CHARACTERIZATION AND ANALYSIS OF HIGH VOLTAGE ATMOSPHERIC COLD PLASMA TREATMENT OF SOYBEAN OIL

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To my family for being there every step of the way.

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ABBREVIATIONS

HVACP	High Voltage Atmospheric Cold Plasma
IV	Iodine Value
SAT	Saturated fatty acids
MUFA	Monounsaturated fatty acids
OES	Optical emission spectroscopy
PUFA	Polyunsaturated fatty acids
SAT	Saturated fatty acids
PV	Peroxide value
UV	Ultra violet
GC	Gas chromatography
FTIR	Fourier-transform infrared spectroscopy
NMR	Nuclear magnetic resonance spectroscopy
HSQC	Heteronuclear single quantum coherence spectroscopy
RH	Relative humidity

ABSTRACT

Yepez, Ximena V. Ph.D., Purdue University, December 2018. Characterization and Analysis of High Voltage Atmospheric Cold Plasma treatment of Soybean Oil. Major Professor: Kevin M. Keener Professor.

In 1900, Paul Sabatier was honored by the Nobel Prize for the invention the catalytic hydrogenation reaction, that changes the liquid oil into a solid fat. Although this reaction was used in many fields, the main application was to produce partially hydrogenated oils for the food industry. Catalytic hydrogenation achieves a reduction of polyunsaturated fatty acids, by a reaction that involves a catalyst, hydrogen gas, high pressure and temperature. However, this process creates the unwanted *trans* fatty acids. In 2015, after a century of research and development, the FDA announced that partially hydrogenated oils cannot be used as food ingredients, because trans fatty acids are associated with detrimental health effects.

Today, emerging technologies are studied as effective tools to modify chemical structures of molecules. High voltage atmospheric cold plasma (HVACP) is a novel technology developed at Purdue University, which generates reactive species from gases such as hydrogen, nitrogen, or carbon dioxide. This technology not only reduces the microbial load of food, but also modifies the chemical structure of food ingredients.

The overall objective of this project is to explore the feasibility of applying high voltage atmospheric cold plasma to modify the chemical structure of soybean oil by partial hydrogenation, without the use of a catalyst, high temperature, and *trans* fatty acids.

The **first study** characterizes the effect of HVACP treatment of soybean oil and identifies changes in iodine value and fatty acid composition. This initial study constitutes a proof of concept, where HVACP treatment of 12h using 5% hydrogen-

95%nitrogen gas produced a reduction to the iodine value from 130 to 90, a value similar to a commercial partially hydrogenated oil.

In the **second study**, the objective is to increase the hydrogenation rate and reduce the treatment time by increasing the percentage of hydrogen gas in the plasma chamber. A processing time up to 1.5 h was evaluated with different concentrations of nitrogen and hydrogen gas. A short treatment time produced a reduction of IV from 130 to 123 in all gases. However, a fraction of the sample inside the plasma chamber was recovered from the top of the box, which had a higher exposure to reactive plasma species. This sample had a semisolid texture and an iodine value within the 80-90 range. These results suggest that a treatment with nitrogen and hydrogen gas modifies the chemical structure of soybean oil at similar rates. Additionally, HVACP is a surface treatment, that can be accelerated by increasing the sample's surface area. The use of nitrogen gas was associated with a yellow color that appeared in samples treated with nitrogen gas, suggesting that nitrogen was absorbed in the triglyceride molecule.

In the **third study**, an extensive analysis of HVACP hydrogenation of unsaturated double bonds by identifying reactants and products is developed, and a plasma hydrogenation reaction mechanism is proposed. Here, soybean oil, pure standards of linolenic acid and trilinolenin were treated with nitrogen, hydrogen, and argon gas. Results showed a hydrogenation reaction with all gases but mostly with nitrogen gas. It was proposed that the hydrogenation reaction may be achieved by hydrogen atoms from (a) water dissociation; (b) intermolecular re-arrangement; or, (c) hydrogen gas.

The **fourth study** evaluated the potential side reactions that may occur during HVACP hydrogenation of soybean oil. Three fractions were identified from 6 h treatment with hydrogen gas treated samples: liquid, gel, and solid. Results showed that polyunsaturated fatty acids are readily susceptible to react with plasma reactive species, not only for hydrogenation, but also for polymerization.

In summary, findings presented in this dissertation show that polyunsaturated fatty acids of soybean oil effectively reacted with high voltage atmospheric cold plasma

gas species of various gases including hydrogen, nitrogen, and oxygen at room temperature without a catalyst. Hydrogenation, nitration, and epoxidation reactions were observed along with polymerization. Unexpectedly, hydrogen gas was not the primary source of atomic hydrogen observed under these experimental conditions. Rather, it is suggested that water vapor, present as an impurity, supplied hydrogen ions to the hydrogenation reactions. Nitrogen gas modified atmosphere is an electron-rich medium that catalyze reactions. Further investigation is suggested for optimizing the process of oil hydrogenation, as well as for exploring the potential to produce bio-based gels, lubricants, and greases.

1. LITERATURE REVIEW

1.1 Lipid chemistry

Lipids are known as non-polar molecules, because they lack of partial positive or negative charges, and they are not capable of forming hydrogen bonds with other molecules. Lipids include fatty acids, glycerides, waxes, sterols, fat soluble vitamins, sphingo-lipids, carotenoids, and glycolipids. In particular, triglycerides are the most abundant lipids, and they are formed by three fatty acids joined together with a glycerol molecule through ester bonds. Triglycerides are the main component of fats and oils, and they are widely used as food ingredients from animal and vegetable sources. Liquid oils and solid fats are used for baking, frying, cooking, as salad dressings and confectionery products. They play an important role by providing flavor, texture, satiety, lubricity, and cohesiveness to food.

Vegetable oils such as soybean, sunflower, and corn oil are liquid because they have a high content of unsaturated fatty acids. In contrast, solid vegetable fats such as palm and coconut oil are produced mainly in the tropic, and they are semisolid at room temperature, due to their high content of saturated fatty acids. Solid fats are valued as a food ingredient because they provide texture, high oxidation stability, and high melting point.

1.1.1 Fatty acids and triglycerides

Fatty acids are hydrocarbon chains with a terminating carboxylic group on one side, and a methyl group on the other side. Fatty acids found in nature are formed mostly by 6-22 carbons. The hydrocarbon chain may include single or double covalent bonds. Single bonds allow rotation of hydrogen atoms along the axis, and they have

a σ bond. Double bonds give a planar configuration of carbon-carbon bond, because they do not allow rotation of the hydrogen atoms, and the double bonds have σ and π bonds.

Saturated fatty acids have all the carbons filled with hydrogen and joined by simple bonds, forming a linear chain. They are solid at room temperature. Conversely, unsaturated fatty acids have simple and double bonds between carbon-carbon. Unsaturated fatty acids are liquid at room temperature because double bonds bend the hydrocarbon chain, and do not stack together.

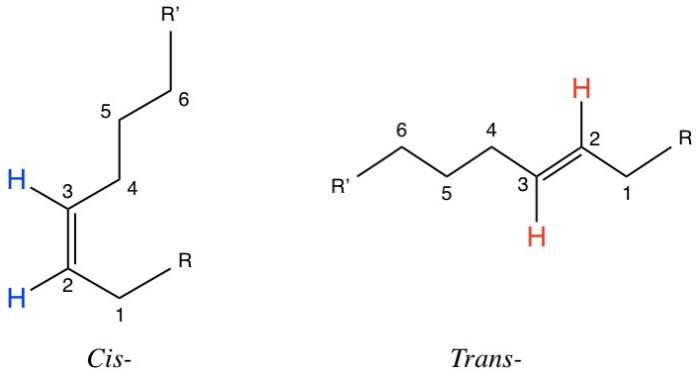

Fig. 1.1. Geometric isomers of alkenes

Double bonds can have a *cis-* or *trans-* geometric configuration, according to the position of the hydrogen linked to the carbon-carbon double bond, as shown in Fig. 1.1. A *cis-* configuration is characterized by hydrogen atoms in the same side of the molecule, therefore *cis-* configurations bend the hydrocarbon chain at the double bond location. In contrast, trans- geometric configurations are formed by hydrogen at an alternative location of the double bond, therefore trans- configurations have a linear chain that behave as a saturated fatty acid, and they can be solid at room temperature, even if they have one or two double bonds. *Trans-* isomers are mainly produced by catalytic hydrogenation, however some trans fatty acids are found naturally in dairy products at low concentrations.

The major vegetable oil produced in the U.S. is soybean oil, with a production of 22.6 million pounds per year, which is equivalent to 64.9% of the total vegetable oil.

Followed by corn and canola oil with a production of 6.1 and 1.7 million pounds per year. Crude soybean oil is formed by 95-97% of triglycerides, 2.5-3% of phospholipids and minor components, including unsaponificable matter (phytosterols, tocopherols), free fatty acids, or trace metals. These impurities are removed to meet quality and stability standards of a refined product. The composition of refined soybean oil is >99% of triglycerides [1]. Soybean oil triglycerides are formed mainly by the following fatty acids: palmitic, stearic, oleic, linoleic, and linolenic acid (Fig. 1.2).

1.1.2 Hydrogenation

Paul Sabatier was honored with the Nobel Prize in chemistry for the invention of the method of catalytic hydrogenation. The reaction consisted in treating a substrate with hydrogen gas, a catalyst, at high temperatures. Initially, this reaction was tested with different compounds such as benzene, aldehydes or nitriles, previous to its application in fats and oils. Wilhelm Norman applied the principle of this reaction to liquid oils, and it was not until 1909 that the first large scale plant was constructed as a food processing facility.

A liquid oil is transformed into a solid fat through catalytic hydrogenation, where hydrogen atoms binds into double bonds of unsaturated fatty acids, in consequence the double bond changes into a simple bond. This reaction requires the use of a catalyst, agitation, high temperature and pressure. This reaction is exothermic, releasing approx. 1 kcal/kg per each unit of iodine value reduction. Full hydrogenation can be achieved if hydrogen is available to react with the double bonds, however the reaction rate becomes slower when there is less quantity of unsaturated fatty acids. Overall, a vegetable oil with less amount of unsaturated fatty acids is more resistant to oxidation, and it provides an extended shelf-life to food products.

Iodine value is one of the main indicators of the hydrogenation reaction, since it represents a measurement of the amount of double bonds. For example, a traditional process of partial hydrogenation of soybean oil reduces the iodine value from 130 to

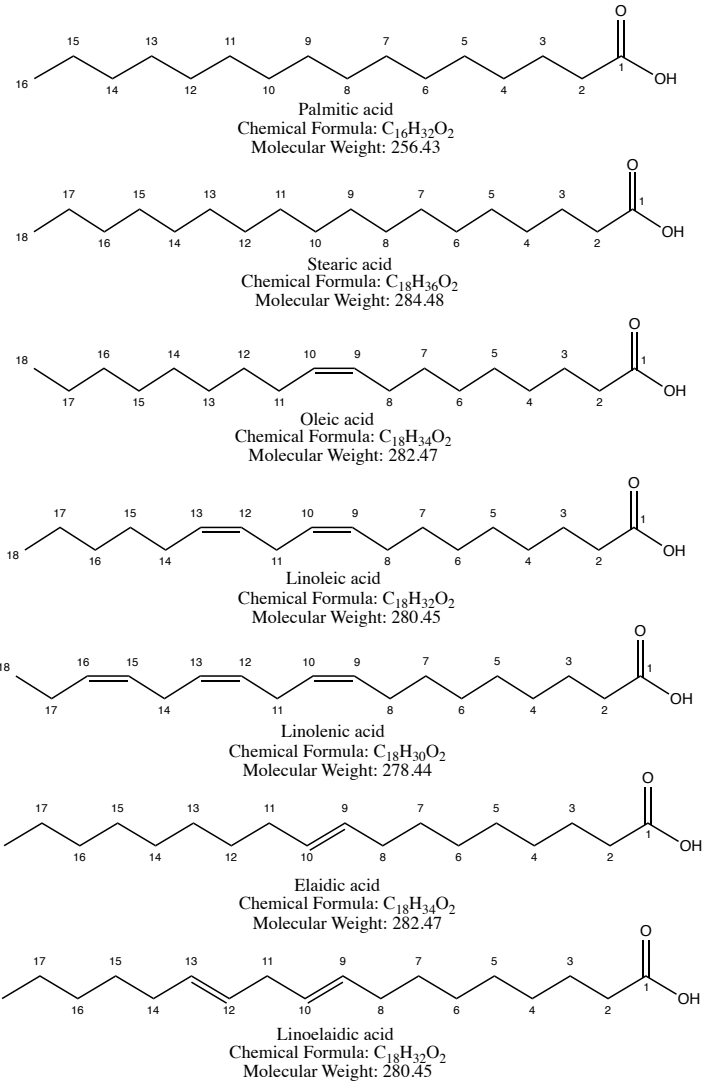

Fig. 1.2. Main fatty acids from refined soybean oil and partially hydrogenated soybean oil

80 (g of iodine/100g of oil), in a process of 1-2 h. Although, depending if the final product requires to have different properties, the iodine value can be in the range of 100-110 for a soft, 80-85 for a flat, 60-75 for a steep, or less than 10 for a hard product [2].

Catalyst

Molecular hydrogen has a low solubility in oil, so it requires a catalyst surface to separate the molecule into atomic hydrogen. Catalysts accelerate the hydrogenation reaction by providing a link between atomic hydrogen and double bonds. In this way, the catalyst accelerates the rate of fixation of atomic hydrogen onto unsaturated fatty acids.

Catalysts used for hydrogenation reaction are mainly metals, such as nickel, platinum, palladium, or copper. The most common catalyst used in industry is nickel, due to its availability, performance, and low cost. It is used in the form of nickel sulfate, nickel nitrate, or nickel chloride [3]. A catalyst is mixed with an inert compound to increase the amount of active sites, with materials such as silica, alumina or diatomaceous earth, as well as to facilitate the removal after treatment. The catalyst can be toxic and must be completely removed after the hydrogenation reaction. The catalyst can be reused until its selectivity gets involved and a higher rate of formation of trans isomers is observed [4].

Mechanism of catalytic hydrogenation

The hydrogenation of vegetable oils is realized in a reactor with a heat exchanger, a gas re-circulation system, agitation, and oil filters. The product inside the reactor requires to have continuous agitation, as well as the presence of the gas in the form of bubbles, and should be kept at a constant temperature. Hydrogen is introduced to the reactor as small bubbles from the bottom of the tank. The reaction starts with the addition of the catalyst. Then, two separate complexes are formed: (a) oil-catalyst complex, where the double bond is absorbed in the catalyst surface and opened in the active sites; (b) gas-catalyst, where the hydrogen gas reaches the catalyst and dissociates into two hydrogen atoms [5]. Afterwards, both complexes react together by the effect of temperature (150-240°C) and pressure (1-4 atm), forming a simple bond when the hydrogen atoms fill the carbon capacity. The final product is cooled

and filtered to extract the catalyst. Hydrogen gas consumption is about 1 m^3/1000kg of soybean oil per unit drop in iodine value [2, 6].

The selectivity of the reaction has been discussed in literature, terms such as linolenic or linoleic selectivity determine the preference of the reaction, and this is information can be useful to determine the reaction rate. The triglyceride composition is also an important factor, as triglycerides with 2 or 3 polyunsaturated fatty acids are more susceptible to hydrogenation that the triglycerides with 1 unsaturated double bond [7].

The hydrogenation process is not straightforward, there are limitations that prevent the addition of hydrogen to the double bond. This occurs when a double bond is opened and form an intermediate state, that returns to the original double bond with modifications. The intermediate products lead to the formation of geometrical isomers. The mechanism of formation of isomers has been established by two main pathways as shown in Fig.1.3 [5], as follows:

1) Incomplete hydrogen absorption. A double bond is opened in the catalyst surface, forming an unstable intermediate state. Then, the hydrogen atom absorbed in one carbon is expelled. Afterwards, the double bond is restored as a *trans-* isomer.

2) Conjugated formation. A double bond is opened in the catalyst surface, and one carbon absorbs a hydrogen atom forming an intermediate state. Then, before the second carbon completely absorbs another hydrogen atom, a third carbon loses a hydrogen atom and forms a *trans-* isomer. This isomer is in a shifted position known as conjugated double bond.

Temperature

Reaction temperature determines the final fatty acid composition of a partially hydrogenated oil, specially the *trans-* fatty acid content. Processes are done in a range of 150-240°C. The use of a high temperature has been associated to an increment in the formation of trans- fatty acids. This is because hydrogen becomes less available

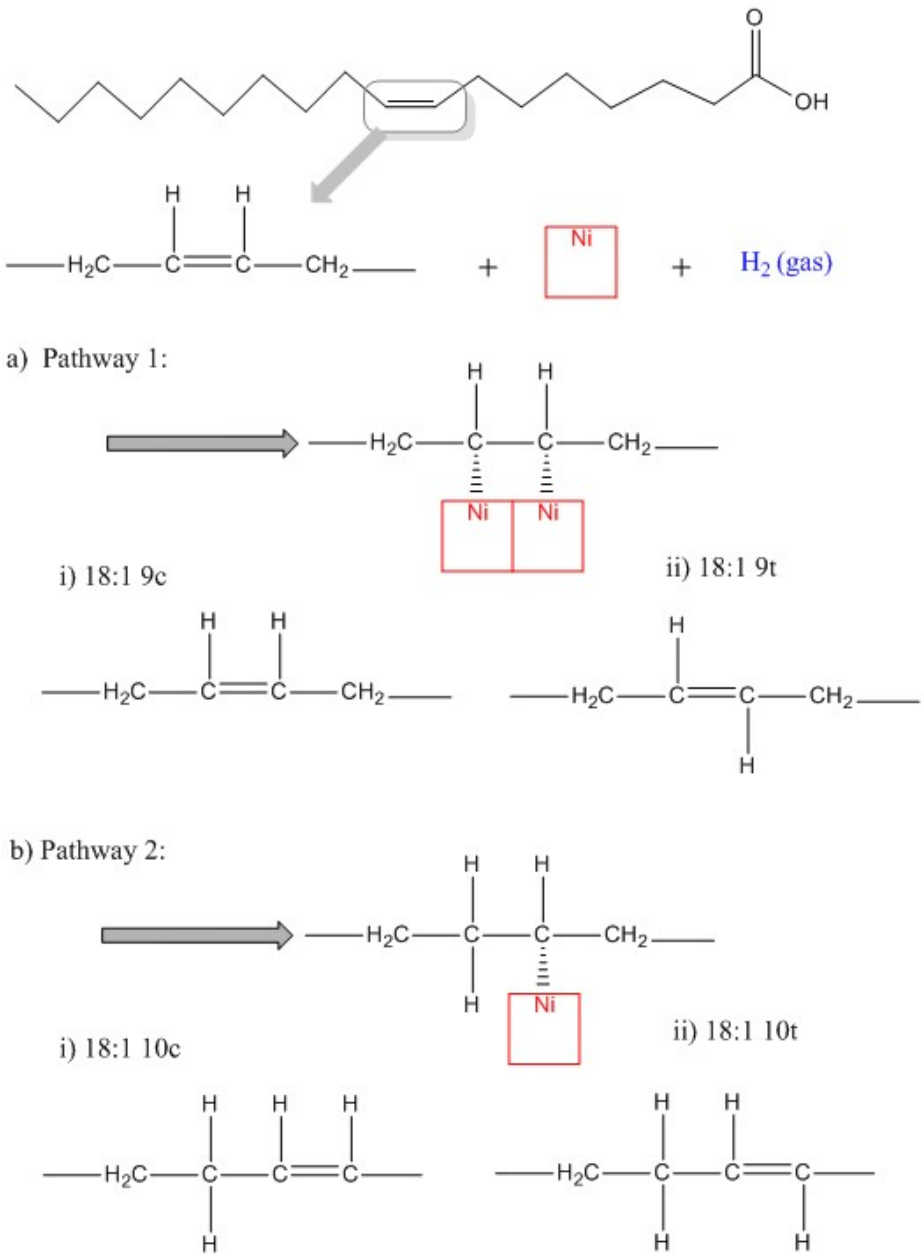

Fig. 1.3. Pathways for isomers formation during catalytic hydrogenation

with higher temperatures, as its solubility is reduced. Therefore, the reaction is limited and becomes incomplete, the double bonds that were opened in the catalyst surface return to the *trans-* geometric location.

