of the researchers cited previously, suggesting that lipid oxidation is encouraged after a pressure of 300–400 MPa. These results also agree with the Kaur et al.<sup>35</sup> study on black tiger shrimps, where a significant increase of lipid oxidation was observed after high-pressure treatments; however, the MDA values remained acceptable for treatments above 300 MPa. Fuentes et al.<sup>36</sup> studied the influence of intramuscular fat content on lipid oxidation after high pressure treatment. They applied 600 MPa on two different parts of a dry-cured ham, namely the flank (lower fat content) and the hip (higher fat content), under subsequent storage at 2 °C over 120 days. The TBARS values obtained were higher for the samples analyzed immediately after treatment in the flank samples. This could be due to the fact that most of the fat content in the flank samples were unsaturated which are more reactive and easier to oxidize. However, at the end of the storage period, the hip samples were more susceptible to oxidation as the lipid concentration was higher. Conversely, several studies report no significant effects

of HPP on lipid oxidation, for example, a storage study of 30 days on dried fermented sausages after different pressure treatments.<sup>37</sup> Similarly, Chouhan et al.<sup>38</sup> did not detect any significant effects of pressure on lipid oxidation immediately after applying 250 and 350 MPa for 10 min with hilsa fish (*Tenualosa ilisha*) but noted an increase of lipid oxidation during storage. No alteration was observed on the lipid compounds or fatty acids composition of cow milk after high-pressure treatments from 250 to 900 MPa.<sup>39</sup> A decrease of TBARs values were observed for treatments of 10 min at 300 MPa at 5 and 40 °C on salmon fillets.<sup>40</sup> Moreover, Lerasle et al.<sup>41</sup> studied the influence of modified atmosphere packaging and high-pressure treatment. They prepared two batches of raw poultry sausage, one packaged with atmospheric air and one with a modified atmosphere composed of 50% CO<sub>2</sub> and 50% N<sub>2</sub>, with both pressurized at 500 MPa for 5 min. After treatment the samples were stored at 4 °C over 22 days. They found that lipid oxidation was significantly encouraged by the storage time and package atmosphere, but not by the pressure, with the treated samples having higher TBARs values.

**Protein.** Protein structure is composed of covalent and hydrophilic bonds, electrostatic, and van der Waals interactions, which make its native structure stable over a specific range of pressures and temperatures. Outside these conditions protein can be denatured.<sup>42</sup> In addition, there is an intermediate state between the native and the denatured state which is called the molten globule state, where proteins can have specific functional properties that do not exist in any of the other two states.<sup>43</sup>

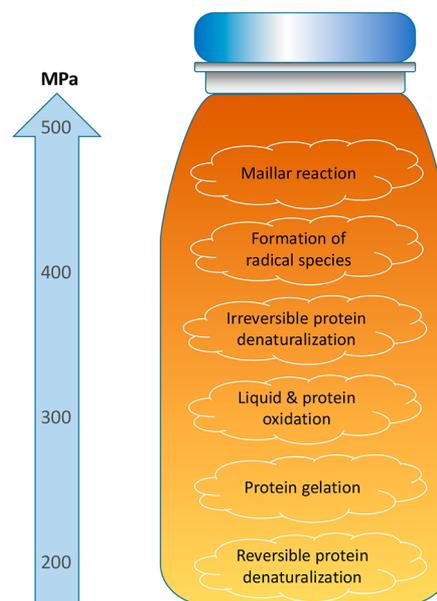
High pressure causes physical compression of the sample and consequently can modify the native structure of a protein, affecting solubility, gelation, emulsion, foaming, and water holding capacities. A reduction in the volume results in a reduction or elimination of the voids initially present in the protein structure as the protein unfolds. Thus, as globular proteins have more cavities than fibrous proteins, they are less stable when a high pressure is applied.<sup>44</sup> In addition, the hydration effect on a protein is related to a reduction of the volume caused by electrostriction around the ionic groups, hydrogen-bonded hydration around the polar groups, and hydrophobic hydration.<sup>45</sup> Proteins are usually denatured after applying a pressure from 100 to 1 200 MPa and the midpoint of pressure-induced transition occurs at 400–800 MPa.<sup>46</sup> It is known that high temperatures can irreversibly denature proteins by transferring nonpolar hydrocarbons from the hydrophobic core toward water. However, in the case of high pressure, protein denaturation is initiated by the intrusion of water into the inner part of the protein matrix, which depends on the level of pressure applied.<sup>47</sup> In general, below 200 MPa there are only conformational changes on the tertiary and quaternary structure of the proteins, which are constituted by weak bonds such as hydrogen and electrostatic bonds, hydrophobic, and van der Waals interactions. These modifications are reversible after treatment and the protein can recover its native structure. However, depending on the type of protein, pressures above 300 MPa can also affect the secondary structure and consequently cause irreversible denaturation.<sup>48</sup> All these changes cited on protein conformational structure can initiate various reactions, causing modifications of the food quality. In addition to the pressure and treatment time, these reactions could be encouraged by temperature, pH, ionic strength, and the presence of other components in the food matrix such as metal ions and other free radicals.