Applications

A hydrogenated oil has a higher oxidation stability and increased melting point. First, the reduced number of double bonds makes this product less susceptible to get oxidized by the presence of light, metal or other agents that initiate oxidation reactions. Therefore, hydrogenation increases the shelf life of vegetable oils. Second, when a double bond is hydrogenated, the hydrocarbon chain becomes linear and can stock together with other saturated fatty acids because of non-covalent interactions. As a consequence, the melting point increases, and the partially hydrogenated vegetable oil turns solid at room temperature.

These properties make the PHOs a suitable ingredient for baking, frying, and confectionery. For example, cocoa butter has a melting point close to the human temperature, and it provides a mouth-feel sense of smoothness and softness, which is difficult to replace with any other low-cost source vegetable oil. Partially hydrogenated oils behave like cocoa butter and it is used as a low-cost alternative for cocoa products maintaining their properties. High melting point PHOs are used for frying applications, as they provide a dry appearance because they turn semi-solid at room temperature. In addition, PHO stability provides an extended shelf life to food products.

1.1.3 Trans fatty acids and health

The traditional hydrogenation process yields approximately 25-40% of *trans* fatty acids [6]. Trans fatty acids are found naturally in low quantities in fats from dairy and meat products (2-5%), but the largest source comes from PHOs [8].The consumption of trans fatty acids have been linked to a significant risk factor for cardiovascular diseases, raising the bad low-density lipoprotein cholesterol and reducing the good high-density lipoprotein cholesterol levels, as well as inflammatory effects and endothelial dysfunction [9]. The Food and Drug Administration (FDA) estimated that 1200 coronary events and 480 deaths from heart diseases can be prevented each year

by reducing the consumption of trans-fat from 0.60% to 0.04% of total energy intake [10].

In 2003, the FDA issued the trans-fat labeling requirement for food products. Since then, food companies started to replace PHOs from their product formulations. In January 2006, food companies were required to declare the amount of trans fat in food on the nutritional fact label. At that time the label could state 0% trans if the content of total trans fatty acid were less than 0.5g per serving, and many companies reformulated their foods or reduced the serving size to label them as zero trans fats. In June 2015, the FDA made a final determination that PHOs were not recognized as safe for any use in human food. The FDA gave a compliance date until June 2018 to remove this ingredient from the marketplace. Currently, the replacement of PHOs were mainly tropical oils such as palm and coconut, interesterified oils, fully hydrogenated oils, and genetically modified vegetable oils [11]. In Europe, PHOs are still allowed as ingredients, but there is currently a proposal to limit their use to a maximum of 2g in 100g of fat.

1.1.4 Oxidation

Oxidation changes the physical properties of food, through the formation of volatiles or other reaction products that change the quality of food products. Animal fats such as butter, lard or edible tallow, and tropical vegetable oils are rich in saturated fatty acids. They have a high melting point and maintain a solid or semisolid state at room temperature. Fats rich in saturated fatty acids are less susceptible to oxidation, therefore the addition of fats rich in saturated fatty acids to food products allow them to extend their shelf life. In contrast, liquid vegetable oils are susceptible to oxidation, especially if they are exposed to light, metals, oxygen, or any other condition that accelerate the oxidation reaction. Linolenic acid (C18:3) oxidizes 2.4 times faster than linoleic acid (C18:2), and the latter 40 times faster than oleic acid (C18:1) [12, 13].

Lipid oxidation is one of the major causes of food quality deterioration, in the form of off flavors, color degradation, and destroying essential fatty acids.

Oxygen reacts with unsaturated fatty acids by different pathways according to its energetic state. The oxygen molecule can exist in two energetic forms known as triplet and singlet. Singlet state (22.4 kcal/mol) is more reactive than the triplet state, and they are formed by the effect of enzymes, light, or chemicals reactions. Triplet oxygen is associated with autoxidation reactions, while singlet oxygen is related with photo-oxidation reactions of olefinic compounds.

The mechanism of lipid autoxidation reaction has 3 stages: initiation, propagation, and termination [14]. The reaction starts when a hydrogen is subtracted from the allylic carbon forming a fatty acid radical (initiation), in the presence of heat, a metal catalyst, and ultraviolet or visible light (Fig. 1.4). Afterwards, the fatty acid radical re-arranges forming a conjugated diene, then oxygen is absorbed to form peroxyl radicals (propagation). Then, a serial chain of reactions occurs at this point, with the formation of peroxides, cleavage of hydrocarbon chains, and formation of non-radical products (termination).

Primary and secondary reaction products are analyzed to measure the extent of oxidation on food products. Primary reaction products include peroxides and conjugated dienes, which are formed during initiation and propagation. Secondary reaction products include peroxides, aldehydes, ketones, and other volatiles compounds, that are the final products of this reaction.

Singlet oxygen is a highly energetic molecule that has both electrons in the π-antibonding orbital with opposite spins (-1/2, +1/2). This molecule is formed by photosensitization and reacts directly with electron rich double bonds. In contrast to autoxidation reactions, where triplet oxygen attacks the allylic carbon between two double bonds forming diene hydroperoxides.

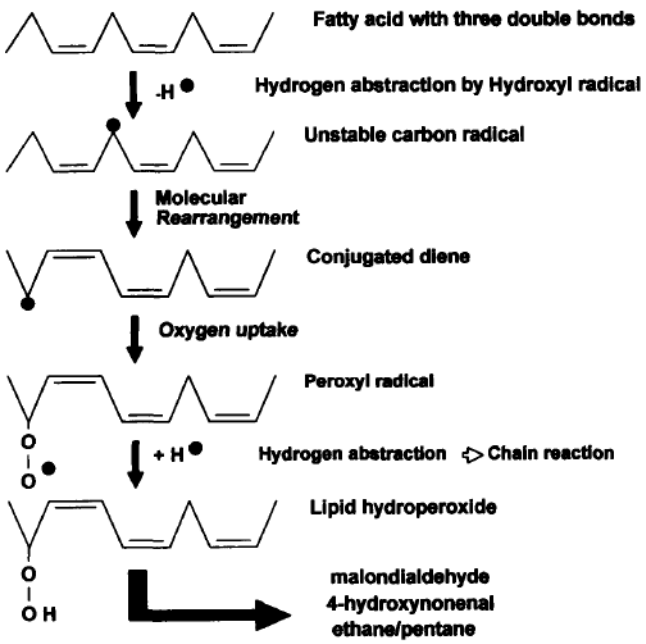

Fig. 1.4. Oxidation mechanism of alkenes [15].

1.1.5 Oleogels

Oleogels or organogels are structured oils that have been investigated as a replacement of PHOs [16, 17]. The structure of oleogels is given by a liquid oil dispersed in a solid continuous phase that traps the oil in a tri-dimensional network and mimics the PHO texture, without changing the fatty acid composition of the vegetable oil. Polysaccharides and proteins have been studied as gelling agents (continuous phase). However, most of them are hydrophilic and require an emulsifier to form oil-water emulsions. When the emulsion is formed, the water is removed to entrap the oil in a polymer complex [18]. The major challenge of producing oleogels has been to select gelling agents that are both food grade and hydrophobic [16, 19, 20].

1.1.6 Epoxides, estolides, and biolubricants

Oils and fats from vegetable sources are renewable raw materials that can replace lubricants, greases, and plasticizers. There is an increased interest to reduce the dependence on petroleum to obtain these products. Vegetable oil lubricants are non-toxic and biodegradable. However, vegetable oils are susceptible to oxidation and they are not stable at high temperatures. Therefore, chemical modifications are done to overcome these disadvantages.

The reduction of the unsaturation degree helps to increase oxidation stability. This can be done by hydrogenation, esterification, or epoxidation. Current methods of epoxidation of vegetable oils include the use of hydrogen peroxide (H_2O_2), acetic or formic acid, catalyst, and reaction times of 12-24 h [21, 22]. A peracid is formed by the reaction of H_2O_2 with the carboxylic end of the acid (R-C(O)-OOH). Then, the peracid reacts with the double bond, forming a 3-ring structure known as epoxide.

Additional reactions such as ring opening, esterification, or acetylation improve the oil stability, viscosity, and lubricity properties [23]. Polymerized vegetable oils are another alternative for the production of bio-lubricants. Studies have shown the beneficial properties of estolides, with improved lubricity, oxidation stability, and decreased pour points. These compounds are formed by chemical modifications, in which the carboxylic end of a fatty acid is bonded to the double bond of another fatty acid [24].

1.2 Analytical techniques for lipid analysis

1.2.1 Gas chromatography

In this study, gas chromatography is used for the identification and quantification of fatty acids (% by weight). This technique is used for fatty acid analysis because fatty acids with the same molecular weight can be separated according to the position of a double bond, and it is also useful for determining the geometric isomers *cis*- and

trans-. Gas chromatography analysis requires the triglycerides to be derivatized into fatty acid methyl esters (FAME), because FAME have a lower molecular weight and are polar. The derivatization is a transesterification reaction, where the glycerol of the triglyceride is displaced by an alcohol in the presence of an acid, to form fatty acid methyl esters and glycerol [25], as follows:

$$\text{Triglyceride} + \text{R-OH} \xrightarrow{\text{HCl}} \text{R}\!-\!\overset{\displaystyle \text{O}-\text{CH}_3}{\overset{\displaystyle \diagup\!\!\diagdown}{\underset{\displaystyle \text{O}}{\diagup\!\!\diagdown}}} + \text{Glycerol}$$

The mixture of fatty acid methyl esters is separated with a temperature gradient in a capillary column, because FAME of vegetable oils differ from each other by their volatility and polarity. However, the elution time of each FAME depends of the nature of the column and experimental conditions. Optimal conditions allow to separate FAME by the carbon chain length, degree of unsaturation, geometrical isomers, and position of double bonds. A flame ionization detector (FID) is adequate for quantification of FAME, and it is the standard method for fatty acid composition.

1.2.2 FTIR

FTIR is an important tool to analyze fatty acids and triglycerides structure, because polar molecules are associated with strong IR absorption. Infrared bands indicate molecular vibrations, such as bonds stretching or bending. The absorption intensities of this vibrations can be strong, medium or weak for different types of bonds. Table A.1 of the Appendix, shows the main IR absorption frequencies for functional groups of fatty acids and triglycerides, as well as frequencies related to oxidation and polymerization reactions.

1.2.3 Nuclear Magnetic Resonance

Nuclear magnetic resonance (NMR) utilizes a magnetic field to align the nucleus of specific atoms that possess magnetic moment (e.g., ^{1}H, ^{13}C), and analyzes their behavior by applying electromagnetic radiation that promotes transitions in nuclear

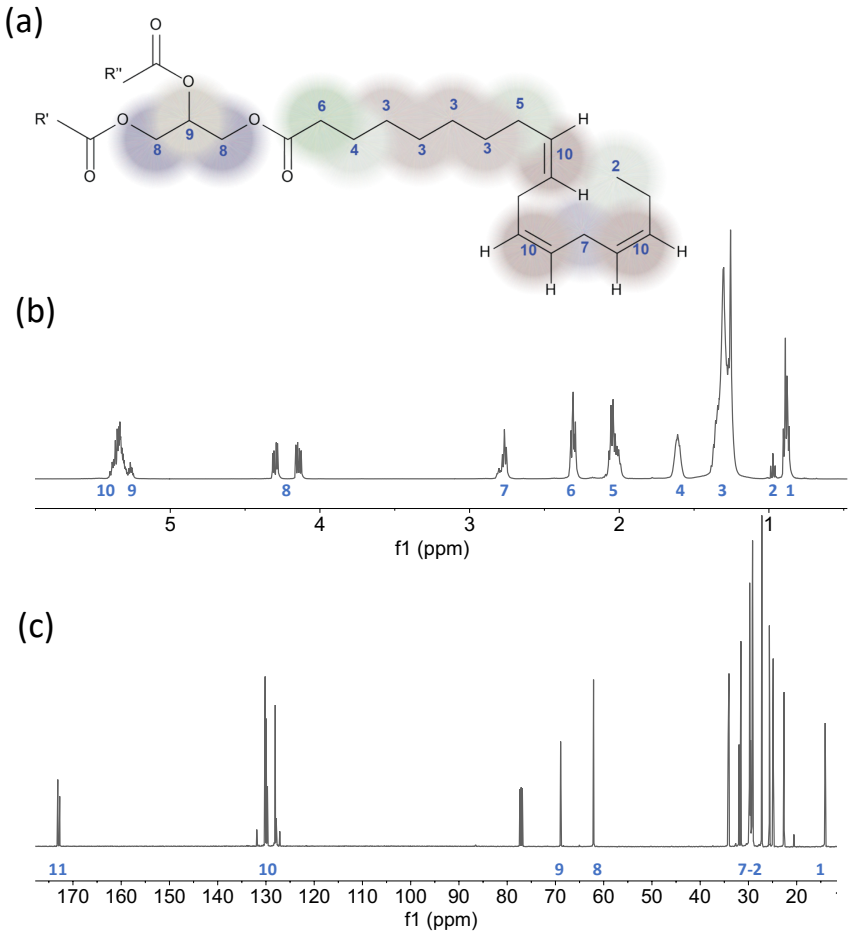

Fig. 1.5. ^{1}NMR assignments for soybean oil. (a) Structure of a triglyceride with a linolenic acid in n-3. (b) ^{1}H-NMR spectra of soybean oil. (c)^{13}C-NMR spectra of soybean oil.

energy levels. As a result, the structure of molecule this technique can be determined through this technique, as an example in ^{1}H-NMR the protons of each part of the organic molecule absorb energy depending on the local chemical structure.

The process to identify the NMR spectrum starts with a relaxation delay, that allows the nuclei to align. Then a short pulse is applied by a high-power radio frequency. Afterwards, a free induction decay is produced, and it is recorded during the acquisition time. The signal is then analyzed accordingly by the Fourier transform,

Table 1.1.
NMR chemical shifts for a triglyceride

Signal	**Chemical shift**		**Structure**	**Name**
	^{1}H-NMR	^{13}C-NMR		
1	0.85-0.90	13-15	-CH_3	Terminal methyl
2	0.09-1.1	22-23	-CH_3	Terminal methyl$_{(C18:3)}$
3	1.2-1.4	24-34	-CH_2-CH_2-CH_2-	Aliphatic alkane
4	1.5-1.7	24-34	-C(=O)-CH_2-CH_2-CH_2-	Aliphatic alkane
5	1.9-2.1	24-34	-CH_2-CH_2-CH=CH-	Aliphatic alkane
6	2.2-2.4	24-34	-C(=O)-CH_2-CH_2-CH_2-	Aliphatic alkane
7	2.7-2.9	24-34	=CH-CH_2-CH=	Aliphatic alkane
8	4.0-4.3	61-63	n-1 and n-3	Glycerol
9	5.2-5.3	68-69	n-2	Glycerol
10	5.3-5.4	127-132	CH_2-CH=CH-CH_2	Aliphatic alkene
11	-	172-174	C=O	Carboxyl

to obtain a plot of the intensity of absorption vs. frequency. This plot is usually expressed by ppm in the x-axis (chemical shift), which is directly proportional to the magnetic field strength. The whole process has a duration of few seconds, but usually the signal of one acquisition is not enough to have a good signal, therefore this process needs to be repeated to improve the signal-to-noise ratio. Functional groups of triglycerides and fatty acids corresponding to ^{1}H-NMR and ^{13}C-NMR are shown in Fig. 1.5 with numbers from 1-11. The corresponding numbers are described in detail in table 1.1. Quality parameters such as fatty acid composition, free fatty acids, or fatty acid structure can be analyzed with ^{1}H-NMR [26, 27]. Oxidation products are characterized by the presence of hydroperoxides (chemical shift 5.72, 8.3-8.9), aldehydes (chemical shift 9.5-9.8, fig 3-c), alcohols (chemical shift 3.43-3.62), epoxides (chemical shift 2.63,2.88-2.90, 3.1), or ketones (chemical shift 6.08, 6.82) [28, 29].

1.3 Cold plasma

Plasma is the fourth state of matter, alongside with solid, liquid and gas. In a plasma state the molecules are not only distant from each other, but also, they break apart into their atomic elements. Therefore, plasma is formed by a partially ionized gas, where excited species, ions, and electrons coexist in a charged electric field. Reactive species are formed by an electrical discharge between two electrodes, where molecules are bombarded with a flow of electrons.

From a macro perspective, a plasma state is formed by electrons and heavy particles. Heavy particles include reactive species, molecules, atoms, radicals, and ions. Electrons are smaller, so they acquire a higher velocity, and reach higher temperature easily. When the electric field is formed, electrons collide with heavy particles, reactions occur constantly in orders from tens to hundreds. In "cold plasma", the temperature of electrons is much higher than the temperature of heavy particles, therefore they are not in equilibrium and the whole system is maintained at room temperature. In contrast, "thermal plasma" can achieve temperatures of 1000-10000°C when the heavy particles heats up, and both electrons and heavy particles achieve thermodynamic equilibrium.

A plasma state is formed when a system gains enough energy to partially ionize a gas. The transition between a gas state and a plasma state is marked by an ignition point, that involves a current increment because of a higher flow of electrons. At this point, atoms and molecules move through different energetic states. Molecules absorb energy when they change from low to high energetic levels, and release energy when they move from high to low energetic levels. In the latter, they emit light and form a uniform glow discharge.

Increasing the intensity of the electric field can change a uniform glow plasma into an unstable system with streamers. In that case, the system changes from an insulating dielectric into a conductive medium. Arc filaments or streamers can reach a breakdown if the intensity of the electric field is increased. In cold plasma treatment,

it is preferred to maintain a uniform glow discharge, and this state is controlled by gas composition, electrode configuration, dielectrics, and pressure [30]. The type of gas used is an important factor, for example helium gas requires less energy to form a uniform glow plasma compared to nitrogen gas. The differences between gases has been acknowledged by the value of Townsend breakdown, that determines the amount of energy required to reach a system breakdown. Parameters such as number of electrons, Townsend breakdown, dissociation energy, and ionization energy are shown in table 1.2.

Table 1.2.
Properties of gases [31, 32]

Element	**Number of electrons**	**Ionization energy (eV)**	**Townsend Breakdown(kV/cm)**	**Dissociation energy(eV)**
Nitrogen	7	14.5	35	9.76
Hydrogen	1	13.6	20	4.48
Argon	18	15.8	2.7	-
Oxygen	8	13.6	30	5.12

1.3.1 Applications of cold plasma

Cold plasma technology involves a combination of fields such as plasma physics, chemistry, and several areas of engineering. Plasma physics and chemistry are required for understanding the processes of transferring energy into a gas state, to produce charged species and their chemical activity and physical interactions. Plasma engineering involves the design and development of plasma systems. All of which have been studied extensively since the 18th century and provide a theoretical support for the underlying physics in the plasma usage food decontamination. Cold plasma has

a wide range of applications in fields such as agriculture, medicine, materials, and environmental. The formation of reactive species is used to modify surfaces, oxidation reactions, and microbial reductions.