One of the most common reactions where proteins are involved is oxidation. They can be oxidized following the same chain reactions as lipids. A free radical can interact with a protein, initiating the oxidation of the latter.<sup>49</sup> Protein oxidation can be measured by the loss of sulfhydryl groups (S–H) or by the formation of protein carbonyls. Sulfhydryl or thiol groups are very reactive and sensitive to oxidation, leading to the formation of disulfide groups. In meat for instance, these disulfide bonds can form a gel-like network, thus changing the texture of the product.<sup>50</sup> Protein carbonyls are the common product of protein oxidation. Carbonyls (aldehydes and ketones) can be formed in proteins through four different pathways, namely, (i) direct oxidation of the side chains from lysine, threonine, arginine, and proline; (ii) nonenzymatic glycation in the presence of reducing sugars; (iii) oxidative cleavage of the peptide backbone via the  $\alpha$ -amidation pathway or via oxidation of glutamyl side chains; and (iv) covalent binding to nonprotein carbonyl compounds such as 4-hydroxy-2-nonenal (HNE) or malondialdehyde (MDA).<sup>51</sup> Some researchers have suggested that there could be a correlation between lipid oxidation and protein oxidation, as both elements are present in the food matrix. The oxidation of both proteins and lipids are initiated by a free radical which can be derived from a lipid or protein, causing the oxidation of other lipids or proteins.<sup>49,52</sup> As HPP can encourage lipid oxidation, protein oxidation could also occur. For example, metmyoglobin Fe<sup>3+</sup> is formed after a pressure treatment is applied beyond 350 MPa, as a result of the oxidation of oxymyoglobin Fe<sup>2+</sup>.<sup>29</sup> In addition, these researchers observed that a pressure of 200 MPa often causes protein gelation, giving a denser structure, which leads to an increase in texture. Similar results were obtained by Reddy et al.<sup>33</sup> after applying 300 and 600 MPa for fresh chevon. They studied the effect of high-pressure processing on myoglobin, oxymyoglobin, and metmyoglobin, which are proteins related to the color of some food products. No changes were observed in myoglobin content. However, there was a correlation between the decrease of oxymyoglobin content and the increase of metmyoglobin content. As a result, a decoloration of the samples was more intense for higher pressures and longer times. Fuentes et al.<sup>53</sup> applied 600 MPa for vacuum packaged dry-cured ham using three different presentations: nonsliced vacuum-packaged dry-cured ham, dry-cured ham slices stretched out in the package, and dry-cured ham slices piled horizontally. To study the protein oxidation, they analyzed the formation of  $\alpha$ -amino adipic and  $\gamma$ -glutamic semialdehydes (AAS and GGS, respectively) which are the results of different amino acids oxidation, such as proline, lysine, and arginine. There was a significant increase of the content of these two protein carbonyls after pressurization. Moreover, they found that lipid oxidation was highly encouraged for the treated samples which depended on the type of packaging used. Ojagh et al.<sup>40</sup> attempted to mitigate the negative effects of high pressure by covering salmon samples with a fish gelatin–lignin film. In this experiment, they compared the formation of protein carbonyls in samples with and without the film at 300 MPa during 5 min treatment at 5 and 40 °C. They found that protein oxidation increased significantly for all the raw samples. However, the values for the samples covered by the film were significantly lower than the uncovered samples. On the other hand, Cava et al.<sup>54</sup> found that HPP does not affect protein oxidation in dry-cured ham and dry-cured loin. However, lipid oxidation increased in the dry-cured lion samples and decreased for the dry-cured ham after

this treatment. High pressure did not have any effect on the concentration of thiol groups after 600 MPa of pressure treatment during 5 min on sarcoplasmic and myofibrillar proteins of brine enhanced pork semitendinosus.<sup>55</sup> However, these values decreased during storage for sarcoplasmic proteins but not for myofibrillar proteins, where there was a formation of S–H groups. The explanation of this increase was based on Omana et al.<sup>50</sup> experiments, who found an increase of S–H groups after a pressure treatment for chicken breast meat. They suggested that this increase was related to the unfolding state of the protein, where the majority of the available sulfhydryl groups were exposed to the environment and that most of the sulfhydryl groups in the inner core were already oxidized to form disulfide bonds. However, Grossi et al.<sup>55</sup> observed in a similar study the formation of protein carbonyl immediately after treatment for sarcoplasmic proteins, but with degradation during storage. The results suggest that high pressure (above 600 MPa) could accelerate the decomposition of the carbonyl group formed after oxidation.

Another common reaction of food proteins is the Maillard reaction, where an amino group reacts with a carbonyl group which is initially present in the food matrix such as reducing sugars, osones, furfural, hydroxymethylfurfural (HMF), and pyrrole derivatives. As a result the protein suffers a modification and brown polymers called melanoidins are formed. It is common in the food industry because it generates pleasant flavors and desired brown color compounds in cooked foods. However, it can lead to the production of acrylamide, a potential human carcinogen element, a result of the interaction between the amino acid asparagine and a reducing sugar.<sup>56</sup> The Maillard reaction involves complex sequences of reactions, including condensation, cyclization, dehydration, rearrangement, isomerization, and polymerization.<sup>22</sup> These reactions are influenced by temperature, metal ions, water activity, and pH. Studies regarding the use of novel technologies to control the Maillard reaction are being currently carried out.<sup>57</sup> It is known that pH is sensitive to pressure, as a dissociation of ionizable substances can occur under high-pressure conditions. Consequently, HPP could have an effect on the Maillard reaction.<sup>58</sup> Martinez-Montegudo and Saldana<sup>22</sup> suggested that due to the complexity of the Maillard reaction, the effect of pressure should be evaluated for individual reaction steps to understand if pressure encourages or delays the overall reaction. Santos et al.<sup>59</sup> proposed that high pressure accelerates the Maillard reaction for wine after a pressure treatment between 400 and 500 MPa for 5 min. The authors measured volatile compounds which are the products of the Maillard reaction, namely, 2-furfural, benzaldehyde, and phenylacetaldehyde, and they found an increase of their content after treatment. On the other hand, Campus et al.<sup>60</sup> found a reduction of several flavor compounds from the Maillard reaction after pressure treatment. However, their content increased during storage, and they suggested that the presence of their precursors such as the substrates, free amino acids can continue reacting, leading to their formation. Figure 1 schematically outlines some of the key chemistries induced due to HPP.