The application of cold plasma for microbial decontamination dates back from the early 2000s, including applications in medicine. Since 2005, this technology has been studied extensively for the reduction of microbes in food, where there is a significant increment in scientific publications on the topic.

Cold plasma has been used to change surface properties, including cleaning, activation, and etching. As well as modifying specific characteristic such as surface composition by making them hydrophobic or hydrophilic. It can be used with different materials such as plastics, metals, clothing, ceramics, or food. Cleaning surfaces from lubricant residues, biofilms, or oxide layers, where reactive species break down molecules deposited in the surface and turning them into volatiles.

Food Science

The importance of cold plasma in the field of food science is about the generation of highly reactive at low temperatures, since reactive species can decontaminate the surface of food without changing the physical properties, as it is done with high temperature. Additionally, reactive plasma species can change the chemical structure of nutrients by attaching moieties, breaking bonds, or cross-linking molecules, with the intention to obtain functional ingredients.

Cold plasma has been studied to decontaminate fruits, vegetables, cereals, meat, fish, and dairy products [33]. The mechanism for microbial inactivation using cold plasma is the production of reactive oxygen species (ROS) and reactive nitrogen species (RNS), such as ozone, hydroxyl, nitrous oxide, and others that causes oxidation of lipids, amino acids and nucleic acids [34, 35].

1.3.2 Plasma chemistry

Plasma is a state in constant movement, where electrons collide with atoms and molecules generating unique reactive species. A dielectric barrier discharge atmospheric pressure plasma was studied by modeling the reactions of helium and water (1-3000ppm), and this study described 577 reactions and 46 different species [36]. The generation of plasma species is complex and involve continuous interactions between molecules, atoms, ions and excited species. This process initiates with electron collisions, and it includes reactions as shown in table 1.3.

Atoms and molecules acquire different electronic states by electron collisions, forming excited species (A*). In addition of electronic states, molecules also acquire rotational and vibrational excitation, increasing the complexity of the system. The main process of plasma generation is the formation of ions. Ionization occur when an electron is knocked off from the atom or molecule. Ionization is an inelastic collision, where there is a change in the internal energy state of the atom or molecule.

Reactive species in the plasma field are formed not only from electron collisions, but also by reactions within each other, such as ion-ion, or ion-molecule. The result is a hundred of reactions that occur in seconds, including recombination, neutralization, fragmentation, or polymerization.

1.3.3 Identification of reactive species

Reactive species from air include ROS, RNS, ultraviolet radiation, electric current, energetic ions and other charged species. Species created from oxygen gas have a high oxidation capacity. The main ROS include atomic oxygen, hydroxyl, hydrogen peroxide, and ozone. Molecular oxygen (O(3P)) acquires excited states, O(1S) and O(1D), and dissociates in atomic oxygen. Hydroxyl radical (OH) is formed from water, by electron impact dissociation. Ozone is formed by reactions of molecular and atomic oxygen, and this reaction require an energy of 6-9 eV [39].

Table 1.3.
Reactions associated with the formation of plasma species [37, 38]

Reaction	**Formula**
ELASTIC SCATTERING	
Coulomb scattering	$e + e \rightarrow e + e$
Elastic scattering	$e + A \rightarrow e + A$
Charge exchange	$A^+ + A \rightarrow A + A^+$
ELECTRON ENERGY LOSS SCATTERING	
Vibrational excitation	$e + A \rightarrow e + A$ (v=1...n)
Electron impact excitation	$e + A \rightarrow e + A^*$
Dissociation	$e + A_2 \rightarrow e + A + A$
Metastable excitation	$e + A \rightarrow e + A^*$
ELECTRON AND ION PRODUCTION AND LOSS	
Dissociative recombination	$e + A_2^+ \rightarrow A + A$
Neutralization	$A^- + A^+ \rightarrow A + A$
Dissociative attachment	$e + A_2 \rightarrow A + A^-$
Direct detachment	$A^- + A \rightarrow A + A + e$
Associative detachment	$A^- + A_2{}^* \rightarrow A_3 + e$
Electron impact ionization	$e + A_2 \rightarrow 2e + A_2^+$
Electron impact attachment	$e + A \rightarrow A^-$
Electron impact detachment	$e + A^- \rightarrow A + 2e$
Ion molecule (collisional detachment)	$A^- + B \rightarrow AB + e$
Ion molecule	$A^+ + B \rightarrow A + B^+$
Ion-ion recombination	$A^+ + B^- \rightarrow A + B$
Penning ionization	$A^* \rightarrow A^+ + e$
Radiation	$A^* \rightarrow A + hv$

Molecular nitrogen is dissociated and react with oxygen, forming nitrogen oxides (NO, NO_2, N_2O_3, N_2O_5). Nitrogen oxides reacts with water forming nitric and nitrous acids (HNO_3 and HNO_2), and then decompose into nitrate and nitrite (NO^{-3} and NO^{-2}). These RNS have been attributed also to increase the nitrite content in water, plant extracts, and processed meat [40].

Plasma systems produce short and long lifetime species. The first type are species formed in the volume within the electrodes, known as direct exposure area. These species have an important function to kill microbes. However, it is difficult to identify them because of their short lifespan. Optical emission spectroscopy has been used to identify the wavelength emitted by these species in the range of UV-VIS spectrum, consequently identifying excited species (A*) that emit photons when they return to a lower energy state. For example, in atmospheric air, there are strong lines in the 300-425 nm wavelength range, identified as transitions of second positive system of N_2, NO, and OH.

The long lifetime species can be identified when the source of energy is disconnected. The main long live specie that has been studied is ozone, that can reach levels of 100-10000 ppm [41]. High concentrations of ozone have been detected until 24h after treatment in sealed packages. Detailed examination of long lifetime plasma species studied by Judee et al (2018), showed the quantification of 11 species by colorimetric methods (ammonia, ammonium, orthophosphates, nitrites, nitrates, hydrogen peroxide), titration (carbonate ions), and pH measurement [42].

Nitrogen and hydrogen gas

The applications of cold plasma in Food Science at the moment have been focused in reducing microbial load. For such application, air has been the main gas used because it is necessary to produce ROS and RNS to inactivate microorganisms. On the contrary, in the present study oxidation reactions are avoided. Therefore, the gases that were used include nitrogen, hydrogen, and argon.

Cold plasma has been used to hydrogenate silicon and thin films, where hydrogen gas was the source of atomic hydrogen added into the molecules [43–46]. Hydrogenation of silicon by a surface reaction of atomic hydrogen generated upon non-thermal plasma has been reported in literature [44].

Nitrogen fixation by cold plasma treatment has been studied recently, for applications such as nitrogen fixation, ammonia and nitrate production [47]. The Haber-Bosch reaction is an energy extensive process to generate ammonia. Cold plasma has been investigated as a technology to produce ammonia with a reduced reaction temperature and pressure. The high energy species from nitrogen and hydrogen gas can be used for ammonia synthesis in the presence of magnesia catalyst [48].

Effect of humidity

The role of humidity in the generation of plasma is important, because water molecules dissociate and produce a large number of reactive species 1.4. These species include positive and negative ions, as well as excited states, radical, and neutral species. Also, metastable species that can be detected after plasma treatment. The generation of hydrogen peroxide, hydroxyl radicals, and atomic oxygen has been demonstrated in literature [49]. These species play an important role in microbial decontamination.

Table 1.4.
Reactive species generated from water [36]

Name	**Reactive species**
Positive ions	$H^+, O^+, OH^+, H_2O^+, H_3O^+$
Negative ions	$H^-, O^-, OH^-, H_3O_2^-, H_5O_3^-$
Metastables	$O(1D), O(1S), O_2, OH$
Ground state neutrals	$H, O, H_2, O_2, OH, OH_2, H_2O_2$

1.3.4 Equipment

There are different sources to generate a partially ionized a gas, according to power characteristics and electrode geometries. The common source of energy is electricity for most of plasma systems, with different voltages and applied frequencies. Plasma sources are classified according to electrode configurations as: dielectric barrier discharge, plasma jet, corona discharge and microwave plasma, as shown in Fig. 1.6.

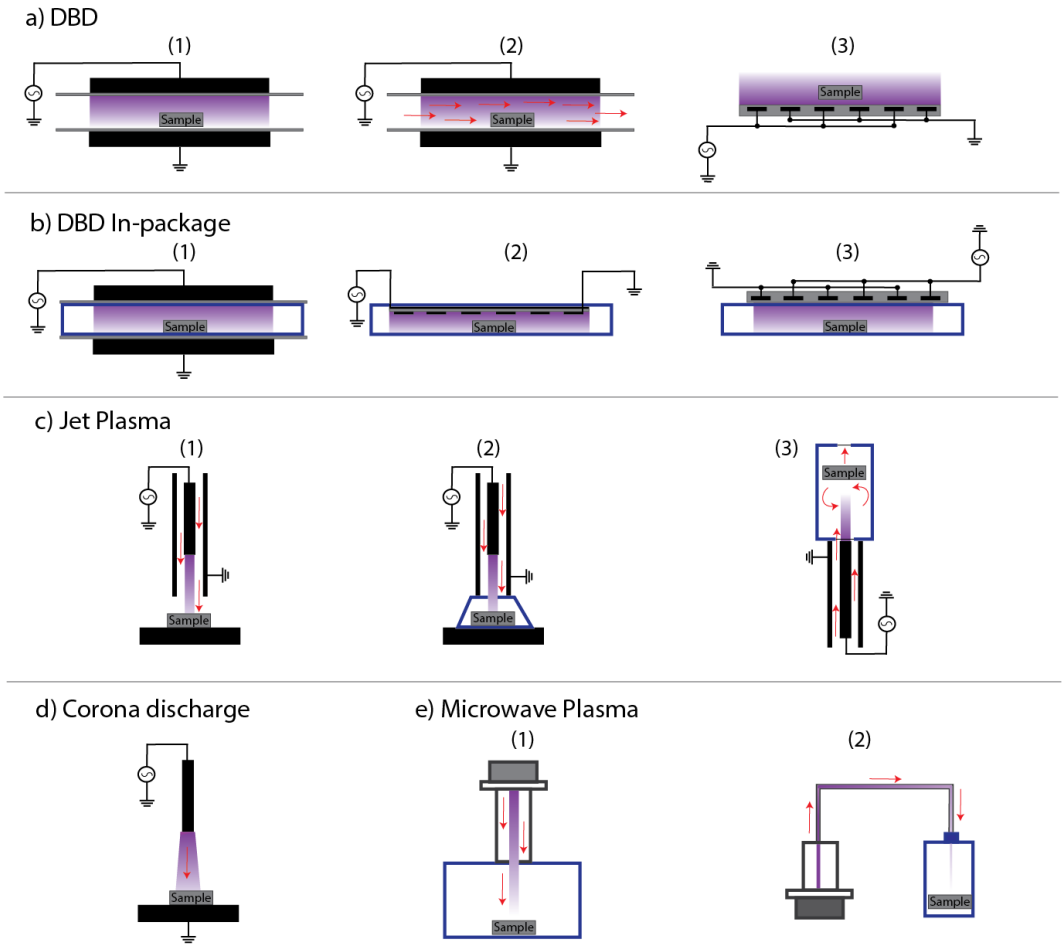

Fig. 1.6. Cold plasma sources

Dielectric barrier discharge

Atmospheric cold plasma is generated mostly with dielectric barrier discharge (DBD) systems, that forms a plasma glow between two electrodes separated by a given distance. The main electrode is connected to the energy source, while the other electrode is grounded. The gas volume between electrodes is submitted to an electrical field, forming a plasma state if the energy surpasses the ignition point. Voltage values reported in literature range from 5-90 kV, and frequency values from 50/60 Hz to 1-60 kHz (low radio frequency).

An important characteristic of DBD is the addition of a dielectric material, which covers one or both electrodes. The function of the dielectrics is to maintain a uniform plasma glow by preventing arc formation and distributing streamers through the surface. Common materials used as dielectric barriers are teflon, plastic, glass, or ceramic.

In-package DBD is a system designed to treat food on its final packaging material. It includes three types of configurations, as shown in Fig. 1.6b. The first setup creates a plasma state between two external electrodes, located at the top and bottom of the package. The second and third setup create a plasma through pairs of electrodes, placed across one side of the package. The difference between them is that the electrode can be embedded in the package or outside the package [33].

Plasma Jet

A plasma jet configuration is characterized by the formation of a plasma through two concentric electrodes, with a gas flowing between them. Different setups applied in food science have been described in literature according to the electrical parameters: voltage and frequency (1kHz -30MHz). Gases used in plasma jet include helium, argon, air, oxygen and nitrogen. The flow rate is used to control the time of exposure of reactive species with the substrate and provide a cooling effect. A plasma jet does not require a dielectric barrier, and it produces a stable and uniform plasma glow.

Different configurations of plasma jet have been reported for food applications (Fig. 1.6c). The first setup includes an open system, where the gas flows through the electrodes and the substrate is exposed to reactive species. The second setup involves the use of a closed container that keeps reactive species inside for a higher exposure with the substrate [50]. The last setup is an inverted configuration, where plasma reactive species are injected in fluidized bed, where the sample is suspended [51]. These configurations evolved to increase the surface exposure of the substrate with to reactive species.

Corona discharge

In a corona discharge configuration, the plasma state is formed between an upper electrode tip and a grounded electrode. It is called corona because of a glowing crown that is formed around the tip. The volume of plasma formed depends on the space between electrodes and the geometry of the grounded electrode. This setup may not be stable, and it can generate streamers and arcs. Therefore, a dielectric barrier discharge was developed to avoid the instability of a corona discharge.

Microwave plasma

Microwave plasma is characterized by a uniform glow produced from a high frequency discharge in the range of RF and microwaves (800 MHz-3 GHz). This is an electrode-free system, that includes a source of energy that generates the microwave field, and a plasma chamber at low or atmospheric pressure [52].

Fig. 1.6e shows describe two types of microwave plasma that have been used in food applications: direct and remote exposure. In the first type, the substrate is exposed directly to plasma species produced by the microwaves [53]. In the second type, plasma species are generated at high temperature, then conducted through a tube to a treatment chamber that receive the plasma reactive species at room temperature [54].

1.4 Cold plasma and lipids

The reactions associated with oils and fats degradation are mainly: oxidation, hydrolysis, and polymerization. These reactions occur during improper storage or processes with high temperatures, resulting in noticeable changes in physical (color, density, viscosity) and sensory properties.

As it was mentioned before, cold plasma has been studied mainly as a technology to reduce microbial load. In this regard, the information available is mostly related to lipid oxidation, where air or oxygen gas mixtures are used to treat food [55]. However, the creation of reactive species that do not involve oxygen have only been studied as a processing aid to create lubricants from vegetable oil polymerization.

Lipid oxidation with cold plasma treatment

Lipids are susceptible to oxidation, particularly in foods with a high content of unsaturated fatty acids, carotenoids, and volatile or essential oils. Herbal spices are rich in lipids that easily oxidize. Atomic oxygen can have two orders of magnitude higher oxidation rate than molecular oxygen, reaching the double bonds of unsaturated fatty acids, which is the target to initiate lipid oxidation [56].

Cold plasma treatment accelerates lipid oxidation of oils and fats rich in double bonds, especially when they are exposed to extended periods of time using oxygen in the plasma chamber. Fish fillets (mackerel or herring) that are rich in unsaturated fatty acids treated with cold plasma showed high levels of oxidation [57,58]. Peroxide values increased up to 37 meq/kg of lipids in 5 min treated mackerel fillets, indicating the formation of primary oxidation products. Tbars increased to 0.53 mg of MDA/kg after cold plasma treatment and 6 weeks of storage, with a detectable off flavor of lipid oxidation.

In contrast, products rich in saturated fatty acids, such as milk, beef, or nuts, are less susceptible to lipid oxidation. A cold plasma treatment of beef and dairy products increases the oxidation rate but without significant changes [59]. Hazelnuts,

peanuts and pistachios treated with cold plasma can reduce the microbial load by 5 $\log_{10}$ of *Aspergillus parasiticus*, and 50% of total aflatoxins [60]. Sensory properties such as color, odor, texture and appearance, were not significantly different after a 20min treatment. Milk fat contain 64% of saturated fatty acids and no changes in lipid composition were detected after a 20 min treatment with a corona discharge at 20 kV [61].

Monitoring oxidation with cold plasma

Oxidation is a parameter often used as a marker of shelf life of food products. Current methods of analysis are criticized because they do not reflect the natural exposure of food to ambient conditions. Accelerated tests are based in the exposure of food to high temperature of storage, producing much higher concentrations of oxidation products, which are not the real conditions of storage. Cold plasma has been studied as an accelerated method of analysis for lipid oxidation at room temperature, using a plasma jet at 6-15kV for 60 min [62–64]. The authors of these studies identified specific aldehydes and ketones that can be used as markers to monitor lipid oxidation. For example, nonanal is an aldehyde that has been identified as a secondary oxidation product of unsaturated fatty acids with 18 carbons, even if they have 1, 2, 3, or 4 double bonds. This aldehyde is formed by the scission of the C9 double bond. The nonanal content for fish oil stored for 11 weeks (natural aging) is 16.8%, with an accelerated test (rancimat, 6h/100°C) it reaches 381.9 μg/g. A cold plasma treatment of 60 min with air/O_2, generate 28.1 μg/g of nonanal [62]. The measurement with cold plasma is similar to a natural aging oxidation, performed in a lower time than the accelerated method. Another experiment was done with oleic acid, which is a fatty acid more stable to oxidation than fish oil. Results of a natural oxidation aging test of oleic acid stored for 15 weeks at room temperature, showed an increase from 0.56 to 17.91 μg/g. In contrast, with a cold plasma treatment of 60 min (Ar/oxygen in Ar atmosphere), it reached 12.88 μg/g [65]. In these studies, the authors concluded that

there is a correlation between natural aging oxidation and cold plasma accelerated oxidation, by the formation of volatiles that are used as markers that may determine shelf life of food products.

Polimerization with cold plasma

Cold plasma has been studied as a processing aid to polymerize vegetable oil to obtain bio-lubricants from renewable sources. Nitrogen gas and air were used to treat soybean oil, in a dielectric barrier discharge equipment with the oil flowing through the electrodes, for a treatment time of 0-9 h at 90°C [66, 67]. Treatment conditions produced an opening of the double bond of unsaturated fatty acids, forming nitrogen cyclic structures and polymers. After a 9h treatment, the percentage of carbon/hydrogen reduced, and nitrogen/oxygen increased. The polymerized oil showed high viscosity, and improved its tribological characteristics.

1.5 Aims of the research

Considering the recently approved regulations pertaining the ban of partially hydrogenated oils, there is a need to develop a PHO replacement with similar fatty acid composition but without the unhealthy *trans* fatty acids. Literature shows that these isomers are formed by an incomplete hydrogenation, and by the effect of high temperature. Cold plasma can accelerate chemical reactions and change chemical structures at room temperature, by creating new materials (deposition), breaking bonds (etching), or forming bonds between molecules (cross-linking).

It is hypothesized that soybean oil in the presence of reactive species may achieve hydrogenation at room temperature and atmospheric pressure without a metal catalyst. Therefore, the overall objective of this project is to explore the feasibility of applying high voltage atmospheric cold plasma to modify the chemical structure of soybean oil by partial hydrogenation, without the formation of trans fatty acids.