Finally, carboxylation is another common reaction in food chemistry which is based on the removal of the  $\alpha$ -carboxylic acid group of a free amino acid. The resultant amines are known as biogenic amines. The presence of these amines in food is of interest from a toxicological aspect and for their role as possible quality indicators.<sup>61</sup> This reaction consists of two mechanisms. The first is performed by a pyridoxal phosphate



**Figure 1.** Key chemical changes induced due to high-pressure processing.

coenzyme, and the second is mediated by microorganisms which contain a covalently bound pyruvoyl residue on their active site.<sup>62</sup> There are few reported studies on the effect of high pressure on biogenic amines.<sup>63</sup> For examples, biogenic amines content was reduced on Caciotta and Pecorino cheeses when raw milk was treated at 100 MPa before cheese production.<sup>64</sup> Reduction of these amines after pressurization was also found for vacuum-packed trout flesh.<sup>65</sup> Simon-Sarkadi et al.<sup>66</sup> applied 500 MPa to sausage finding an inhibition of the formation of two biogenic amines, namely, cadaverine and putrescine, whereas, this treatment encouraged the formation of tyramine and spermine. Spermidine and spermine were found in soya milk and they remained stable after 200 and 300 MPa pressurization at 55, 65, and 75 °C.<sup>67</sup> However, Ruiz-Capillas et al.<sup>68</sup> applied 400 MPa at 30 °C for 10 min to frankfurters finding an increase of the biogenic amines content. Table 1 summarizes some key factors and effects of HPP on food products reported in the literature.

## ■ PULSED ELECTRIC FIELDS

Pulse electric fields (PEF) are a group of nonthermal food process technologies which have made the transition from the lab to the food industry.<sup>69,70</sup> This technology consists of the application of short electric pulses (1–100  $\mu$ s) of high- and low-intensity electric fields. The food product is located between two electrodes inside a chamber, and the pulse electric field is applied over a short period. Different PEF systems have been created and optimized for efficacy and scale depending on the application and can be employed alone or in combination with thermal technology.<sup>71–73</sup> The main two functions of this technology are inactivation of microorganisms and extraction of intracellular components. PEF is primarily based on a phenomenon called electroporation, which consists of the formation of pores on cellular membranes. As a result, there is a transitory or permanent modification of the permeability of the cell membrane and typically limited increases in the bulk temperature. The effectiveness of the electro-permeability process depends on various parameters, including, the characteristics of the PEF system (holding time,

**Table 1. Effect of High-Pressure Processing on Chemical Changes in Food Products**

food product	treatment	changes <sup>a</sup>	ref
beef loin and chicken	0.1, 200, 400, 600, and 800 MPa at 5, 25, and 40 °C for 5 and 10 min	lipid oxidation: ↑ 400 MPa at 25 °C, 10 min ↑ 500 MPa at 5 °C, 10 min	31
fresh chevon	300 and 600 MPa at 28 °C for 5 and 10 min	from 300 MPa at 28 °C, 5 min ↑ lipid oxidation ↑ protein oxidation	33
yak	0.1, 100, 200, 400, and 600 MPa at 4 and 15 °C for 5 and 30 min	lipid oxidation: ↑ 400 MPa at 4 and 15 °C, 30 min	34
black tiger shrimp	0.1, 300, 400, 500, and 600 MPa at 27 °C for 3, 6, 9, 12, and 15 min	lipid oxidation: ↑ 300 MPa at 27 °C, 12 min ↑ 400 MPa at 5 °C, 3 min	35
dry-cured hams	600 MPa at 12 °C for 6 min	↑ lipid oxidation	36
dry fermented sausage	202 MPa at 10 °C for 960 s 260 MPa at 10 °C for 390 and 1530 s 400 MPa at 10 °C for 154, 960, and 1800 s 540 MPa at 10 °C for 390 and 1530 s 600 MPa at 10 °C for 960 s	no effect	37
hilsa	250 and 300 MPa at 27 °C for 10 min	= lipid oxidation	38
salmon	300 MPa at 5 and 40 °C for 10 min	↓ lipid oxidation ↑ protein oxidation	40
dry cured ham	600 MPa at 12 °C for 6 min	↑ protein oxidation	53
dry-cured ham and dry-cured loin	200 and 300 MPa at <14 °C for 15 and 30 min	↑ lipid oxidation = protein oxidation	54
milk	100 MPa	↓ biogenic amines content	64
trout flesh	300 and 500 MPa for 10 min at 20 °C	↓ biogenic amines content	65
soya milk	200 and 300 MPa at 55, 65, and 75 °C	= biogenic amines	67
frankfurters	400 MPa at 30 °C for 10 min	↑ biogenic amines	68

<sup>a</sup>↑, increase; ↓, decrease; =, no effect.

energy, temperature, frequency, electric strength, pulse shape, and width), the food product (pH and conductivity), and the features of the bacteria cell (membrane, shape, size, and envelope structure).<sup>74</sup> The process can damage the membrane leading to cell inactivation as well as modification of the inner part of the cell and extraction of different substances. This process technology can effectively inactivate microorganisms which are related to the deterioration of food such as vegetative forms of bacteria, yeast, and molds.<sup>75</sup> The application of high-voltage pulsed electric fields can lead to some electrochemical reactions, affecting the quality of specific food products.<sup>76</sup> It is known that many chemically active species can be produced by an electric discharge in a food and also by electrode reactions with the product, such as decomposition of the chemical structure of liquids close to the electrode surfaces (electrolysis), eventually producing toxic chemical species, such as oxygen peroxide, hydroxyl radicals, or chloride ions.<sup>77,78</sup>

Arroyo et al.<sup>79</sup> studied the lipid oxidation of fresh and frozen turkey breast treated by PEF. In this experiment, three different frequencies (10, 55, and 110 Hz), pulses (100, 200, and 300 pulses) each with a pulse width of 20 μs, and voltages (7.5, 10, 12.5 kV, fresh samples; 14, 20, and 25 kV, frozen samples) were tested. No significant differences were observed for the MDA values in both batches. Cortes et al.<sup>80</sup> noted a partial inactivation of peroxidase activity and no modification of the TBARs values after PEF treatment of horchata samples. These findings are in agreement with those of Suwandy et al.,<sup>81</sup> who concluded that PEF does not induce lipid oxidation of beef. Peanut oil was treated by PEF using a square-wave pulse generated with a pulse duration ( $\tau$ ) of 40 μs and pulse frequency ( $f$ ) of 1008 Hz.<sup>82</sup> The authors suggest that this technology could restrain the rate of the lipid oxidation reaction thus extending the shelf life of lipid rich products.