Consequently, the objectives of this research are: 1) characterize the effect of HVACP treatment of soybean oil and identify changes in iodine value and fatty acid composition, 2) increase the hydrogenation rate by increasing the amount of hydrogen species available to react with unsaturated fatty acids, 3) identify reactants and products, and propose a plasma hydrogenation reaction mechanism, 4) evaluate the potential of parallel reactions that may occur during HVACP hydrogenation of soybean oil.

2. HIGH VOLTAGE ATMOSPHERIC COLD PLASMA (HVACP) HYDROGENATION OF SOYBEAN OIL WITHOUT TRANS-FATTY ACIDS

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2.1 Abstract

Fats and oils are essential ingredients in the food industry. Partially hydrogenated oils (PHOs) have a high content of trans-fatty acids, which have been linked to cardiovascular diseases. High-voltage Atmospheric Cold Plasma (HVACP) is investigated to produce partially hydrogenated soybean oil without the formation of trans-fatty acids. In this study, soybean oil was exposed to highly energized species from hydrogen and nitrogen. HVACP treatment of soybean oil reduced iodine value from133 to 92 over a 12-h treatment. Such iodine value reductions are similar to the traditional soybean oil hydrogenation process. Saturated fatty acids increased 12%, mono unsaturated increased 4.6%, and unsaturated fatty acids decreased 16.2%. No measurable trans-fatty acids were detected. Atomic hydrogen species are likely responsible for the HVACP hydrogenation, and were identified with optical emission spectroscopy. Through this innovative technology, chemical synthesis of PHOs can be achieved in a non-traditional manner without trans-fatty acids. Industrial relevance: HVACP has been extensively studied to achieve food decontamination using nitrogen, oxygen, and carbon dioxide gas blends. The use of this technology with hydrogen gas demonstrates further possibilities in room temperature chemical synthesis. In this

study, the application of HVACP was aimed to affix hydrogen atoms at the double bonds of unsaturated fatty acids. The advantages of the HVACP technology over the current hydrogenation processes are that the former can be performed with the following conditions: (1) at room temperature, (2) under atmospheric pressure, and (3) catalyst-free. Bakery products, frozen meals, desserts, and several snacks are the most challenging products for reformulations replacing PHOs. This technology is relevant because it may lead to an increase of the use of local vegetable oils, whose unsaturated fatty acid contents can be chemically modified without the formation of trans-fatty acids. Furthermore, this technology could be broadly used to selectively hydrogenate for various industrial purposes, producing new chemical structures without the requirements of heat, pressure, or catalyst. This study is the first study demonstrating an alternative processing technology to traditional catalytic hydrogenation. Additional research is needed to optimize the treatment process, and evaluate the performance of the HVACP partially hydrogenated oil.

2.2 Introduction

In the food industry, fats and oils are an important ingredients to enhance flavor and texture in foods. Vegetable oils are liquids, and characterized by a high content of unsaturated fatty acids between 75-90%. Conversely, fats are comprised mostly of semi-solids and contain high saturated fat content. Saturated fats provide a number of desirable characteristics to food including mouth-feel, oxidation resistance, and thermal stability [1]. In pastry products, such as pie crust, cookies, or biscuits, fats with a high content of saturated fatty acids are preferred. The straight hydrocarbon chains of saturated fatty acids help to interrupt the gluten structure formation. Also, a high melting point allow a dry surface in products such as donuts or croissants [68]. The reduced number of double bonds in saturated hydrocarbon chains make fats less susceptible to oxidation, hydrolysis, or polymerization Patterson2010. The use of highly saturated fats serves to maintain freshness in food products and extend its shelf life.

Hydrogenation is a manufacturing process that allows one to convert a liquid oil into a solid fat [69]. This process can be applied to both food and non-food lipids. For food oils, hydrogenation modifies the oil chemical structure by the incorporation of a hydrogen molecule within the double bonds of the unsaturated fatty acids resulting in a partially hydrogenated oil (PHO). PHOs obtained by the traditional hydrogenation process, use hydrogen gas and a nickel catalyst that react together with the oil under high temperature and pressure. The mechanism of the hydrogenation catalytic reaction is based in the capacity of the catalyst surface to adsorb hydrogen molecules and double bonds of the hydrocarbon chain. Hydrogen molecules approach the metal surface, and break apart in hydrogen atoms. In the other hand, unsaturated double bonds split up in the metal surface. Then, the double bond pick up hydrogen atoms forming a saturated bond [70]. The nickel catalyst is released, remains unchanged after the reaction, and removed at the end of the process. The level of hydrogenation achieved can be controlled, resulting in a PHO with desired functional properties for use in a specific food application [71]. Unfortunately, oil hydrogenation involves triglycerides with polyunsaturated carbon chains which react with hydrogen atoms, and create intermediate states that end up in positional trans- isomers [5]. Trans-fat formation is influenced by temperature, hydrogen pressure, type of catalysts, and mixing efficiency used for hydrogenation [72]. A higher hydrogenation temperature leads to a greater percentage of trans-fat in the oil. The traditional soybean oil hydrogenation process lessens the nutritional value by the formation of undesirable trans- fatty acids up to 45% [9]. Trans-fatty acids are isomers of unsaturated fatty acids, and also non-essential nutrients. They have proven to be a significant risk factor for cardiovascular diseases, raising the “bad” low-density lipoprotein (LDL) and reducing the “good” high-density lipoprotein (HDL) cholesterol levels FDA estimates that 1180-7510 coronary events, and 490-3,120 deaths from heart diseases can be prevented each year by replacement of industrially produced fatty acids with saturated, cis-mono- or poly unsaturated fatty acids [10]. As of June 18th, 2015, the FDA released its final determination to remove PHO from GRAS status. Starting

in June 18th, 2018, it only can be used as a food additive, which requires an FDA petition review and letter of non-rejection [10]. Trans- fatty acids are found naturally in very low quantities in dairy and meat products, but the largest source comes from partially hydrogenated vegetable oils (PHO) that contain 25-45% [5, 6, 10, 73]. Soybean oil has traditionally been the main vegetable oil used for hydrogenation due to its low cost and availability. In 2012, 49% of edible fats and oils were consumed in the U.S. as shortening and margarine, with PHO being its main ingredient (ASA, 2013). One of the main consequences of the updated PHO status, is the reduction of the use of soybean oil by the food industry. In the last 10 years, the domestic utilization of edible soybean oil consumption decreased from 80% to 55% [74]. There are limited domestically produced vegetable oil crops alternatives available to replace PHO. The main alternatives for PHO currently include tropical oils, interesterification, and plant breeding. These alternative oils also contain a high level of saturated fat up to 60% [75] In the early 1900s, a process known as "Voltolization" was investigated to treat oils in working gases such as air, oxygen, helium or nitrogen [76–78]. This process employed electric discharges up to 25 kV to produce high viscosity oils from vegetable, animal, or mineral oil [77]. The original application of this process was not focused in food products and required days of treatment. However, it suggested a potential pathway to achieve hydrogenation of vegetable oils using HVACP treatment. HVACP consists of a dielectric barrier discharge (DBD) system with the distinctive characteristic that maintains the reactive gas species (RGS) inside a sealed package [79]. HVACP is a novel processing technology that is characterized by an environmental friendly process that may produce partially hydrogenated oil without the formation of trans- fatty acids. The use of catalyst and high temperature are associated with the formation of trans- fatty acids [5, 80]. A low temperature process such as HVACP avoid the catalyst absorption/desorption process of molecular hydrogen that leads up to the formation of trans- isomers. The use of plasma to modify the chemical structure of substrates is known by three main reactions: deposition, cross linking, and etching [81]. Hydrogenation is classified as a deposition reaction in which

hydrogen atoms interact with the oil, changing its structure. There is evidence of the use of hydrogen plasma to modify materials surfaces, such as graphene hydrogenation, that transforms it from a conductive material into an insulator [82], or amorphous hydrogenated silicon as a semiconductor using 10-30% hydrogen gas [83, 84] Optical emission spectroscopy (OES) allows for the identification of excited gas species as a function of the emitted energy released when the electrons move from an upper to a lower energy level. The optical emission spectrometer distinguished a wavelength range of 200-1100nm. For hydrogen atoms, this wavelength range includes transitions corresponding to Balmer and Paschen series. The Balmer series established a range from an upper level of n=40 to a lower level of n=2 in a range of 365-656 nm (3.3-1.8eV). The Paschen series established a range from an upper level of n=40 to a lower level of n=3, with a range of 824-1093 nm (1.5-1.1eV) [85].

The objective of this study is to determine if highly energized hydrogen species generated from HVACP treatment of a nitrogen or hydrogen working gas can modify the chemical structure of soybean oil, resulting in a hydrogenated soybean oil without the "undesirable" trans-fatty acids. The effect of HVACP treatment has been investigated using iodine value, fatty acid composition, and viscosity to determine if the treatment increase the saturated fatty acid content and reduce the number of double bonds. Optical emission spectroscopy was used to identify RGS that interact with the oil surface, and therefore provide an insight of the reaction mechanism.

2.3 Materials and Methods

2.3.1 Soybean Oil

Soybean oil Kroger brand was purchased from a local supermarket (Kroger, USA) and stored at room temperature (20-22°C) prior to use. A volume of 10 ml was filled in a plastic (polypropylene) container (104 x 125 x 30 mm). This container was placed inside the plasma chamber, as shown in Fig. 2.1.

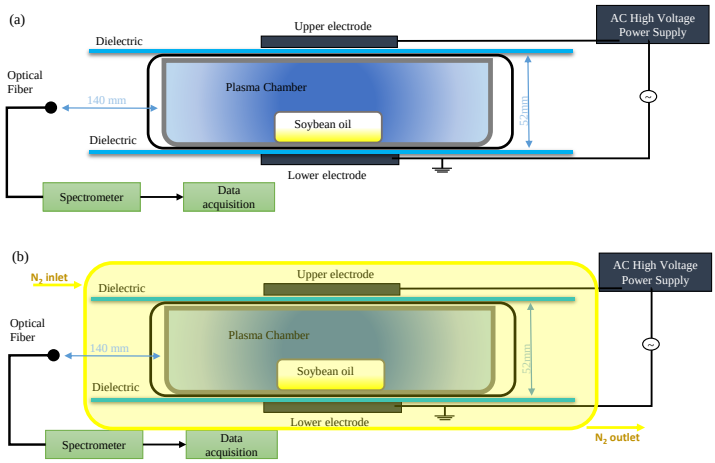


Fig. 2.1. Experimental arrangement of HVACP treatment of soybean oil, for (a) 5% hydrogen/ 95% nitrogen gas blend (NH), and (b) 100% hydrogen (H) modified atmosphere inside the plasma reactor.

2.3.2 HVACP Set Up

The system arrangement is presented in Fig. 2.1. The HVACP system utilized a transformer BK-130 (Phenix Technologies, Accident, MD) with an electrical energy input voltage of 120V (AC) at 60Hz. The BK-130 unit has the capacity to deliver a high voltage output up to 130 kV. A polypropylene plastic container (370 x 355 x 52 mm) was used as the treatment vessel (model 9100AB, Artbin, Middlefield, OH). The container was placed inside a B2630 high barrier film (Cryovac Sealed Air Corporation, NJ) to maintain the gas inside the container. The oil sample was positioned in the center of the container (direct field exposure). The container (volume 6.8 L) inside the B2630 film was then purged with the selected gas using a flow rate of 10 L/min for 3 min and then sealed. Preliminary experiments confirmed this gas flushing process reduced oxygen content to below 0.5% inside the container. Two

circular shaped aluminum electrodes (150 mm diameter) were placed on the top and bottom of the container along with two additional dielectric layers (acrylic sheets 315 x 380 x 6 mm). The two dielectric layers were between the electrode and the container on the upper and lower side to provide stability to the plasma discharge inside the container. The plasma experiments were performed at 90kV for a treatment batches of 2 h, and up to 12 h total with a power setting of 200W. The initial temperature of the oil was room temperature (20-22°C), however after 2 h of treatment it increased maximum to 60°C.

2.3.3 Gas composition inside the plasma chamber

Two gases were used in the experiments, HN gas blend (5% hydrogen / 95% nitrogen) and H gas (100% hydrogen). They were purchased from American Welding Inc., Billings, MT. Hydrogen gas is highly flammable in a concentration above 5% in air [86]. Thus, the HN gas blend was selected to avoid potential of ignitability, especially for treatments that involve treatment periods longer than 2 hours. HVACP treatments times of 2 h showed an increase in temperature up to 60°C. The current set-up was limited to operating temperatures below 80°C, as dielectric materials start to degrade. OES measurements with 100% hydrogen gas were only performed for 90 minutes to identify hydrogen species. The set-up for hydrogen gas included inserting the experimental setup into a 100% nitrogen gas enclosure to maintain the electrodes (located outside of the plasma treatment container) in an oxygen free environment, as shown in Fig. 2.1.

2.3.4 Experimental design

Seven samples of soybean oil were treated with HVACP for 0, 1, 1.5, 2, 4, 6 and 12 hours. The samples were located in direct field exposure, flushed with HN gas, and then treated with HVACP. Direct plasma exposure is where the soybean oil is placed inside the treatment container between the electrodes. All experiments

were replicated three times. Optical emission spectroscopy (OES) measurements were collected on the plasma field produced. Iodine Value (IV), fatty acid composition, and viscosity measurements were collected on the HVACP treated soybean oil samples, and compared to non-treated control samples.

2.3.5 Iodine value

Iodine value was determined according to AOCS official method Cd 1b-87 [87]. Treated and untreated soybean oil samples were maintained in a water bath at 40-45°C for 15 minutes, and mixed vigorously to allow for homogeneous sampling. A sample weight of 0.22-0.26 g was dissolved with cyclohexane (20ml). Standard iodine solution (25ml) according to Wijs 0.1N (Acros organics, Belgium) was added in excess to halogenate double bonds, and stored in darkness for 1 h. The remaining iodine monochloride from Wijs solution was reduced with potassium iodide (20ml) to free iodine and measured by titration with a known concentration of sodium thiosulfate. Iodine value was calculated from the weight of sample and volume of sodium thiosulfate.

2.3.6 Fatty acid composition

Fatty acid composition was determined according to AOAC Official Method 996.06 (AOAC, 2005). Fatty acids methyl esters were obtained by derivatization with a solution of 3M hydrochloric acid in 3N methanol (Sigma-Aldrich, St. Louis, MO) as described by Kiefer [25]. Analysis were performed in a gas chromatograph (Hewlett Packard model 5890, Palo Alto, CA), using a polar ionic liquid column SLB-IL60 30 m x 0.25 mm x 0.20 um (Sigma-Aldrich, St. Louis, MO) with a flame ionization detector (FID) at 260°C. Helium was used as the carrier gas with a flow rate of 1.2 ml/min and a pressure of 60 kPa. The column temperature was programmed from 40°C to 280°C, at a heating rate of 5°C/min. Injection volume was 1ul, with a split mode 100:1. An external fatty acid standard was used to identify components, Supelco 37-component

FAME (fatty acid methyl ester) mix 10 mg/ml (Sigma-Aldrich, St. Louis, MO, USA). Dodecanoic acid (Sigma-Aldrich, St. Louis, MO) was added as an internal fatty acid standard for quantification measurements.

2.3.7 Viscosity

Viscosity measurements were performed in a rotational rheometer (Discovery model HR-3, TA Instruments, New Castle, DE). A sample volume of 1ml was measured at room temperature (25°C), with a plate diameter of 40 mm that provide an operated gap of 1000 um. The reported viscosity values were taken with a shear rate of 5-95s^{-1}.

2.3.8 Optical Emission Spectroscopy

Plasma emission spectra was scanned using a high resolution spectrometer (Ocean Optics, model HR2000+, Dunedin, FL) equipped with a 1000 m optical fiber in the range of 200-1098 nm to monitor UV, VIS, and IR wavelengths. The distance between the optical fiber and the plasma chamber was 140 mm. The OES spectra were corrected for background noise and recorded each 30 sec. for a time-lapse of 90 min for a total of 180 spectra. These were then averaged (six spectra) over each fifteen minute time period. The spectra was recorded for H and the HN gas blend. The latter was recorded with and without a sample of 10ml of soybean oil. The OES peaks were compared and identified with NIST Atomic Spectra Database [85].

2.3.9 Statistical analysis

The data was analyzed using SAS (version 9.3) for Windows (SAS Institute Inc, 2008). Analysis of variances was conducted using GLM procedure. Tukey test was used to determine significance differences at 95% level of confidence ($p<0.05$).

2.4 Results and discussion

Traditional soybean oil hydrogenation using 150-235°C temperature, 1-3 atm pressure, and 0.01-0.08% nickel catalyst requires 1-4 h to achieve a reduction in IV from 130 to 90 [6, 71]. HVACP hydrogenation achieved a reduction in IV from 131 to 92 in 12h treatment (Table 2.1). This reduction in IV is of a similar as the commercial partially hydrogenated soybean oil.

Table 2.1.
Iodine value of soybean oil treated with HVACP at 90 kV on direct field exposure, using a gas blend of 5% hydrogen/ 95% nitrogen (NH) in a container with a gap of 52mm.

Treatment Time (h)	**Iodine Value**
0^{a}	130.9 ± 1.3
$1^{a,b}$	127.4 ± 1.4
$1.5^{a,b}$	127.4 ± 1.4
$2^{b,c}$	124.8 ± 1.4
4^{c}	118.4 ± 1.8
6^{c}	107.5 ± 1.3
12^{d}	91.9 ± 1.6

Changes in fatty acid composition (Fig. 2.2) over treatment time are correlated with the reduction in iodine value. Linolenic (18:3) and linoleic (18:2) acid significantly decrease during treatment time, while oleic (18:1), stearic (18:0) and palmitic (16:0) acid significantly increase. In 2003, Karabulut et al. reported a traditional soybean oil hydrogenation process at 220°C from an IV of 133.6 to 90, that resulted in a reduction of 33% polyunsaturated fatty acids (18:3 and 18:2), formation of 36.8% of trans fatty acids (18:2t and 18:1t), and a small increment of 3.1% of saturated fatty acids [73]. In contrast, HVACP treatment of HN working gas is characterized by a significant, 11.6% increase, in saturated fatty acids without the formation of any trans- fatty acids found in the traditional hydrogenation process. The 2 h HVACP

treatment achieved a 22% of saturated fatty acids with an IV of 124.5, equivalent to a PHO with a saturated fatty acid content of a soybean oil with an IV of 108 using the traditional hydrogenation process [73]. In HVACP treatment total saturated fatty acids content increased to 27.5% and 32.3% after 6 h and 12 h, respectively.