Additionally, Ma et al.<sup>83</sup> found that PEF did not affect lipid oxidation immediately after treatment on three different parts of chilled and frozen–thawed cooked lamb (shoulder, rib, and loin). However, after 7 days of storage, the MDA values of the treated samples were higher than the control but still acceptable from a quality point of view (lower than 2 mg MDA/kg sample). No significant changes were found in the concentration of saturated and unsaturated fatty acids after PEF treatment using bipolar square-wave pulses of 4 μs, at 35 kV/cm field strength and a frequency of 200 Hz for a fruit juice–soymilk beverage during 800 and 1400 μs treatments.<sup>84</sup> The authors reported that the treatment led to a greater reduction of the total fatty acid content. Faridnia et al.<sup>85</sup> found a significant increase in lipid oxidation both immediately after treatment and during storage for frozen–thawed beef semite-ninosus muscles using process conditions of constant pulse width of 20 μs, electric field strength of 1.4 kV/cm, constant frequency of 50 Hz, pulse number of 1032, and total specific energy input of 250 kJ/kg. Volatiles which resulted from lipid oxidation are responsible for off-flavors and were significantly higher for the treated samples. These results could be related to the significant decrease in the concentrations of myristic acid (C14:0), palmitic acid (C16:0), palmitoleic acid (C16:1n7), oleic acid (C18:1n9c), and linoleic acid (C18:2n6c). The consequences of pulsed electric field treatment on food proteins has been reviewed by ref 86, who suggest that PEF has less impact on the food proteins than thermal technologies. Although, they mention that PEF can inactivate some proteins and change their structure and properties. Zhao et al.<sup>87</sup> demonstrated that PEF induces sulfhydryl–disulfide interchange reactions leading the formation of protein aggregates and a decrease in the solubility of the protein. Deactivation of peroxidase (POD) and polyphenoloxidase (PPO) for apple juice, which can cause undesirable browning reactions, were achieved using PEF.<sup>88</sup> Similarly, Bi et al.<sup>89</sup> showed that the inactivation efficacy for these two enzymes with the same matrix increases as a function of electric field strength, resulting in product lightening. Protein oxidation was not detected for egg white protein solution after treatment at 200, 400, 600, and 800 s under a constant electric field intensity of 25 kV/cm.<sup>90</sup> The only observed change was a slight increase in the content of sulfhydryl groups, which could be related to a gentle modification of the structure of the protein. Some effects of pulsed electric field on food products are summarized in Table 2.

Table 2. Effect of Pulsed Electric Field on Chemical Changes in Food Products

food product	treatment	changes	refs
fresh and frozen turkey breast	frequencies: 10, 55, and 110 Hz pulses: 100, 200, and 300 pulses voltages for fresh samples: 7.5, 10, 12.5 kV voltage for frozen samples: 14, 20, and 25 kV pulse width: 20 $\mu$ s	no lipid oxidation:	79
horchata	electric field intensity 20–35 kV/cm treatment times 100–475 $\mu$ s	no lipid oxidation	80
cold-boned beef loins and topsides	voltage: 10 kV frequency: 90 Hz pulse width: 20 $\mu$ s	no lipid oxidation	81
peanut oil	voltage: 20, 30, 40, and 50 kV pulse frequency: 1008 Hz pulse width: 40 $\mu$ s	restrain lipid oxidation:	82
chilled and frozen–thawed cooked lamb	electric field strength: 1–1.4 kV cm <sup>-1</sup> specific energy: 88–109 kJ kg <sup>-1</sup> pulse width: 20 $\mu$ s frequency: 90 Hz pulse number: 964	lipid oxidation was found after 7 days but not just after treatment	83
fruit juice-soymilk beverage	electric field strength: 35 kV/cm pulse: 4 $\mu$ s frequency: 200 Hz treatment time: 800 or 1400 $\mu$ s	no effect on fatty acid content	84
frozen–thawed beef semitendinosus muscles	electric field strength: 1.4 kV/cm specific energy input: 250 kJ/kg pulse width: 20 $\mu$ s frequency of 50 Hz pulse number: 1032	↑ lipid oxidation	85
egg white	electric field intensity: 25, 30, and 35 kV/cm of for treatment time: 400 $\mu$ s pulse repetition rate: 200 Hz pulse width: 2 $\mu$ s	↑ protein aggregates  ↓ protein solubility	87
apple juice	flow: 5 L/h electric field strengths of 15, 25, and 35 kV/cm pulse width: 3 $\mu$ s energy inputs: 8.5 and 65.5 kJ/kg	deactivation of peroxidase (POD) and polyphenoloxidase (PPO)	88
egg white	electric field intensity: 25 kV/cm pulse repetition rate: 100 Hz pulse width: 2 $\mu$ s treatment time: 200, 400, 600, and 800 $\mu$ s	no protein oxidation	90