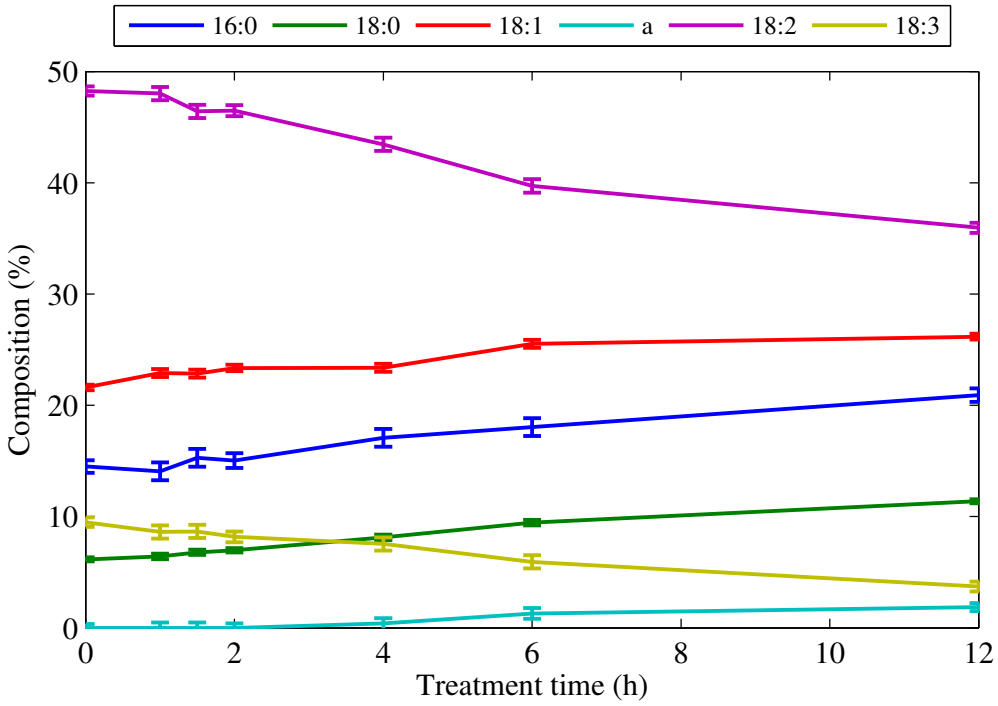


Fig. 2.2. Fatty acid composition of HVACP soybean oil treated at 90 kV with 5% hydrogen/ 95% nitrogen (NH) gas blend

PUFA (polyunsaturated fatty acids) decreases from 57.7% to 41.5%, with a 12 h HVACP treatment. This reduction involve changes of 18:3, from $9.5 \pm 0.4\%$ to $3.7 \pm 0.4\%$, and for 18:2 from $48.2 \pm 0.4\%$ to $36 \pm 0.5\%$, both are significantly less compared to the untreated soybean oil. The reduction of PUFA in a traditional hydrogenation process promotes the formation of trans- fatty acids. Trans- isomers are created from the isomerization of 18:1 and 18:2, as a consequence of high temperature, and the use of nickel catalyst [5]. Note: The nickel catalyst must also be removed after

hydrogenation as it is toxic. In HVACP treatment of soybean oil at room temperature and atmospheric pressure with a HN working gas, o acid (18:2 9t, 12t) and elaidic acid (18:1 9t) were not detected. However, a new peak (a-component) was observed with a $1.9 \pm 0.4\%$ of total oil content with a 12 h HVACP treatment (Fig. 2.3), this is 20 times less than 35-40% of trans- fatty acids reported in a traditional PHO with similar iodine value [6, 73]. Its retention time was between the peaks of 18:1c and 18:2c. It likely is an isomer of 18:2 due to the retention time; however, this component could not be identified within the 37- FAME standard. Elaidic acid (18:1t) elutes before 18:1c. Linolelaidic acid (18:2 9t,12t) elutes at 41.46 min, and a-component at 41.37 min. Isomers such as 18:2 9c,12t or 9t,12c elute between 18:2 9t,12t and 18:2c, and not before, study of this new component is underway.

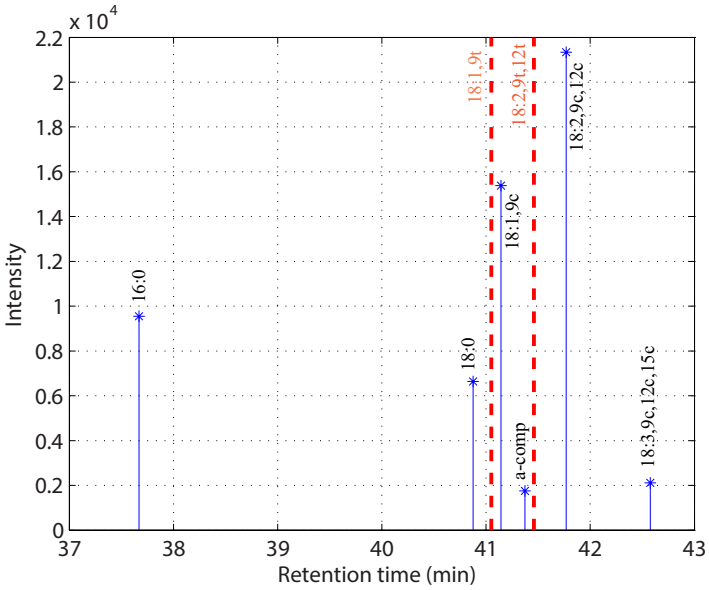


Fig. 2.3. Chromatogram of HVACP treated soybean oil at 90 kV for 12 h.

HVACP treated soybean oil samples treated up to 6 hours exhibited Newtonian behavior with viscosity increasing from 0.05 to 0.23 Pa.s, same as the untreated soybean oil. HVACP treated soybean oil samples treated for 12 h showed a non-Newtonian behavior in which viscosity decreased as the shear rate increased, from

11.7 to 2.4 Pa.s at a shear rate of $5 - 95s^{-1}$, behaving with a Casson model with a R^2=0.9915 (Fig. 2.4). An increment in saturated fatty acid content changes the physical properties of a vegetable oil, increasing its viscosity as a hardening effect similar to a solid fat.

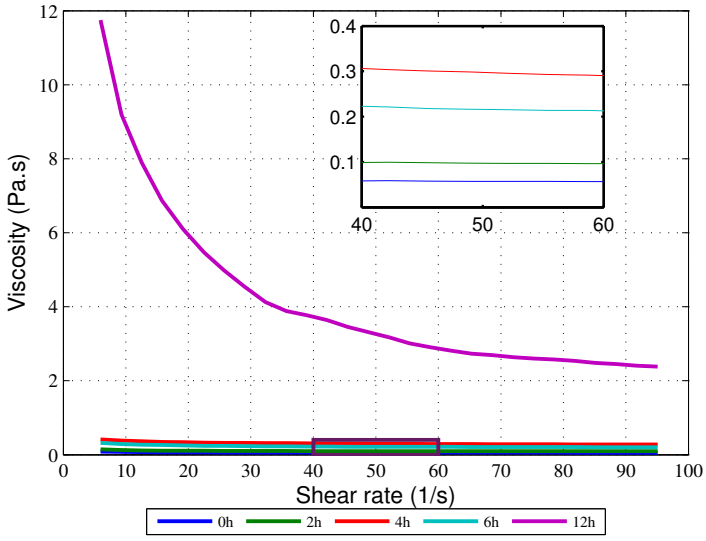


Fig. 2.4. Viscosity of HVACP soybean oil, treated at 90 kV with a gas blend of 5%hydrogen-95%nitrogen in a container with 5.2cm of gap

The characterization of the plasma species are necessary to understand the energy levels required to carry out the HVACP hydrogenation reaction. It is hypothesized that energy levels of hydrogen species increase over time in HVACP treatment. In a plasma reactor, electrons collide with molecules, and they separate into atoms. Hydrogen gas (molecular hydrogen) breaks apart in two hydrogen atoms (atomic hydrogen), by dissociative excitation (2.4) with electron impact [88]. This reaction require 15 eV (cross section of $1.4x10^{-16}cm^2$) [89].

$$H_2 + e \rightarrow e + H + H \tag{2.1}$$

Electron within the hydrogen atom, jump to higher levels, and make species more susceptible to react with the oil surface. OES spectra from HVACP treatment of H gas found hydrogen species with the highest intensity in the range of Balmer and Paschen regions, named A, B, C, D, and E (Table 2.2. The observed species were identified using the NIST atomic spectra database [85].

Table 2.2.
Emission spectra wavelengths and vibrational levels from 100% hydrogen in a HVACP treatment at 90 kV.

	Observed wavelength (nm)	**Attributed wavelength* (nm)**	**Vibrational levels Upper level**	**Lower level**
A	367.54	367.64	22	2
B	398.45	397.00	7	2
C	873.21	875.05	12	3
D	916.64	922.90	9	3
E	1034.5	1004.94	7	3

In a traditional hydrogenation process, the source of hydrogen atoms is obtained by the adsorption of molecular hydrogen in the catalyst surface. The catalyst reduce the activation energy of the reaction by facilitating the contact between the hydrogen atoms and double bonds. Thus, an advantage of HVACP is to shift the electron energy distribution to a higher mean electron energy, and increase the generation rate and concentration of atomic hydrogen. In 2012, Connolly et al described a helium/air plasma treatment similar to HVACP system, using 15 kV at 50 Hz with an electron temperature of 4980 K, and electron density of 1.0 x $10^{17} m^{-3}$ [90]. Andre and co-workers (2001) studied a method to determine the composition of hydrogen and nitrogen plasmas at atmospheric pressure, they established that nitrogen gas dissociate and ionize at 6000-6500 K in a higher rate, in contrast with hydrogen gas

that dissociate and it doesn't ionize until it reach a higher electron temperature [91]. Therefore, hydrogen gas is more difficult to ionize compared to nitrogen gas, due to nitrogen containing a larger number of electrons in the outer shell. As can be seen in Fig. 2.5, where nitrogen species have higher intensity than hydrogen at the same voltage (90 kV). Assuming the same values of electron density (6000-6500 K) for HVACP treatment, it is suspected that hydrogen gas is dissociated into hydrogen atoms, while nitrogen is dissociated and ionized. Hydrogen atoms are therefore available to react with unsaturated fatty acids.

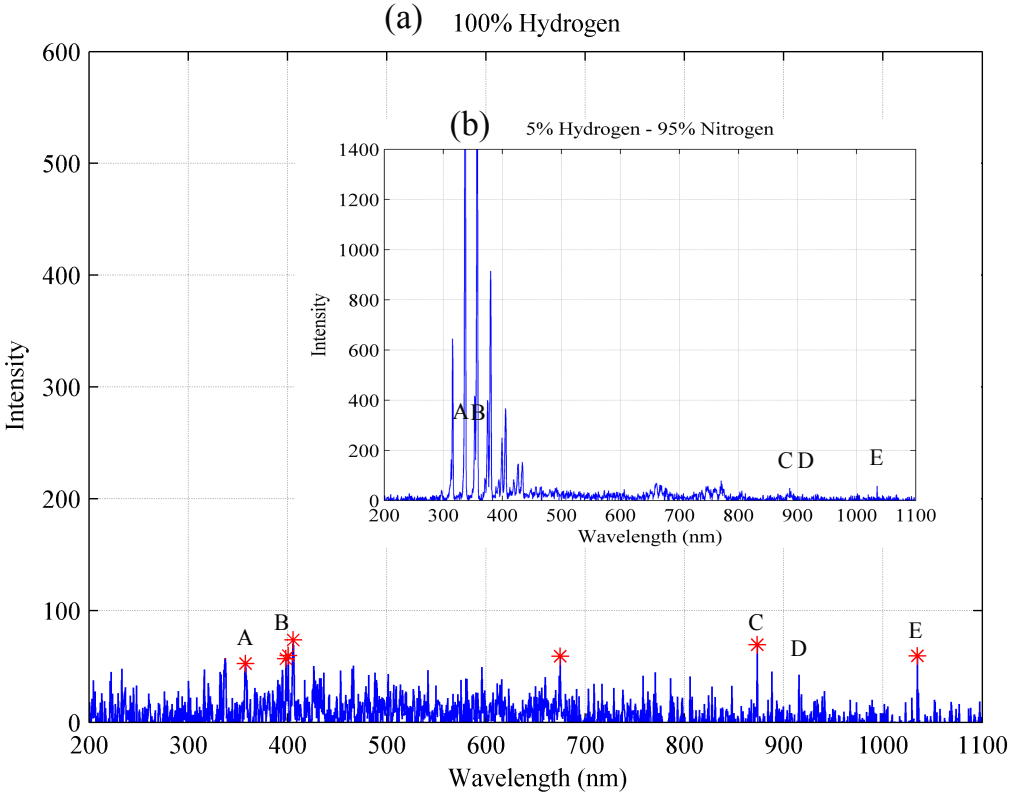


Fig. 2.5. Optical emission spectrum of (a) 100% Hydrogen, and (b) 5% hydrogen/ 95% nitrogen gas blend with HVACP at 90 kV for 90 minutes, no soybean oil sample.

Differences were observed in the OES spectra from HVACP treatment with NH gas blend, when oil was present (Fig. 2.6). Specifically, the presence of soybean oil during HVACP treatment showed a reduction of species A (367.54 nm) and species B (398.45 nm) from 23.9 to 8.2, and 148.1 to 65.1, respectively. This reduction suggests that these atomic hydrogen species were consumed by the HVACP hydrogenation reaction of soybean oil. It is suspected that hydrogen species A and B, have the energy required to modify the double bonds of unsaturated fatty acids, increasing the saturated fatty acids of a HVACP soybean oil.

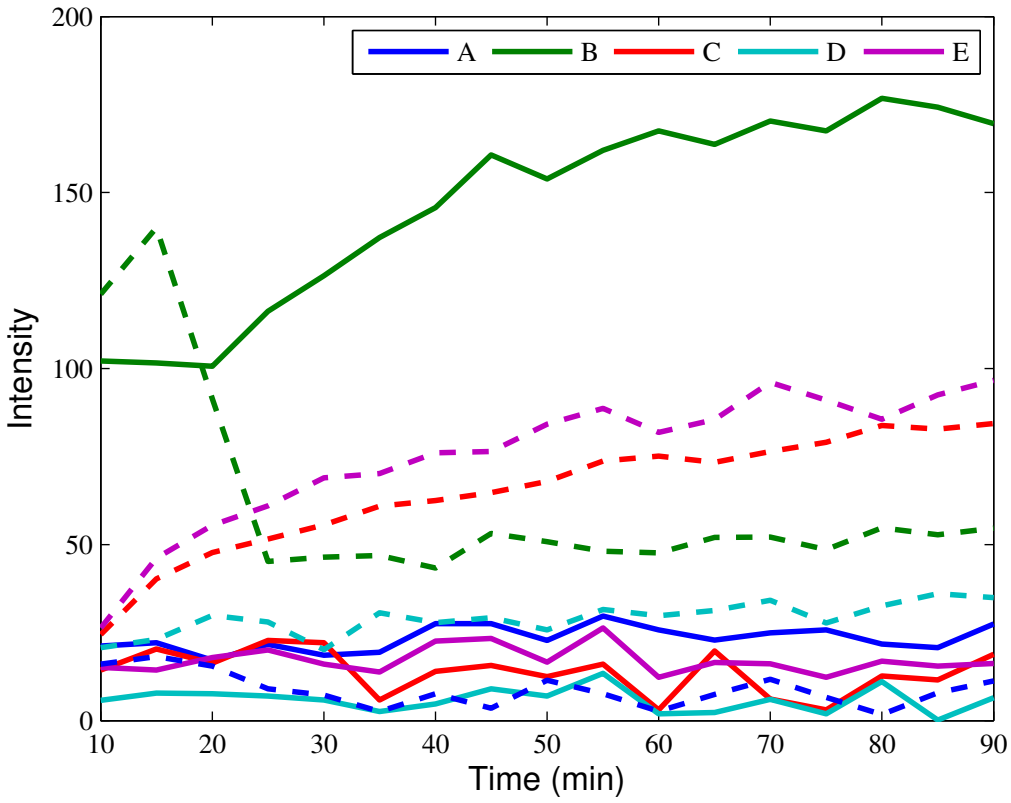


Fig. 2.6. Selected hydrogen species (Table 2) in a NH gas spectra without a sample of soybean oil (solid lines), and with a sample of soybean oil (dashed lines). HVACP treatment at 90 kV for 90 minutes.

2.5 Conclusions

Soybean oil was modified by the action of HVACP within a direct plasma field using a HN working gas blend. Results suggest that atomic hydrogen species are incorporated in the double bonds of unsaturated fatty acids, changing the chemical structure of soybean oil. The high voltage of the plasma field, allow the break-down of molecular hydrogen into atomic hydrogen species. It is proposed that these species selectively populate the C=C double bonds converting them to simple bonds, without the production of trans- fatty acids. The traditional hydrogenation reaction utilizes nickel catalyst in combination with high temperatures and high pressures to separate the molecular hydrogen and allow its absorption in the unsaturated fatty acid. HVACP treatment has the capability to replace traditional hydrogenation with a room temperature, atmospheric pressure, catalyst-free processes that results in a hydrogenated oil comparable to a commercial PHO without trans-fat. This technology is relevant to long-term sustainability of U.S. agriculture and food systems by ensuring an adequate demand for U.S. soybeans.

3. MODIFYING SOYBEAN OIL CHEMISTRY USING HIGH VOLTAGE ATMOSPHERIC COLD PLASMA (HVACP) TREATMENT WITH HYDROGEN AND NITROGEN GAS

3.1 Abstract

Partially hydrogenated oils are not considered safe to use as food ingredients due to its high content of trans- fatty acids. High Voltage Atmospheric Cold Plasma (HVACP) has been investigated as a novel technology to hydrogenate soybean oil without the formation of trans- fatty acids. Soybean oil was exposed to direct HVACP treatment using a modified atmosphere with nitrogen and hydrogen gas at four different ratios. Results showed a decrease in iodine value over treatment time, reaching an iodine value of 122-123 in the liquid fraction, and 90-100 for the solid fraction. The fatty acid composition showed a higher content of saturated fatty acids, and lower unsaturated fatty acids. NMR studies showed a triglyceride chemical structure in both fractions. Plasma species were identified with Optical Emission Spectroscopy. HVACP has the potential to generate highly reactive species that change the oil chemistry, without the formation of trans fatty acids.

3.2 Introduction

Soybean oil is the main vegetable oil in the U.S. with a production of 10.2 million metric tons per year [74]. It is composed mainly with polyunsaturated fatty acids (PUFA), linolenic acid (18:3) and linoleic acid (18:2), at 58-60%. Monounsaturated fatty acid (MUFA): oleic acid (18:1), at 22-24%. The remainder percentage consist of saturated fatty acids (SAT): palmitic acid (16:0) and stearic acid (18:0), at 18-20%.

The hydrocarbons chains of unsaturated fatty acids contain a considerable amount of double bonds, which are more susceptible to modification than simple bonds. The shelf life of soybean oil has been extended by the reduction of PUFA with industrial processes such as partial hydrogenation or inter-esterification [92]. These processes also change the melting behavior, and solidification properties.

The traditional hydrogenation process incorporates hydrogen in the double bonds of unsaturated fatty acids, by bubbling hydrogen gas into the oil with the presence of a metal catalyst, and high temperature. The catalyst is a mediator that increases the solubility of hydrogen gas in oil. It dissociates molecular hydrogen into atoms that react successively with the double bonds, under a temperature range of 200-230C [93]. The composition of a partial hydrogenated soybean oil (PHO) is 8-12% PUFA, and subsequently high content of MUFA and SAT (80-90%). The negative effect of this reaction is the formation of trans- fatty acids, elaidic acid (18:1 9t), and linolenaidic acid (18:2 9t, 12t), reaching 25-45% [7, 10, 73]. In 2015, FDA ruled against the use of PHO as a food ingredient, due to the risk associated with trans-fatty acid consumption [10].

Several approaches had been studied to reduce the amount of trans fatty acids, such as a complete hydrogenation, reducing reaction temperature, or the use super-critical conditions, with few successes [5]. Now, tropical oils such as palm and coconut oil are replacing PHOs. The use of soybean oil in food products has decreased in the last 5 years, while palm oil imports has increased 7 times since 2002, in the U.S. [94].

HVACP is a novel technology that has been studied mostly to reduce the microbial load of food products [95, 96], or modifying the chemical structure of polymers and films [97,98]. However, the generation of highly energized species in a plasma chamber can have further applications, such as accelerate chemical reactions without the use of catalysts.

The objective of this study was to determine if an increased hydrogenation rate will occur by increasing hydrogen gas content in the plasma chamber. Therefore, nitrogen and hydrogen gases at four different ratios were used to treat soybean oil

with HVACP. This treatment is investigated as an alternative technique to catalytic hydrogenation which has been used for decades to obtain partially hydrogenated oil. This technology may have the benefit of hydrogenate unsaturated fatty acids, without the formation of trans- fatty acids. Iodine value, and gas chromatography were used to determine changes in double bonds, and fatty acid composition. Nitrogen content was used to analyze the effect of nitrogen gas. ^{1}H-NMR was used as a tool to analyze the chemical structure of treated samples. Plasma reactive species from nitrogen and hydrogen gas were identified with optical emission spectroscopy.

3.3 Materials and Methods

3.3.1 Experimental arrangement

HVACP treatment is classified as a dielectric barrier discharge (DBD) system. It is comprised by two aluminum parallel electrodes (150mm diameter) located above and below a sealed box (Fig. 3.1). A high voltage 40-130 kV is supplied from a transformer (BK-130 model, Phenix Technologies, Accident, MD), that create an electric field between electrodes, and partially ionize the gas contained between them. The electrical input of the system is 120V (AC) at a frequency of 60Hz. A sample was introduced inside a box (370x355x52mm, model 9100AB, Artbin, Middlefield, OH), or plasma chamber. Then, filled with gas, and sealed with a high barrier film (B2630, Cryovac Sealed Air Corporation, NJ). Two dielectric layers (acrylic sheets, 315 x 380 x 6 mm) were placed at the top, and bottom side of the box. The dielectric layers were used to safeguard a steady plasma state, and avoid electric breakdowns. The experiments were done at 80-90 kV.

3.3.2 Experimental design

Kroger brand Soybean oil was purchased from a local supermarket (Kroger). A sample (5g) was placed inside the plasma chamber, between the electrodes for a direct

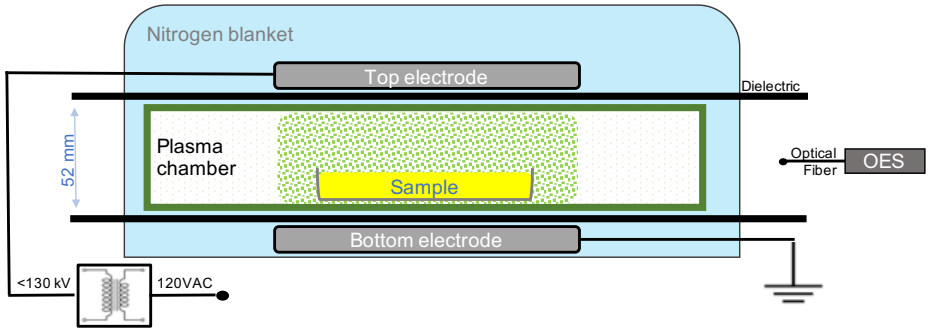


Fig. 3.1. Experimental set up of high voltage atmospheric cold plasma (HVACP).

plasma treatment. Four samples of soybean oil were treated with HVACP for 0, 0.5, 1, and 1.5 hours, for each gas. Experiments were replicated three times.

3.3.3 Hydrogen and Nitrogen gas

The gases used to fill the box (plasma chamber) were: 100% nitrogen (100N), gas blend of 5% hydrogen / 95% nitrogen (5H), gas blend 50% hydrogen / 50% nitrogen (50H), and 100% hydrogen (100H). All gases were 99.995% high purity dry, and purchased from American Welding Inc. (Billings, MT). The box was flushed, and filled with the gases at a flow rate of 10 L/min for 3 min. This step was taken to evacuate the air inside the box, and have a uniform composition in all treatments. 50H and 100H were treated with a special set-up that included a nitrogen blanket (Fig. 3.1) which completely envelopes the electrodes eliminating oxygen, and reduce the risk of flammability of hydrogen gas [86].

3.3.4 Iodine Value

Samples were heated in a water bath at 40-45°C for 15 minutes, and then analyzed according to AOCS official method Cd 1b-87 [87]. Iodine value (IV) of solid samples were calculated with a formula according to AOCS official method Cd 1c-85 [87].

3.3.5 Fatty acid composition

Samples of soybean oil were derivatized to fatty acid methyl esters (FAME) as described by Kiefer [25]. Then analyzed with gas chromatography according to AOAC Official Method 996.06 [99]. A gas chromatograph (Hewlett Packard model 5890, Palo Alto, CA) was used to analyze fatty acid composition, with a polar ionic liquid column SLB-IL60 30 m x 0.25 mm x 0.20 μm (Sigma-Aldrich, St. Louis, MO), and a flame ionization detector (FID) at 260°C. Helium was used as the carrier gas with a flow rate of 1.2 ml/min. The column temperature was programmed from 40°C to 260°C, at a heating rate of 5°C/min. A sample volume of 1μl was injected, with a split mode 100:1. Supelco 37-component FAME mix 10mg/ml (Sigma-Aldrich, St. Louis, MO, USA) was used to identify components, and dodecanoic acid (Sigma-Aldrich, St. Louis, MO) as an internal standard.

3.3.6 Nitrogen content

Nitrogen content was determined according to AOAC Official Method 968.06. The samples were analyzed by an external laboratory, A&L Great Lakes Laboratories (Fort Wayne, IN).

3.3.7 Nuclear Magnetic Resonance

Analysis were performed on a Bruker Avance 500MHz spectrometer. Spectra were acquired at 300K, observing 1H at 500.128 MHz, and processed with Topspin software package version 1.3. NMR samples were prepared by dissolving in approximately 0.6 ml of CDCl3+0.1% of TMS, and transferring the solution to a 5-mm NMR tube for analysis. Spectra were acquired using 16 scans, 32K data points, 90 pulse angle (10.5μs), relaxation delay 1 s, acquisition time 2.47 s, and spectral width 13.2ppm (6613.7 Hz). Spectra were accurately phased, and their baseline adjusted accordingly.

TMS was used as internal standard, and chemical shifts were reported in ppm from TMS (δ=0).

3.3.8 Optical emission spectroscopy (OES)

Analysis were done in a high resolution spectrometer (Ocean Optics, model HR2000+, Dunedin, FL), with a 1000μm optical fiber (200-1098 nm, UV-VIS-IR wavelengths). The detector was located at 140 mm from the box (Fig. 3.1). The OES spectra were corrected for background noise, and recorded each 30 s for a time-lapse of 90 min. The spectra were collected for all gases, and a total of 180 spectra on each gas. OES peaks were compared, and identified with NIST Atomic Spectra Database [85].

3.3.9 Statistical Analysis

Results were analyzed using SAS (version 9.3) for Windows (SAS Institute Inc, 2008). Analysis of variances was conducted using GLM procedure. Tukey test was used to determine significance differences at 95% level of confidence ($p<0.05$).

3.4 Results and discussion

Iodine Value (IV)

IV is a measurement of unsaturation degree, and reductions in IV reflect a decreasing number of double bonds that occur mainly in a hydrogenation reaction, where hydrogen saturate a double bond into a simple bond. Results showed a reduction of IV when samples were exposed to longer treatment times (Fig. 3.2). Reductions of IV at 1h treatment were significantly different in all gas mixtures (122.8-129.2IV) compared with untreated soybean oil (1330.6 IV), but they were not different between each other. Treatments at 1h (129 IV) and 1.5 h (127 IV) with 100H gas, showed less reduction in IV, compared with other gases. Samples treated for 1.5h with nitrogen gas (100N, 5H, 50H) have similar IV, in a range of 122-123. For gas blends 5H and

50H, IV decreased rapidly from 0.5h to 1h. Reductions in IV from HVACP treatment of soybean oil are small compared to a commercial PHO oil that reaches an IV of 80-100. However, it has been shown that HVACP can reach those levels with longer treatment time [100] . Further improvements in HVACP treatment will be needed to reach commercial values of IV in shorter times.

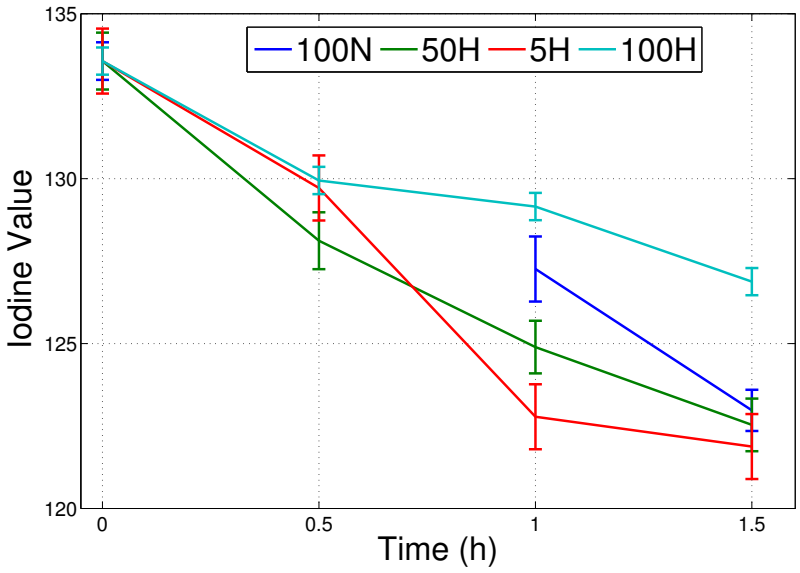
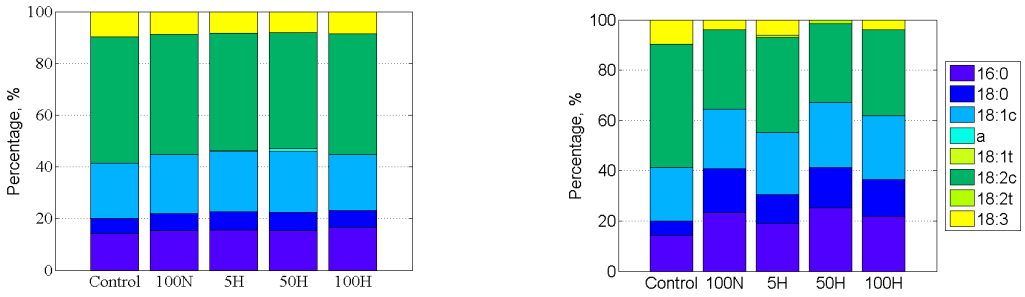


Fig. 3.2. Iodine value of soybean oil treated with HVACP at 80-90 kV on direct field exposure.

Based upon results, the presence of nitrogen and hydrogen species lead to chemical modification of soybean oil. Hydrogenation is the main reaction associated with the reduction of IV. Nevertheless, oxidation and polymerization are also reactions that may lead to a reduction of double bonds. Oxidation create re-arrangements of double bonds, and serial reactions that ends up in the cleavage of hydrocarbons chains. Polymerization occur when two fatty acids are joined by a crosslink, that may occur between double bonds, in this way there is also a reduction of the number of double bonds [15].

Fatty acid composition

Samples exposed to nitrogen and hydrogen gas species change the fatty acid composition of soybean oil. According to results, fatty acids with higher number of double bonds reduce, while saturated fatty acids increase with longer treatment times. Fig. 3.3 shows results of fatty acid composition, for "liquid" (1.5 h treatment), and "solid samples". This "solid samples" was an additional analysis done with a small volume of oil (4-5%), that was solidified at the top of the box. It is possible that the intensity of the electric field was high enough to physically move the sample within the container, and reach the top of it, leaving it more exposed to plasma species. It is known that cold plasma is a surface treatment [101], sample in the surface will interact with highly energized plasma species, and may react in less time than the bulk sample. This "solid samples" was a minimal amount generated for each treatment, and was characterized by a semisolid-gel texture. "solid samples" from 0.5, 1, and 1.5 hours were combined for fatty acid composition testing on each gas/replicate.



(a) Liquid sample.

(b) Solid sample.

Fig. 3.3. Fatty acid composition of liquid and solid samples of soybean oil treated with HVACP for 90 minutes at 90kV.

The fatty acid composition for the solid samples showed the same tendency as the liquid samples, meaning a reduction of PUFA, and an increment in SAT. The IV of solid samples obtained from the fatty acid composition, showed values of 90-100, like a commercial PHO. A notable difference in 18:0 and 18:1 content was found

between traditional PHO, and solid sample. For 18:1, HVACP treatment showed a low increment (2.3-4.4%) for all gases, whereas PHO increases in approx. 26%. On the other hand, HVACP treatment increases 18:0 content by 5.7-11.7%, whereas PHO increases just 1-2%. HVACP treatment may increase the saturated fatty acid content, instead of creating trans- fatty acids as the traditional hydrogenation process. Therefore, the main difference between HVACP soybean oil and PHO is the absence of elaidic acid (18:1-9t).

Trans- fatty acids were not detected in samples treated with 100N and 100H for 1.5h. For samples treated with 5H and 50H, it was found a small amount of linoelaidic acid (18:2 9t,12t), $1.53 \pm 0.53\%$, and $1.02 \pm 0.48\%$, respectively. This amount is low compared to the traditional catalytic hydrogenation, where a PHO with an IV of 90 contains 36.8% trans-fatty acids, mostly as elaidic acid [73]. HVACP treatment under these conditions still produced a greater than 95% reduction in trans-fatty acids. The formation of trans- fatty acids in a traditional, high temperature, catalytic hydrogenation is primarily the result of the absorption and desorption of double bonds in the catalyst surface, acquiring a trans- isomer position. Molecular hydrogen gas is bubbled in the oil, and it is separated into atoms by the effect of the catalyst. These atoms get in contact with the double bonds of unsaturated fatty acids, react together and form intermediate states that not only achieve hydrogenation, but also convert cis- into trans- configurations [5, 6]. This isomerization reaction produces elaidic (18:1, 9t) acid, and linoelaidic acid (18:2t, 9t,12t). HVACP treatment change the fatty acid composition, but there is a small quantity of trans- isomers. Therefore, the mechanism of the reaction between unsaturated FA and hydrogen species allow the formation of a simple bond, without the absorption/desorption process of the catalytic hydrogenation.

It shall be noted that there was a non-soluble fraction in the "solid samples", that corresponds to a 30-40%. The presence of hexane (solvent) insoluble components may be related to the formation of polymers, and this may have a relationship with the increased amount of 16:0. A hydrogenation reaction alone will not change

16:0 composition, because there are no detected unsaturated fatty acids with 16 carbons in untreated soybean oil. A significant difference of 16:0 was detected in solid samples treated with four gases. Zhao and collaborators, studied the effect of cold plasma in soybean oil to obtain a lubricant with improved tribological properties, using nitrogen gas and air [66, 67]. They reported the formation of nitrogen heterocyclic structures, and polymers. Thus, it is possible that unsaturated FA may form polymers by crosslinks between double bonds, that may not be soluble in hexane.

Nitrogen content

Nitrogen gas was used initially as an inert gas to reduce the risk of flammability of hydrogen gas. However, samples treated with nitrogen gas acquired a yellow color, in contrast with samples treated with pure hydrogen gas (Fig. 3.4). Results showed an increased content of nitrogen in samples treated with 100N and 5H gas for 1.5h experiments, however this dissimilarity was not significantly different compared with other samples. Color changes may be attributed to nitrogen fixation. Based upon results, it is suspected that nitrogen species are absorbed by the oil, reacting with double bonds of unsaturated fatty acids [21]. Nitrated fatty acids were not detected with GC or 1H-NMR. Both analysis require the use of a solvent, which are hexane and chloroform, respectively. In both solutions, an insoluble fraction was observed, that may be attributed to nitrated or polymerized compounds. Seemingly, the nitrated compounds are not soluble in these solvents, and therefore not detected by GC or ^{1}H-NMR.

Nuclear Magnetic Resonance

^{1}H-NMR assignments of chemical shifts for soybean oil are appointed with numbers 1-10, as shown in figure 3 [23-26]. These numbers corresponds to: 1) Methyl terminal group, 0.8-0.9; 2) Methyl terminal group for linolenic acid, 0.9-1.0; 3) Methylene protons -CH2-CH2-CH2-, 1.2-1.4; 4) Methylene protons -C(=O)-CH2-CH2-CH2-

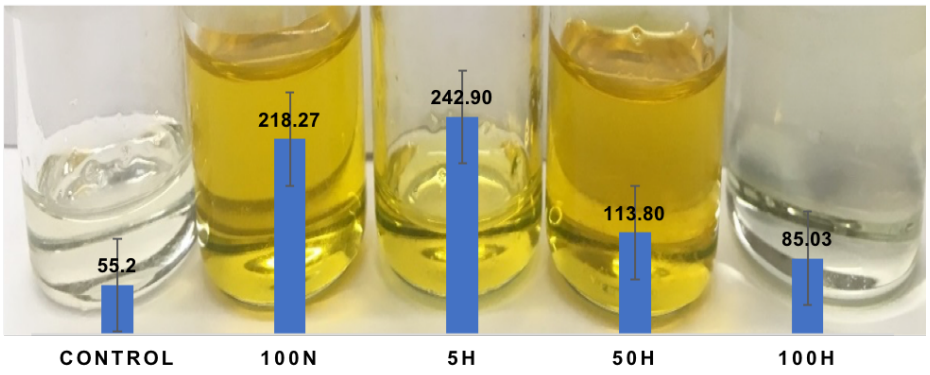


Fig. 3.4. Nitrogen content of samples treated with HVACP for 1.5h at 90 kV (ppm).

1.5-1.7; 5) Methylene protons -CH2-CH2-CH=CH-, 1.9-2.1; 6) Methylene protons -C(=O)-CH2-CH2-CH2-2.2-2.3; 7) Allylic group =CH-CH2-CH=, 2.7-2.9; 8) Glycerol protons n-1 and n-3, doublet: 4.0-4.3; 9) Glycerol protons n-2, 5.2-5.3; 10) Olefinic protons CH2-CH=CH-CH2, 5.3-5.4. Samples treated with HVACP have the same number of peaks as the control. ^{1}H-NMR is a useful tool to monitor a hydrogenation reaction, because peak area have a direct relationship with the relative number of protons [102]. Changes in peak area will determine if a moiety increase/decrease their number of hydrogens/protons. Specific changes in peak area was observed for all treated samples. Results from 1H-NMR showed, a decreasing percentage of peak 10 (olefinic protons) and 7 (allylic protons), and an increasing percentage of peaks 3 (methylene), in all treated samples. These changes may be attributed to a hydrogenation reaction, where unsaturated double bonds change to simple bonds, by hydrogen di-substitution. Peak 6 didn't change, and it is expected because any reaction occurs at the end of the hydrocarbon chain, where the double bonds are located, and not in the methylene group near the glycerol moiety. Peak 2 is specifically related to linolenic acid, for liquid samples peak was reduced, and for solid samples it almost disappears (Fig. 3.5). These results coincide with fatty acid composition, where linoleic acid also reduces with an increased treatment time, and mostly in the "solid samples".