## ■ ULTRASOUND PROCESSING

Acoustic energy has also been investigated as a novel technology for food processing. High-frequency ultrasound (low intensity or low power) is commonly used as a nondestructive quality assessment technique. Whereas low frequency (high power) employs high intensity sound waves which can have significant impact on food properties, offering a technologically driven solution for various food processing operations including sterilization, extraction, emulsification, freezing, sonocrystallization, drying, defoaming.<sup>91</sup> Cavitation is the main mechanism of ultrasound when applied to liquid foods using contact type systems such as ultrasound baths and probe-based systems. Here, ultrasound is propagated via a series of compression and rarefaction waves through the liquid, which at sufficient power can produce cavitation. These bubbles can reach an unstable size and collapse, generating physical and chemical effects, such as localized high temperatures and

pressures, radiation forces, microstreaming, shock waves, microjets, and free radicals.<sup>92,93</sup> A second approach is to use airborne acoustic ultrasound aimed primarily at treating solid foods. Although there is a lack of knowledge regarding the mechanisms involved, the following factors can be considered: high acoustic pressures, standing waves, radiation pressure, and microstreaming. This technology has been used for drying, defoaming, and decontamination.<sup>94</sup> As for the other technologies, antimicrobiological efficacy (log reduction), sensory parameters (color, flavor), and physicochemical characteristics (gelation, viscosity) appear to be the most studied factors following a sonication treatment. There are relatively few studies focusing on the effect of ultrasound processing on biomolecules. A summary of the modifications of this technology on different food products is summarized in Table 3.

Torkamani et al.<sup>95</sup> studied the effect of ultrasound on different quality parameters of cheddar cheese why including

Table 3. Effect of Ultrasound Processing on Chemical Changes in Food Products

food products	conditions	main findings	refs
cheddar cheese whey	frequencies between 20 to 2 000 kHz and specific energies between 8.0 to 390 kJ/kg for 10 and 30 min at 37 °C	lipid oxidation occurred for both treated and untreated samples	95
mackerel, cod, hake and salmon	ultrasonic bath at 30 kHz for 5, 15, 25, 35, and 45 min	no effect of ultrasound on lipid oxidation for mackerel, cod and hake slight decrease in TBARS values for treated salmon	96
pasteurized milk	probe-based system at 24 kHz for 2.5, 5, 10, 15, and 20 min	increase in secondary volatile products derived from lipid oxidation	97
raw, thermized, and pasteurized milk	Probe-based system at 24 kHz for 2, 4, 8, and 16 min at 15–25 °C, and 0, 1, 2, 4, 6, and 8 days of storage before sampling	volatile compounds derived from lipid oxidation increased in concentration with sonication and storage time	98
raw and pasteurized skim milk	frequencies at 20, 400, 1,000, 1,600 and 2,000 kHz at 4, 20, 45, and 63 °C up to 20 min	highest amount of volatiles detected at 400 and 1,000 kHz, where the cavitation yield and hydroxyl radical production were greatest decreasing the processing temperature and sonication time can help to control the lipid oxidation	99
refined sunflower oil	ultrasound probe at 20 kHz; power at 150 W for 2 min at 20 °C	increase of peroxide value after sonication treatment	105
beef	probe-based system at 20 kHz at power intensity of 2.39, 6.23, 11.32, and 20.96 W cm <sup>-2</sup> for 30, 60, 90, and 120 min	greater lipid oxidation during ultrasound-assisted brining compared to static brining protein oxidation increases with high ultrasound intensities and treatment times	106
soy protein isolate	probe-based system at 20 kHz at 200, 400, and 600 W for 15 and 30 min	increase of sulfhydryl content upon sonication	108
bovine serum albumin solutions	probe-based system at 20 W cm <sup>-2</sup> for 7 to 90 min	reduction of sulfhydryl groups	111
soy protein isolate, egg white protein and whey protein	ultrasonic processor at 20 kHz, 4.27 and 0.71 Q for 20 min	increase of the protein surface hydrophobicity	109
soy protein isolates	probe-based system at 25 kHz at 200, 400, and 600 W for 15 min	increase of the protein surface hydrophobicity	112
black bean protein isolates	probe-based system at 20 kHz at 150, 300, and 400 W for 12 and 24 min	increase of the protein surface hydrophobicity	113

polar lipids, free and bound fatty acids, and lipid oxidation derived compounds. Lipid oxidation occurred for both untreated and treated samples notwithstanding the ultrasound treatment over time. Pedrós-Garrido et al.<sup>96</sup> did not report any effects of ultrasound on lipid oxidation for mackerel, cod, and hake, whereas treated salmon showed a slight decrease in TBARS values. A probe-based system at 24 kHz was used by Riener et al.<sup>97</sup> to treat pasteurized milk for 2.5, 5, 10, 15, and 20 min. Secondary volatile products derived from lipid oxidation including carbonyl compounds, pentanal, hexanal, and heptanal, were detected. According to the researchers, the increase in these compounds was caused by the radical species formed under the high temperatures and pressures conditions created by cavitation. Similar results were obtained by Chouliara et al.<sup>98</sup> for ultrasound-treated raw, thermized, and pasteurized milk. In another study, various types of milk (raw milk, pasteurized skim milk) were submitted to sound waves at different frequencies (20, 400, 1 000, 1 600, and 2 000 kHz) and temperatures (4, 20, 45, and 63 °C) up to 409 kJ/kg, and the volatile compounds generated by lipid oxidation analyzed. The highest amount of volatiles was detected at 400 and 1 000 kHz, at the same frequencies where the cavitation yield and the hydroxyl radical production were the greatest.<sup>99</sup> It was observed that the production of radicals did not increase linearly with the acoustic frequency. The sonochemical yield depends on the number of active bubbles, the average temperature within these bubbles and the mass transfer effects. The first two parameters are themselves dependent on the acoustic power and frequency. An increase in the acoustic power raises the number of active cavitation bubbles, the size of these bubbles, and the temperature during collapse. Greater negative pressures can be reached during the rarefaction phases and greater positive pressures during the compression phases.<sup>100</sup> An increase in the frequency also raises the number of active cavitation bubbles but reduces the time for these bubbles to grow during