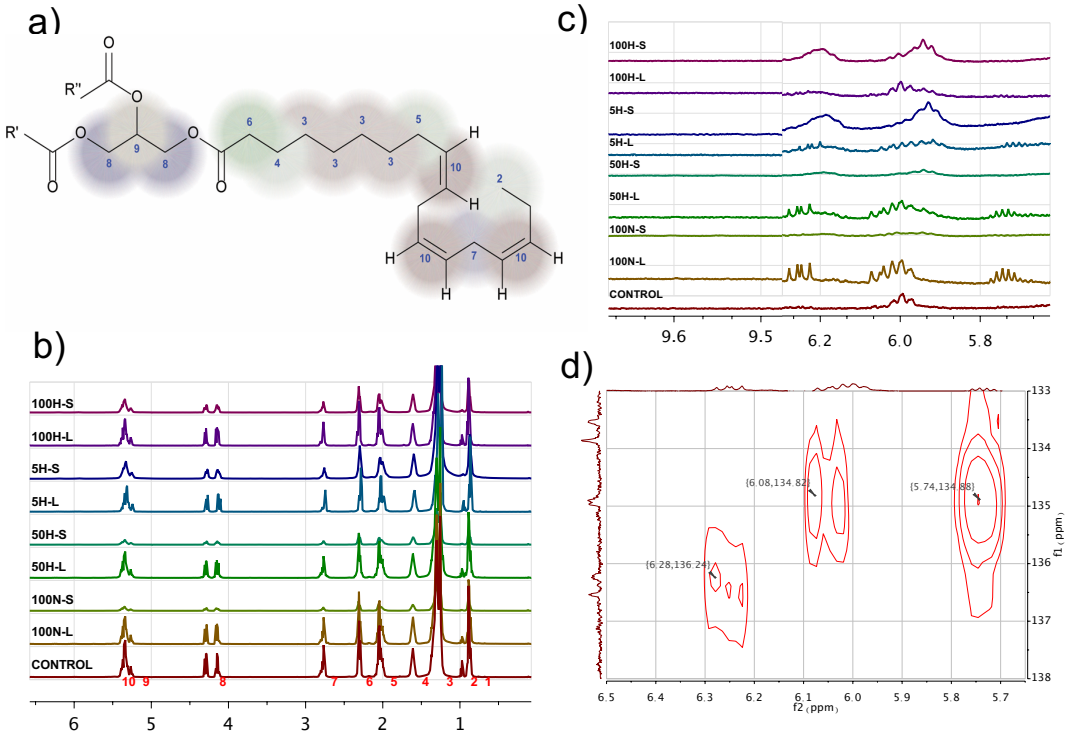


Fig. 3.5. ^{1}H-NMR spectra for soybean oil samples, control and treated with HVACP at 90kV for 1.5h, liquid (L) and solid (S) samples. a) example of a triglyceride structure with fatty acids R' in n-1, R" in n-2, and linolenic acid (18:3) in n-3 position, including numbers for chemical shifts assignments, b) ^{1}H-NMR spectra with chemical shifts assignments (red numbers), c) expansion of the spectra for specific ranges, d) 2D-HMBC spectra for sample treated with 100N for 1.5h

Oxidation products are characterized by the presence of hydroperoxides (chemical shift 5.72, 8.3-8.9), aldehydes (chemical shift 9.5-9.8, fig 3-c), alcohols (chemical shift 3.43-3.62), epoxides (chemical shift 2.63,2.88-2.90, 3.1), or ketones (chemical shift 6.08, 6.82) [28, 29]. These chemical shifts were analyzed in the expanded spectra, and hydroperoxides were identified in the treated samples and not in the untreated. Fig. 3.5c, show the expanded spectra of 5.7-6.3, and 9.4-9.8, to monitor aldehydes,

hydroperoxides, and conjugated double bonds. Peaks x and y are product of HVACP treatment, and showed a higher area for samples treated with high content of nitrogen gas (100N, 5N), and lower for 100H samples. Fig. 3.5c include an expansion of the aldehyde range (9.5-9.7), and there are no peaks related to the formation of aldehydes, which are secondary products of oxidation. According to Martinez and collaborators, peaks x (5.70-5.73) and y (6.23-6.28), correspond to conjugated double bonds associated with hydroperoxides (-CH(OOH)-CH=CH-) [103], that were identified in corn oil samples submitted to thermal treatments. These peaks show that double bonds migrate to a conjugated form, and may form a ring structure that can result in dimerization of triglycerides [104]. The 2D-NMR spectra show a relationship between these 2 moieties and carbons of the double bonds of unsaturated fatty acids (135-138ppm). The glycerol moiety is defined by peaks 8 and 9, with a proton ratio of 4:1. Peak 8 with four protons, and peak 9 with one proton. The 4:1 ratio was observed in untreated soybean oil, and in the "liquid samples". However, the glycerol moiety ratio for the "solid samples" were: 4.2:1.2 for 100N, 4.1:1.2 for 5H, 3.7:1.2 for 50H, and 3.9:1.1 for 100H. The peak area can be affected by changes in the glycerol moiety, and in their surroundings. For example, the formation of di-glycerol and mono-glycerol is characterized by peaks with a chemical shift of 3.72 and 5.08 [105], and may affect this ratio. Nevertheless, peaks of mono- and di-glycerol were not detected in treated samples, indicating that the triglyceride structure of the samples were maintained after HVACP treatment. Changes in the number of protons in the glycerol or nitrogen fixation may appear to cause changes in the glycerol moiety ratio. However, peaks related to nitrated fatty acids were not detected by this analysis.

Treated samples analyzed with ^{1}H-NMR were diluted with deuterated chloroform. An insoluble fraction of the sample was observed when using this solvent, therefore results shown in Fig. 3.5 correspond to the soluble fraction of the sample. As a means to identify the structure of the insoluble fraction, several solvents were tested including: bromo benzene, pyridine, hexane, dimethyl sulfoxide, acetic acid, and

trifluoroacetic acid. Neither of these solvents were successful. Further analysis is required to identify the structure of the insoluble fraction (see chapter 5).

Optical emission spectroscopy

Two main reactions may produce reactive species: dissociative (3.4), and atomic (3.4) excitation [89, 91]. First, molecules may separate into atoms, and then get into an excited state, where electrons jump to higher energy levels, and return to a lower energy level, releasing energy that is emitted as photons, and detected by OES.

$$A_2 + e^- \rightarrow e + A + A \tag{3.1}$$

$$A + e^- \rightarrow e + A^* \tag{3.2}$$

Spectral lines identified in HVACP treatment, from nitrogen and hydrogen gas, are listed in Table 3.1. Excited states of hydrogen are identified as H1-H8. Species H1 through H5 were selected as the highest emission peaks in the 100H spectra. Additionally, H6 through H8 were identified as hydrogen species in the Balmer series, and commonly recognized in literature [106–108]. Molecular hydrogen species (H9) are reported by several authors with a spectral line wavelength of 609 nm [106, 108].

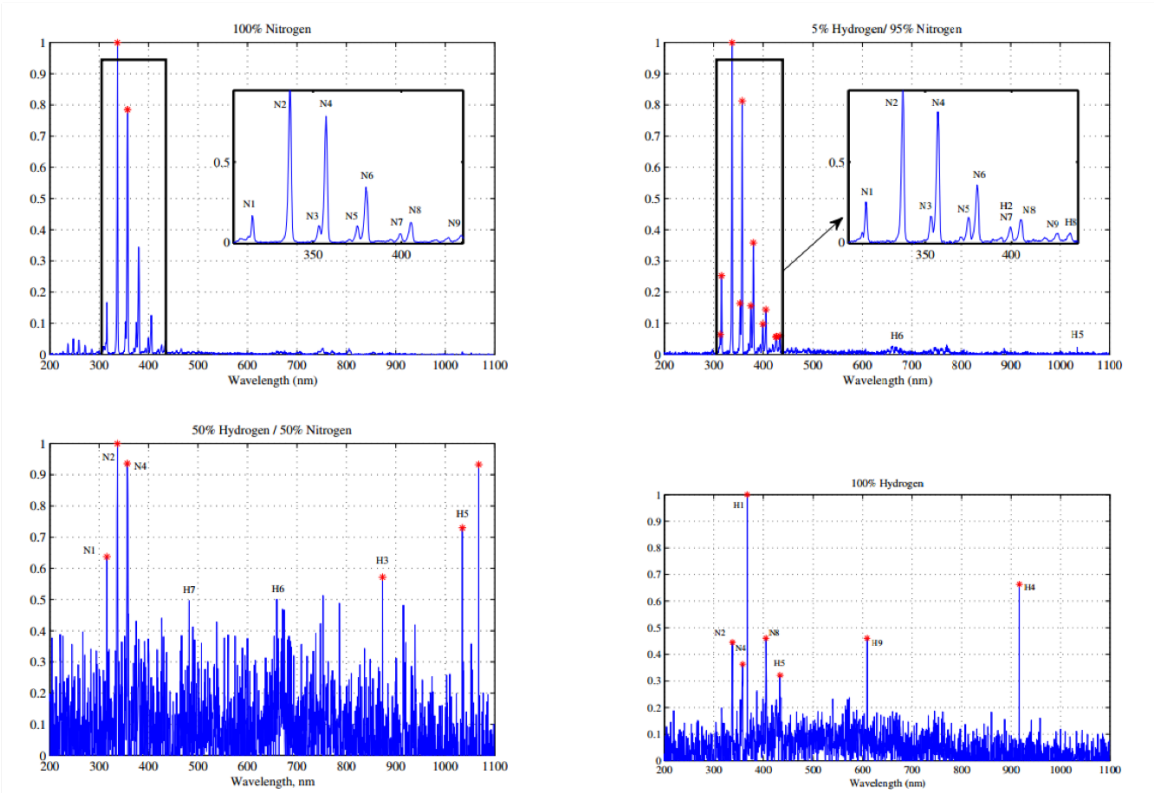
Sharp peaks of nitrogen species were observed in experiments 100N and 5H (Fig. 3.6), and their intensity were constant over 90 min' treatment. Spectral lines N1-N8 in the UV region are commonly associated with the second positive system of the neutral nitrogen molecule [90]. Peaks of N2 and N4 were the most intense species in 100N, 5H, and 50H. They were also detected in 100H treatment, even when apparently, there was no nitrogen inside the plasma chamber. However, their intensity was much lower than in treatments that have a higher content of nitrogen gas. These species may be formed in the nitrogen blanket or as gas impurities.

Nitrogen gas is rich in electrons, which can be observed by the formation of defined excited states detected with OES, in contrast to hydrogen gas which may generate

Table 3.1.
Emission spectra wavelengths and vibrational levels from hydrogen (H) and nitrogen (N) species in a HVACP treatment at 90 kV. * [106, 108] ** [90].

	Observed wavelength (nm)	**Attributed wavelength (nm)**	**Vibrational levels Upper level**	**Lower level**
H1	367.54	367.64	22	2
H2	398.45	397.00	7	2
H3	873.21	875.05	12	3
H4	916.64	922.90	9	3
H5	1034.5	1004.94	7	3
H6 $*H\alpha$	659.54	656	$3d^2D$	$2p^2P^0$
H7 $*H\beta$	481.86	486	$4d^2D$	$2p^2P^0$
H8 $*H\gamma$	433.38	434	$5d^2D$	$2p^2P^0$
H9 $*H_2$	609.00	602	$3p^3\Pi_u$	$2s^3\Sigma_g$
N1 **A	315.64	315.98	$1s^25p$	$1s^26s$
N2 **1	336.99	337.41	$2s2p(^3P^o)3s$	$2s2p(^3P^o)3p$
N3 **B	353.2	352.34	$1s^27p$	$1s^210s$
N4 **2	357.37	359.36	$2s^22p3p$	$2s^22p4s$
N5 **C	374.93	374.75	$1s^22p(^2P^o_3/2)3s$	$1s^22p(^2P^o_3/2)3p$
N6 **3	380.01	379.30	$2s2p(^3P^o)3p$	$2s2p(^3P^o)3d$
N7 **D	399.37	399.50	$2s^22p3s$	$2s^22p3p$
N8 **4	405.35	405.78	$1s^2s3p$	$1s^2s3d$
N9 $**N_2^+$	426	427.81	0	1

less amount of excited states because of the low intensity of hydrogen peaks. A decay in the intensity of the nitrogen peaks was observed with the addition of 5% hydrogen gas (Fig. 3.7), as well as an increment in the current of the system. Therefore,



* Intensity is showed in a normalized scale.

Fig. 3.6. Optical emission spectra for HVACP treatment at 90kV.

adding hydrogen gas possibly increases electron density that may promotes ionization by electron impact collisions [109]. Other possible reactions are dissociation, recombination, or ionization occur with a hydrogen modified atmosphere rather than the formation of excited species.

Optical emission lines are utilized to identify species from nitrogen and hydrogen gas, and at this point they are not applied to quantify and correlate to changes in the oil chemistry, because the peak intensity is susceptible of treatment parameters and spectrophotometer conditions.

Hydrogen species produced at 90kV /52mm are less efficient at modifying the oil chemistry compared with nitrogen species, as seen in results from fatty acid composition, iodine value, and ^{1}H-NMR. The spectra of 5H and 100N, showed higher peaks

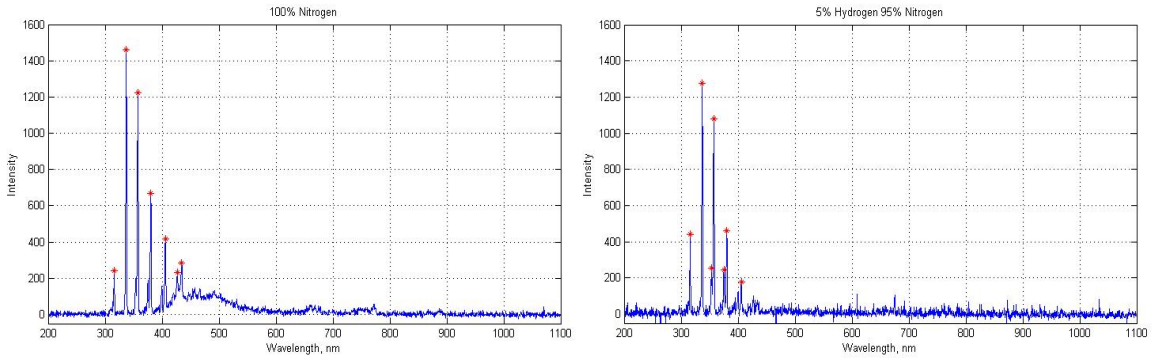


Fig. 3.7. Optical emission spectra for 100N and 5H (intensity in arbitrary units).

intensity than 50H or 100H, which is evidence of the amount of reactive species inside the plasma chamber.

The mechanisms of the cold plasma reactions are not completely understood, however HVACP treatment has a different approach to reach double bonds compared with the catalytic hydrogenation. No catalyst absorption or desorption process occurs in this reaction. Nevertheless, side reactions may occur in the plasma chamber, such as polymerization that requires further analysis. The information reported in this chapter does not provide enough evidence to identify the chemical composition of the insoluble fraction formed with HVACP treatment. It is suspected that this fraction is related with the reduction of PUFA, which is investigated in more detail in chapter 5.

3.5 Conclusions

HVACP treatment is proposed as a processing technology to modify the chemical structure of soybean oil. The application of this technology is focused in replacing catalytic hydrogenation, to produce partially hydrogenated soybean oil without trans-fatty acids, by adding hydrogen atoms produced in a high voltage electric field.

Liquid and solid samples of treated soybean oil were analyzed, reaching an IV of 122-123 and 90-100 IV, respectively. Samples treated with 100H increase the saturated fatty acid content from 20% to 36%, and reducing the PUFA from 58.6% to 38%, without detecting trans- fatty acids. An increased content of nitrogen was found in samples treated with nitrogen gas, but nitrated fatty acids were not detected with GC.

An increased content of hydrogen gas in the plasma chamber did not increase the hydrogenation rate. At the moment, it is not understood the relationship of nitrogen species with the reduction of unsaturated/increment of saturated FA, and this reaction will be analyzed further. ^{1}H-NMR results revealed that soybean oil maintain the triglyceride structure after HVACP treatments. However, the use of HVACP treatment involve complex interactions of reactive species with the oil structure, that may include parallel reactions such as polymerization. Hexane and chloroform were used to dissolve untreated and treated soybean oil for GC and ^{1}H-NMR analysis, respectively. These solvents didn't dissolve completely the treated samples, and a further investigation is suggested to determine the structure of the oil treated with plasma. Further exploration of this reactions will be investigated in more detail in chapter 5.

4. IDENTIFICATION OF REACTIONS OCCURRING UPON HIGH VOLTAGE ATMOSPHERIC PRESSURE COLD PLASMA TREATMENT OF SOYBEAN OIL

4.1 Abstract

High-voltage atmospheric pressure cold plasma (HVACP) treatment has been developed as a processing technology to partially hydrogenate soybean oil. However, the identity of the plasma reactive species and the mechanism(s) of their reactions with liquid soybean oil is (are) not well understood. This study investigates the identities of the reactive species and the mechanism(s) of hydrogenation of soybean oil with different gas compositions, including nitrogen, hydrogen or argon, upon exposure to HVACP. Pure standards of unsaturated fatty acids were used to identify the reaction products and delineate reaction mechanisms. HVACP treatment was demonstrated to decrease the extent of unsaturation in the fatty acids in soybean oil when nitrogen gas was used. Nitrogen gas was also found to hydrogenate linolenic acid more effectively than the other gases, forming oleic and stearic acids. These products were identified by high-resolution tandem mass spectrometry. HVACP is a processing technology that has the promise to be able to hydrogenate vegetable oils without a metal catalyst and at low temperature.

4.2 Introduction

Saturated fatty acids have a linear configuration that allows them to undergo stabilizing non-covalent interactions with each other, forming a semi-solid state at room temperature. Fats and oils rich in saturated fatty acids have suitable properties as food ingredients, such as an adequate mouthfeel, appropriate firmness and soft-

ness, long shelf life, and a pleasing appearance. Unfortunately, the sources of highly saturated oils and fats are limited.

Liquid vegetable oil can be chemically modified to increase its saturated fatty acid content in order to form a semi-solid fat. Catalytic hydrogenation has been employed to incorporate hydrogen into the double bonds of unsaturated fatty acids by bubbling hydrogen gas into the oil in the presence of a metal catalyst under a temperature range of 200-230°C [5, 93]. This reaction reduces the amount of polyunsaturated fatty acids by 30-35% by mass of total fat content, hence increasing the amount of monounsaturated and fully saturated fatty acids [6, 73]. Partially hydrogenated oils (PHO) are oxidative stable and have a higher melting point. These partially hydrogenated oils (PHO) are stable toward oxidation and have a higher melting point than the original oils. However, PHOs are considered unsafe to use as food ingredients due to their high content of trans-fatty acids that can be up to 35-40% by mass [10]. Since 2003, food industries have been slowly replacing PHOs with imported tropical oils, interesterified oils, or genetically modified vegetable oils, and in June 2018, FDA stopped allowing any use of PHOs in food.

Plasma is a partially ionized gaseous state containing reactive species, such as ions, radicals, reactive molecules and atoms, in their ground or excited states, generated upon exposure to a strong electric field [31,110]. Exposure of chemicals to cold plasma has been studied as a technology to accelerate chemical reactions at low temperatures with promising results. It has been used to catalyze reactions such as oxidation of volatile organic compounds [111], ammonia synthesis [112–115], hydrogen generation from methane [116], and other applications [117]. The reactive species generated in plasma interact with a substrate by creating materials (deposition), breaking bonds (etching), or forming bonds between molecules(crosslinking) [118]. These reactions occur at temperatures below 50°C and they can be very fast.

The use of cold plasma technology to hydrogenate unsaturated hydrocarbons have not been reported in literature. High-voltage atmospheric pressure cold plasma (HVACP) is a technology developed at Purdue University [119]. It involves a dielec-

tric barrier discharge system characterized by the formation of reactive species such as ozone or nitrogen oxides in a sealed package or plasma chamber [33]. This technology has been of interest mainly as a microbial decontamination process for fruits, poultry, and cereals, with the advantage of the in-package treatment that reduces the risk of cross contamination. HVACP can achieve 60-90 kV, which is higher than other similar configurations [120].