rarefaction phases and to collapse during compression phases.<sup>101</sup> Consequently, fewer acoustic cycles are required for bubbles to reach their active resonance size, and transient cavitation occurs at faster rates.<sup>102</sup> As the maximum potential energy of the bubble is reached at its maximum size, at high frequencies the collapse is occurring with low energy. However, a greater number of active bubbles facilitates a high production of free radical species. Moreover, the effects of mass transfer during cavitation should be taken into account. During the rarefaction phase the pressure inside the bubble decreases, inducing evaporation of the solutes from the bubble/liquid interface, thus increasing the amount of water vapor. During the compression phase, the pressure inside the bubble increases, inducing condensation at the bubble wall, thus reducing the amount of water vapor. The shorter the acoustic cycle, the lower the amount of water vapor within the cavitation bubble.<sup>103</sup> Hence, in the case of high frequencies, the amount of evaporated water decreases. This could explain the reduction in hydroxyl radical generation at the higher frequencies. It has also been demonstrated by the authors that low temperatures and sonication times help to reduce the volatiles compounds derived from lipid oxidation. This underlines the role of temperatures in the production of hydroxyl radicals, as the dissociation of water into •OH radicals and •H atoms is encouraged by high temperatures.<sup>104</sup> In another study on refined sunflower oil samples, free acidity, total polar compounds, peroxide value, conjugated dienes concentration, and fatty acid composition were identified and quantified before and after ultrasound treatment. The peroxide value was the only parameter with a significant difference between nonsonicated and sonicated samples.<sup>105</sup> The oxidation of beef proteins and lipids during an ultrasound-assisted brining process has been investigated by Kang et al.<sup>106</sup> TBARS analysis showed that ultrasound promoted greater lipid oxidation compared to static brining (0.2 MDA (mg/kg meat) at 120

min for static brining, 1.2 MDA for the same processing time at 20.96 W cm<sup>-2</sup>). This result could be explained by the same consequence of cavitation described previously, namely, sonolysis. When the transient cavities undergo violent collapse, high temperatures and pressures are generated (4 000 K and 1 000 atm, respectively), and the dissociation of water produces hydroxyl free radicals and hydrogen atoms. In addition to temperature, ultrasound intensity plays a governing role in the production of free radical species. Indeed, Jana and Chatterjee<sup>107</sup> found a linear correlation between ultrasound intensity and the generation of •OH radicals, up to a threshold of 3.5 W cm<sup>-2</sup>, which is likely due to saturation with cavitation bubbles. These free radicals could be responsible for lipid oxidation.

Besides lipid oxidation, protein reactions upon sonication treatment have also been studied. In a study on pea protein, a rise in sulfhydryl groups was observed after ultrasonic treatment. The results reported by Hu et al.<sup>108</sup> on soy protein isolate are similar. The increase of SH groups content could be related to the conformation changes of the proteins upon sonication treatment. Under the effect of acoustic cavitation, the thiol groups are more exposed as the protein unfolds; conversely, the cleavage of the disulfide bonds S–S of the native proteins leads to increases in the content of SH groups. A few studies have shown that sonication does not have any effect on the content of sulfhydryl groups.<sup>109,110</sup> However, different conclusions have been reached regarding the effect of ultrasound on the sulfhydryl group content in other studies. Indeed, the increase in carbonyl group content and decrease in sulfhydryl group content in ultrasound treated beef proteins indicate that protein oxidation increases with high ultrasound intensities and treatment times.<sup>106</sup> Lipid oxidation has also been observed in this study, and it has been hypothesized that the products of this reaction (i.e., malondialdehyde, carbonyl compounds) can react with proteins and form carbonyl groups. Gülseren et al.<sup>111</sup> treated bovine serum albumin solutions (BSA) with an ultrasound probe at 20 W cm<sup>-2</sup> for 7–90 min. They observed a reduction in sulfhydryl groups by 31% after 90 min of sonication. The reactive species formed during sonication (i.e., •OH and •H) cross-react and produce hydrogen peroxide (H<sub>2</sub>O<sub>2</sub>) which can alter the chemical structure of the molecules. Consequently, upon a sonication treatment, the SH groups localized in the inner structure of the molecules are more exposed to the interface, thus are more susceptible to oxidation. This could explain the decrease of SH groups after sonication treatment. Protein surface hydrophobicity is another commonly analyzed parameter which is related to the stability, conformation, and functional properties of proteins. Ultrasound treatment increases the protein surface hydrophobicity, as demonstrated in several studies including, soy protein isolates,<sup>112</sup> black bean protein isolates,<sup>113</sup> soy protein isolate, egg white protein, and whey protein concentrate.<sup>109</sup> The hydrophobic groups of the proteins initially localized in the interior of the molecule are exposed to the more polar surrounding environment due to the mechanical effects of cavitation. Several studies have analyzed the protein structural changes.<sup>106,113</sup> The results point toward a common conclusion, namely, that the  $\alpha$ -helix content decreases as oxidation occurs (upon sonication), and the  $\beta$ -sheets content increases. As a consequence of the unfolding of the proteins, internal hydrophobic regions are unmasked, and sulfhydryl group content decreases as S–S bonds are created. These disulfide bonds influence the functional properties of the

proteins and play an important role in the gel network structure and mechanical strength. A significant reduction in particle size treated by ultrasound was reported for soy protein isolate and whey protein concentrate.<sup>109,114,115</sup> This reduction appeared to be greater during the first minute of treatment while the particle size is bigger. Cavitation, microstreaming, and turbulent forces occurring during a sonication treatment violently agitate the aggregates, thus reducing the size distribution. Unlike in a homogeneous solution, cavitation bubbles collapse asymmetrically in the presence of solid particles. High-speed jets of liquid are expelled from the imploded bubble toward the solid surface. This physical mechanism, called microjets, together with shockwaves can lead to pitting, erosion, and corrosion of the solid surface as well as increasing sonochemical production following a breakage of the chemical bonds within the molecule.<sup>116</sup> Microstreaming is another consequence of cavitation which is characterized by microscopic turbulences at the surface of the solid, increasing the rate of mass transfer.<sup>117</sup> The probability of larger particles to be subject to cavitation energy is higher than for smaller particles; therefore, the rate of the size reduction for high weight molecules is faster than for smaller molecular weight species.<sup>115</sup> However, different behaviors were observed for egg whites proteins,<sup>109</sup> bovine serum albumin solutions,<sup>111</sup> and hydroxypropylmethylcellulose,<sup>118</sup> where an increase in the particle size after ultrasound treatment was noticed. In these cases, any partial degradation could be overcome by the formation of small aggregates due to noncovalent bonds, such as electrostatic and hydrophobic interactions. This would be one consequence of the high hydrophobic index rise upon sonication.

## ■ COLD ATMOSPHERIC PLASMA

In 1928, the American scientist Irving Langmuir proposed that the electrons, ions, and neutrons in an ionized gas could be considered as a corpuscular material entrained in a fluid medium and termed this medium “plasma”.<sup>119</sup> Nowadays, the term plasma refers to a partially or wholly ionized gas composed essentially of photons, ions, and free electrons as well as atoms in a fundamental or excited state possessing a net neutral charge. A new technology coined cold atmospheric plasma has been developed for sterilizing and modifying material with application in the food, agriculture, textile, electronic, biotechnology, and medicine sectors.<sup>120</sup> Depending on the application, different plasma sources have been built such as corona discharge, dielectric barrier discharge (DBD), microwave discharge (MD), gliding arc, and plasma jet.<sup>121</sup> Recently, cold plasma has emerged as a nonthermal technology with potential applications for food decontamination.<sup>122</sup> The main interest in this technology is that it can efficiently inactivate bacteria, yeasts, molds, spores, biofilms, and other hazardous microorganisms, including potential bioterrorism agents at low/ambient temperatures and at atmospheric pressure.<sup>123,124</sup> The efficiency of the treatment depends of different parameters including the flow of the electric charge (direct or alternating), voltage, plasma source, distance between the source and the product, treatment time, packaging material, and atmosphere. Plasma can be a tool to maintain the microbial safety of fresh food products, thus increasing the self-life of the products, with limited impact on food quality.<sup>125</sup> Besides plasma being a potential technology to ensure food safety, it is necessary to study in depth how it is affecting food quality. During cold atmospheric plasma treatment negative and positive ions, free radical molecules, electrons, UV-photons,

nitrogen and carbon oxides, and ozone are formed depending of the gas used.<sup>126,127</sup> The production of reactive species (Figure 2) in cold atmospheric plasma can promote hundreds

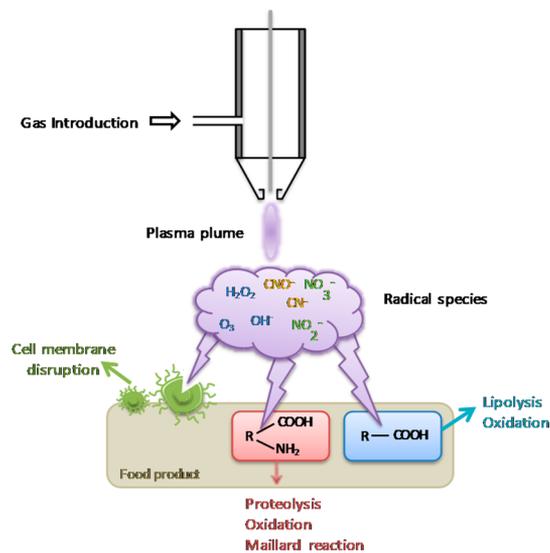


Figure 2. Cold plasma induced chemical changes.

of reactions involving several components: He, Ar, N, O, and H atoms and molecular species composed of these atoms (in ground and excited states), various atomic and molecular ion species, and electrons.<sup>126–128</sup> Such reactions can affect the components of the food product, representing a risk for the key quality parameters of the food product.<sup>129,130</sup>

However, plasma treatment does not have the same effect on solid or liquid matrixes. In the first case, plasma is not able to penetrate significantly inside the product and is generally classified as a surface treatment, usefully where most of the microorganisms reside. Thus, the process will have limited

effects on the bulk components of many solid foods. Obviously, the degree of penetration of the plasma varies depending on the food product. Whereas when a liquid food is treated, plasma can be diffused or infused in the liquid, potentially leading to more significant impacts.<sup>131</sup>

It is necessary to highlight the formation of ozone by atmospheric plasma devices. Ozone is a triatomic oxygen molecule formed by the interaction of a diatomic oxygen molecule with free oxygen radical. The breakdown of the bond O–O to form this radical requires an energy that could be provided by the plasma system. Ozone is very reactive and unstable species, decomposing into hydroxyl, hydroperoxy, and superoxide radicals which have high oxidation potentials. The use of ozone in the food industry has been widely investigated.<sup>132</sup> Ozone can reduce mycotoxins and microbial contamination of food products and has also been shown to be efficient in removing pesticide residues from different food matrixes. However, because of the high reactivity of ozone many reactions can occur, which can potentially affect the quality of the food products.<sup>133–135</sup> Table 4 lists some modifications on food nutrients by cold atmospheric plasma.

The formation of radical species could suggest the hypothesis that plasma increases lipid oxidation. Wheat flour was treated by atmospheric plasma for 1 and 2 min at 15 and 20 kV by Bahrami et al.,<sup>136</sup> who analyzed the level of lipid oxidation by measuring the hydroperoxides and volatile compounds produced after plasma treatment and found a significant increase of their content for all the treated samples. The difference was greater for longer treatment times and higher voltages. Moreover, they found an important reduction in the fatty acid profile, particularly linoleic acid which is an essential fatty acid for humans, with a complete reduction after 120 s of treatment at 20 kV. Thirumdas et al.<sup>123</sup> observed an increase of 20% of peroxides content for plasma treated peanuts and walnuts at 60 kV, which can lead to the oxidation of lipids. Joshi et al.<sup>137</sup> demonstrated that plasma encourages lipid oxidation

Table 4. Effect of Cold Atmospheric Plasma on Chemical Changes in Food Products

food product	plasma source	treatment	changes	ref
wheat flour	–	frequency: 9 kHz power 40 W and voltage 15 V power 90 W and voltage: 20 V time: 1 and 2 min	↑ lipid oxidation fatty acid degradation	136
walnuts and peanuts	–	frequency: 13.56 MHz pressure: 0.5 mbar power: 40, 50, 60 W time: 5, 10 and 15 min	↑ lipid oxidation	123
raw milk	corona discharge	power: 9 kV current: 90 mA time: 3, 6, 9, 12, 15, or 20 min	↑ lipid oxidation = fatty acids content	138
vegetable oil	radio-frequency driven plasma jet	power: 25 kV radio frequency power: 13.56 MHz gas flow: 2 sl/m time: 2 min	↑ lipid oxidation	139
fish oil	dielectric barrier discharge and plasma jet	voltage: 6 kV current: 128 mA gas flow: 2 sl/m time: 60 min	↑ lipid oxidation	140
beef jerky	radio frequency driven atmospheric plasma unit	flow rate: 20 000 sccm power: 200 W time: 0–10 min	= fatty acid composition	142

on the cellular membrane of *Escherichia coli* using a dielectric barrier discharge (DBD) system. From these studies, it can be concluded that the longer the treatment time the higher the radical species concentration resulting in higher levels of lipid oxidation and more pronounced impacts on food quality. In another study, a corona discharge plasma system was used for milk samples at intervals of 0, 3, 6, 9, 12, 15, and 20 min.<sup>138</sup> The researchers did not notice noteworthy differences in the fatty acid profile among all the samples. They suggested that long chain fatty acids such as C18:00 could suffer a hydrolysis process, as their content was slightly reduced after treatment, while C10:00 and C12:00 showed a low increase. However, a significant increase was observed for the volatile compounds content, which could be related to lipid oxidation. In similar studies, Van Durme et al.<sup>139</sup> and Vandamme et al.<sup>140</sup> used a plasma jet device and a DBD system for vegetable and fish oils, respectively, and report that plasma accelerates lipid oxidation. However, Korachi and Aslan<sup>141</sup> did not find any evidence of lipid oxidation nor changes of the fatty acids profile on the cellular membrane of *Escherichia coli* and *Staphylococcus aureus* treated by an atmospheric plasma corona discharge device. In addition, no significant changes in the fatty acid composition of beef jerky were observed after radio frequency (rf) atmospheric pressure plasma with 5 min of treatment.<sup>142</sup>

Apart from lipids, the effect of plasma on proteins has also been studied.<sup>143</sup> Takai et al.<sup>144</sup> investigated the effect of a low frequency plasma jet on an aqueous solution of 14 amino acids. They noticed that these biomolecules suffered some modifications by using high-resolution mass spectroscopy. After treatment, new molecules were observed, resulting from different reactions such as oxidation, sulfonation, amidation, sulfoxidation, hydroxylation, dehydrogenation, nitration, and dimerization depending on each specific amino acid. Segat et al.<sup>145</sup> studied the inactivation kinetics of an alkaline phosphatase solution at different treatment times between 5 and 300 s and at three different voltages: 40, 50, and 60 kV using a DBD. The inactivation of this enzyme was detected after a few seconds of treatment and it was attributed to the loss of  $\alpha$ -helical and  $\beta$ -sheet secondary structures of the protein. Tappi et al.<sup>146</sup> found that polyphenol oxidase activity significantly decreased for apples after plasma treatment. In another study, polyphenol oxidase (PPO) and peroxidase (POD) were denaturalized by losing the  $\alpha$ -helical structure using a plasma jet.<sup>147</sup> These enzymes are part of the undesirable browning reactions related to a reduction of the nutritional and sensorial quality of vegetables and fruits. In another experiment, Park et al.<sup>148</sup> studied the effect of plasma on hemoglobin and myoglobin (Mb) using different gases, observing that plasma treatment leads to the modification of the secondary structure of both proteins, from  $\alpha$ -helical to  $\beta$ -sheet, and a degradation of the heme group. Conversely, Alves et al.<sup>149</sup> studied the effect of plasma on fructooligosaccharides (FOS) and found no significant changes in FOS concentrations after applying 70 kV using a DBD system. These carbohydrates are used in the food industry as sweeteners and are interesting from a health perspective point of view due to their prebiotic activity.

Cold plasma is an early stage with regards to food application and data on the induced chemistries from the array of plasma technologies and process conditions available are only emerging. A challenge here is the complexity of the "cocktail" of active species generated, the widely different time-scales over which they may act and diagnostics of the interaction with the target product. Of course it is this complexity with provides the

advantages observed in terms of process efficacy and lack of apparent resistance to the approach by microbial populations. A priority area of research will be unravelling both the mechanisms of antimicrobial action along with induced chemistry for treated foods and linking them to the key reactive species generated.

## CONCLUSION

Nonthermal food processing technologies have shown great promise as microbial decontamination tools, with a large body of work found in the literature. However, the effects of these novel technologies on food chemistry have not been studied deeply (see Table 5). These promising technologies can

**Table 5. Effect of Novel Technologies on Chemical Changes in Food Products<sup>a</sup>**

effects	high pressure	pulse electric field	ultrasound	plasma
<b>Protein</b>				
oxidation	+	–	+	+++
denaturation	+++	++	+++	++
aggregation	++	++	++	++
proteolysis	+	–	+	+
<b>Lipids</b>				
oxidation	+	+	++	+++
lipolysis	++	++	–	++

<sup>a</sup>+++ , significant effect; ++, moderate effect; +, low effect; –, no reported.

damage some nutrients, such as proteins and lipids. Consequently, more research is required into the effects and mechanisms of action of each technology on food chemistry. Such insights is key to ensure that these emerging technologies are accepted by industry, regulatory agencies, and consumers alike.

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