In this study, HVACP was investigated as a novel technology to modify the fatty acid composition of soybean oil. Previous work has demonstrated that this approach can be used to increase the content of fully saturated and monounsaturated fatty acids in soybean oil by 12% and 30% by weight, respectively [100]. However, the mechanism(s) of the reaction(s) is(are) not well understood. Thus, the objective of this study was to determine the identities of the reactants and products during HVACP treatment when using nitrogen, hydrogen and argon gas. First, soybean oil was treated with HVACP using each of above gases to identify changes in the fatty acid composition by using gas chromatography. Second, pure linolenic acid and trilinolein standards were used as model compounds to characterize the mechanism(s) of the hydrogenation reaction(s) when different gases are used. High-resolution tandem mass spectrometry was used to identify the reactants and products. FTIR was used to identify modifications in the bonds of the standards compounds. The use of plasma as a processing technology is still expensive due to low conversion efficiency and high energy consumption. However, understanding of the reactive species and reaction mechanism(s) is a critical step in an effort to improve this methodology.

4.3 Materials and Methods

4.3.1 Samples

Three samples were treated with high-voltage atmospheric pressure cold plasma (HVACP): (a) soybean oil, (b) linolenic acid, and (c) trilinolein. Refined soybean oil (10 g) from a local supermarket was treated for 4 h with three gases: nitro-

gen (99.995% purity), hydrogen (common grade, 99.8% purity), and argon (common grade, 99.997% purity), by triplicate. An 80-100 mg sample of pure linolenic acid standard (>99% purity, Nu-check, Elysian, MN) was treated with HVACP using nitrogen, hydrogen, and argon gas. The gas atmosphere inside the plasma chamber had a relative humidity level of 22% that corresponds to an absolute water concentration of 4.03 ppm (room temperature 21±1°C) as a result of a 5 min gas flushing. Relative humidity was measured inside the plasma chamber after 10 min of sealing the bag with a hygrometer (Traceable, Fisher Scientific) with a resolution of 1%. In addition, oxygen content after a 5 min flushing was less than 0.3%, measured with a gas analyzer (MOCON Inc., Minneapolis, MN, USA). An 80-100 mg sample of pure trilinolenin (>99% purity, Nu-check, Elysian, MN) was treated with argon gas under low and high humidity. The high humidity set-up was obtained by bubbling argon gas through distilled water before entrance into the plasma chamber, reaching a relative humidity level of 63% inside the plasma chamber, that corresponds to an absolute water concentration of 11.6 ppm (at room temperature).

4.3.2 HVACP Treatment

Samples were introduced into the plasma chamber (polypropylene box with the dimensions 175 x 275 x 44 mm, Artbin, Middlefield, OH) filled with gas at a flow rate 1 L/min, flushed for 4 min and sealed with a high barrier flexible film cryovac bag (Sealed Air Corporation, NJ). The oxygen transmission rate for this film was 1.5-3.5 $cm^3/(m^2 - 24h - 1atm)$ at 5 °C-0% RH. The moisture vapor transmission rate was 0.3-0.6 g/(100 in^2 - 24 h -1 atm) at 38 °C-100% RH. The plasma chamber was exposed to a strong electric field (80 kV, 150 200 W). The energy was supplied by a transformer (BK-130 model, Phenix Technologies, Accident, MD) that converted a source of 60 Hz/120 V AC into 0 - 130 kV energy output. Two polypropylene layers were placed above (4.4 mm thickness) and one polypropylene layer below (2.2

mm thickness) the plasma chamber as additional dielectric barriers. HVACP setup is shown in Fig. 4.1.

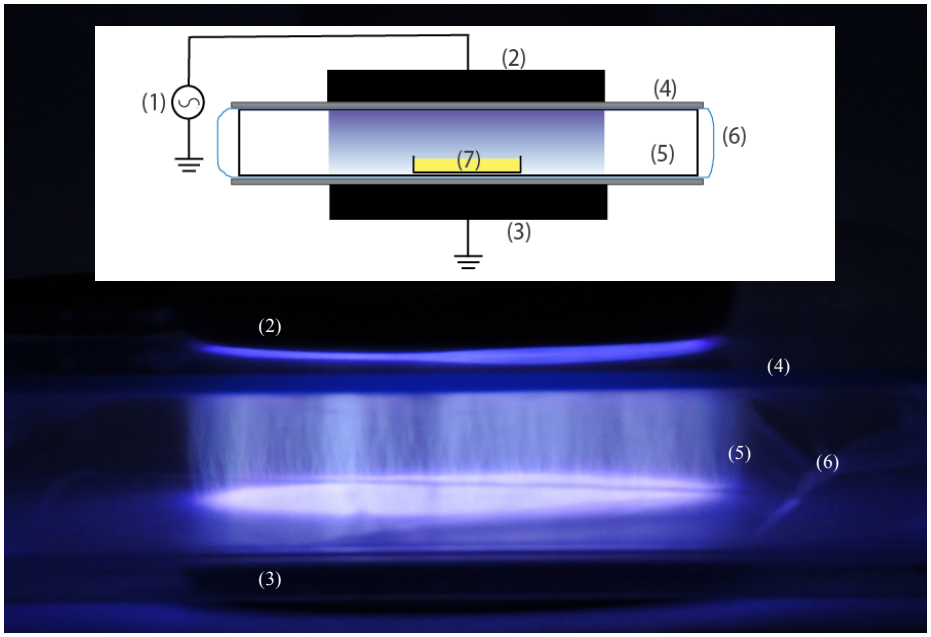


Fig. 4.1. Experimental setup for High Voltage Atmospheric Cold Plasma (HVACP) with argon gas. Source of power (1), main electrode (2), grounded electrode (3), dielectric barriers (4), plasma chamber (5), cryovac bag (6).

4.3.3 Fatty acid composition

Soybean oil was derivatized to fatty acid methyl esters (FAME) as described by Kiefer [25]. The fatty acid composition was measured by gas chromatography with flame ionization detector (Shimadzu, GC-2010, Japan), according to AOAC Official Method 996.06 (AOAC, 2005). Under the following conditions: polar ionic liquid capillary column SLB-IL60 (Sigma-Aldrich, St. Louis, MO) temperature from 150°C to 280°C, at a rate of 5°C/min. Split mode 50:1. A mixture of 37 FAME standards (Supelco 37-component FAME mix, Sigma-Aldrich, St. Louis, MO, USA) was used for

the identification of the fatty acid methyl esters and an internal standard (dodecanoic acid, Sigma-Aldrich, St. Louis, MO) was added for quantification.

4.3.4 Mass spectrometry

A linear quadrupole ion trap mass spectrometer coupled with a high-resolution orbitrap detector (LTQ-Orbitrap XL, Thermo Scientific, Waltham, MA), equipped with an electrospray ion source (ESI), was used to analyze untreated and treated samples of linolenic acid and trilinolenin. Thermo Xcalibur 2.2 software was used to identify HVACP reaction products and determine their molecular weights. Linolenic acid was dissolved into 50:50 acetonitrile/water solution at a concentration of 0.5 mg/mL and then directly injected into the (-)ESI source at a 15 l/min flow rate. Trilinolein was dissolved into 50:50 methanol/chloroform solution at a concentration of 0.5 mg/mL and doped with ammonium formate at 0.1 mg/mL, and then directly injected into the (+)ESI source at a 15 l/min flow rate. ESI source conditions were as follows: spray voltage 4.5 kV, nitrogen sheath gas flow rate: 30 arbitrary units, auxiliary nitrogen gas flow rate: 15 arbitrary units, temperature 250°C.

4.3.5 Fourier transform infrared spectroscopy (FTIR)

A Nicolet 470 spectrophotometer (Thermo Fisher Scientific, MA) equipped with a MCT detector was used to collect FT-IR spectra with a resolution of 4 cm^{-1} by using 128 scans. The samples were deposited directly onto the Attenuated Total Reflectance (ATR) crystal, where the sample formed a thin film to cover the surface. Duplicate absorbance mode spectra were recorded from 4000 down to 800 cm^{-1} and processed with Omnic software.

4.3.6 Statistical analysis

The fatty acid composition results obtained for soybean oil were subjected to statistical analysis. Significant differences were detected among mean values with Duncan multiple range test, using SAS 9.4 software with a confidence level at $P < 0.05$. Mean values and standard errors are reported.

4.4 Results and discussion

The in-package configuration of HVACP treatment allows the use of any type of gas inside the plasma chamber. In this study, nitrogen, hydrogen, and argon gas were used to treat samples. Hydrogen gas is flammable. For this reason, a nitrogen gas blanket was created around the electrodes in order to isolate them from air. The electrodes were separated by a gap of 44 mm, and a voltage of 80 kV was used for all experiments. Therefore, the current and power depend on the gas. For nitrogen, hydrogen, and argon gas, the current was 0.40-0.45 mA, 0.60-0.64 mA, and 0.70-0.78 mA, respectively. A uniform glow with random microdischarges was observed during treatment, as shown in Fig. 4.1. The microdischarges were connected between the electrodes, and they are weakly ionized channels with a high current that may form sparks. For this reason, the dielectric layers allow to maintain a uniform plasma and thereby avoiding the formation of a spark breakdown [31].

4.4.1 Soybean oil

Table 4.1 shows the fatty acid composition and iodine and peroxide values measured for untreated soybean oil and soybean oil exposed to HVACP under different conditions. These results demonstrate that the fatty acid composition changed upon all the treatments. A reduction of iodine value was observed for all treated samples. These values are in the range of 121-127, with the nitrogen treated sample having the lowest value. A commercial partially hydrogenated oil has an iodine value between

80-90, from soybean oil treated at 180-230°C, with nickel as a catalyst and hydrogen gas [6,73]. A further treatment with HVACP can reach these numbers [121]. However, in this study, a 4 h treatment of a 10 g sample was performed to identify statistically significant differences between untreated and treated soybean oils and to identify any differences caused by the different gases.

Table 4.1.
Fatty acid composition (% by mass) of soybean oil exposed to HVACP for 4 h at 80 kV while using nitrogen, hydrogen, and argon gas. Monounsaturated fatty acids (MUFA), polyunsaturated fatty acids (PUFA), total trans represent the sum of C18:1-9t and C18:2-9t,12t.

Sample	**Untreated**	**Nitrogen**	**Hydrogen**	**Argon**
C16:0	12.6 ± 0.2^{a}	14.3 ± 0.2^{b}	13.2 ± 0.2^{a}	14.0 ± 0.1^{b}
C18:0	5.2 ± 0.3^{a}	7.5 ± 0.5^{b}	5.7 ± 0.4^{b}	5.4 ± 0.3^{a}
C18:1-9t	0^{a}	1.6 ± 0.1^{b}	0 ± 0.1^{a}	0 ± 0.1^{a}
C18:1-9c	20.3 ± 0.2^{a}	20.7 ± 0.4^{ab}	22.0 ± 0.3^{c}	21.8 ± 0.2^{bc}
C18:1-11c	1.6 ± 0.2^{a}	1.8 ± 0.3^{a}	1.0 ± 0.2^{a}	1.5 ± 0.2^{a}
C18:2-9t,12t	0 ± 0.1^{a}	0 ± 0.1^{a}	0.3 ± 0.1^{a}	0 ± 0.1^{a}
C18:2-9c,12c	51.4 ± 0.6^{a}	45.9 ± 1.0^{b}	50.4 ± 0.8^{a}	50.0 ± 0.6^{a}
C18:3-9c,12c,15c	9.0 ± 0.3^{a}	8.2 ± 0.5^{ab}	7.4 ± 0.4^{ab}	7.2 ± 0.3^{b}
Iodine Value	131.3 ± 0.7^{a}	121.7 ± 1.0^{c}	126.9 ± 0.9^{b}	125.6 ± 0.6^{b}
Saturated	17.8	21.8	19.0	19.4
MUFA	21.8	24.1	23.0	23.4
PUFA	60.4	54.1	58.1	57.2
Total trans	0	1.6	0.3	0
Peroxide Value (meq/kg)	0.73 ± 0.46^{a}	3.97 ± 0.46^{b}	2.18 ± 0.46^{ab}	3.90 ± 0.46^{b}

Values represent mean ± standard error of measurements made on three replicates. Values within a row followed by the same superscript letter do not differ significantly ($p>0.05$)

Changes in fatty acid composition included a reduction of the amount of the original unsaturated fatty acids, and an increased amount of monounsaturated or

completely saturated fatty acids for the nitrogen treated sample. Nitrogen treated soybean oil contained increased amounts of saturated (4% by mass) and monounsaturated fatty acids (2.3% by mass), but reduced amounts of polyunsaturated fatty acids (6.3% by mass). Hydrogen treated samples contain the same amount of saturated fatty acids as the untreated soybean oil. However, they contained somewhat more oleic acid (1.7%) and slightly less linolenic acid (1.6%). Argon treated samples showed a similar composition as hydrogen treated samples. These results suggest that nitrogen gas is the most effective gas in HVACP treatment.

4.4.2 Pure standards

Linolenic acid (C18:3) and trilinolein were used as model compounds to identify the reaction products and delineate reaction mechanisms for HVACP treatment because the multiple double bonds in these compounds are susceptible to reactive species. The rate of catalytic hydrogenation of linolenic acid is known as 3 times higher than linoleic (C18:2), and 16-30 faster than oleic (C18:1) acid [1, 122]. The content of linolenic acid (C18:3) in soybean oil is 8-9%, and it nearly disappear in a catalytic hydrogenation [15, 73]. Similarly, the oxidation rate of linolenic acid (C18:3) is 14 times faster than linoleic acid (C18:2), and 100 faster than oleic acid (C18:1) [15].

Samples of linolenic acid (C18:3) were exposed for 1 h to HVACP at 80 kV with nitrogen, hydrogen, and argon gas. The products were analyzed with ESI MS (Fig. 4.2). Deprotonated linolenic acid of m/z 277 $[M-H]^-$ was the most abundant ion observed for all treated samples. Ions of m/z 279 ($[M+2H-H]^-$), m/z 281 ($[M+4H-H]^-$), and m/z 283 ($[M+6H-H]^-$) indicate the addition of 2, 4, and 6 hydrogen atoms into linolenic acid. These ions correspond to ionized linoleic (C18:2), oleic (C18:1), and stearic (C18:0) acid, respectively. The relative abundances of these ions are shown in Table 4.2. An increase in the relative abundance of linoleic acid (C18:2) was observed for all treated samples, being the largest for nitrogen, then argon, and the least for hydrogen treated samples. The nitrogen treated sample showed a relative

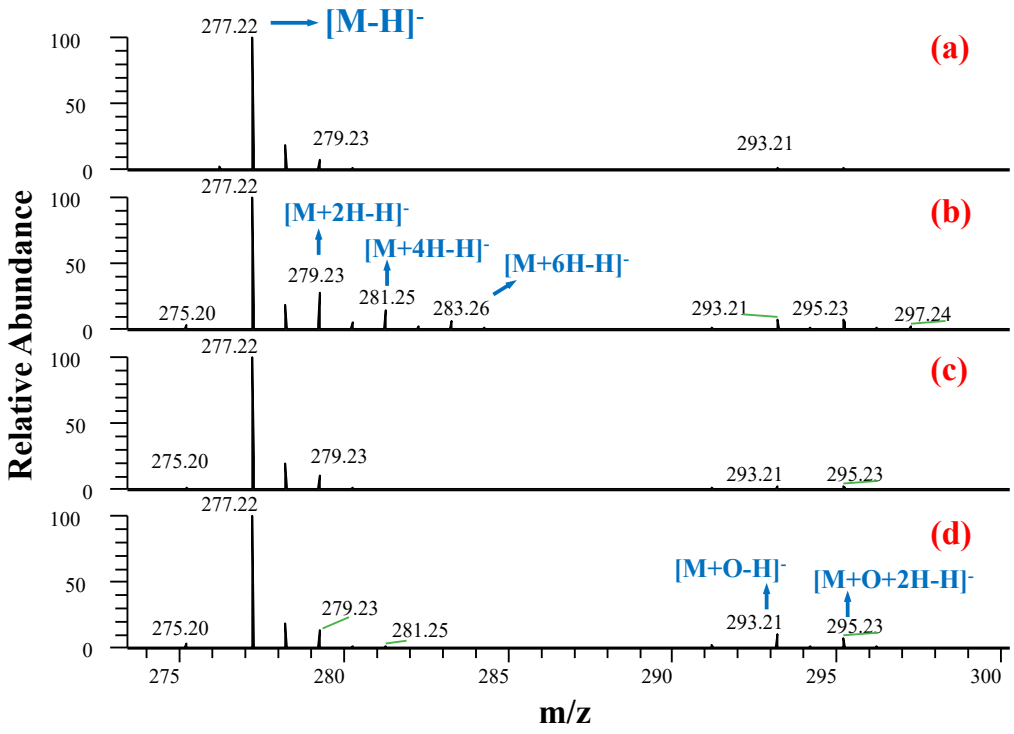


Fig. 4.2. Negative mode ESI mass spectra for (a) untreated linoleic acid, and linoleic acid exposed to HVACP for 1 h at 80 kV when using (b) nitrogen, (c) hydrogen, and (d) argon gas.

abundance of 15.0% and 6.3% for oleic (C18:1) and stearic (C18:0) acid, respectively, compared to the relative abundances of these compounds being below 1% for the untreated sample. These results suggest that hydrogenation reactions had occurred at the double bonds of linolenic acid (C18:3). However, this is not a conclusive evidence for hydrogenation, because the treated sample had an insoluble material that may remove part of linolenic acid (C18:3) from the sample and may cause an increment of the linoleic acid (C18:2) peak intensity.

At the same time, it is important to highlight that the untreated sample does not show peaks of oleic and stearic acid, with m/z of 281.25 and 283.26 respectively. This is an important point to demonstrate that HVACP treatment generates hydrogen

atoms that hydrogenate the double bonds of unsaturated fatty acids. Under these circumstances, the pi-electrons of the double bond that are loosely bound to the carbon are pulled away, and two hydrogen atoms are absorbed into the carbon-carbon. However, the source of atomic hydrogen may not come from hydrogen gas, because hydrogen treated samples do not show the presence of peaks corresponding to oleic or stearic acid.

Table 4.2.
Relative abundances of ions derived from compounds in untreated linolenic acid and linolenic acid exposed for 1 h to HVACP at 80 kV when using nitrogen, hydrogen, and argon gas.

m/z	Compound	Untreated	Nitrogen	Hydrogen	Argon
275.22	C18:4	0.6	3.3	1.1	3.4
277.22	C18:3	100	100	100	100
279.23	C18:2	7.8	27.5	10.5	13.3
281.25	C18:1	0.9	15.0	0.9	2.0
283.26	C18:0	0.2	6.3	0.2	0.5
295.23	$C_{18}H_{30}O_3$	1.3	7.4	2.2	8.0
297.24	$C_{18}H_{32}O_3$	0.2	2.1	0.3	0.9

HVACP treatment also led to the addition of oxygen to linolenic acid as indicated by the observation of ions of m/z 293.21, 295.23 and 297.24, corresponding to $[M+O-H]^-$, $[M+O+2H-H]^-$ and $[M+O+4H-H]^-$, respectively. The $[M+O-H]^-$ ion had the highest relative abundance for a sample treated with argon (Fig. 4.2). Reactive oxygen species can be formed from impurities such as oxygen and water from gases, materials, or sealing method. Oxygen content in the plasma chamber before treatment was below 0.3%, however the oxygen transmission rate of the bag can be enhanced by the oxygen partial pressure difference between the inside and outside of the chamber over treatment. The relative humidity values inside the sealed plasma chamber are below 4 ppm, and it is difficult to reduce this value even with a longer flushing time

because water molecules got absorbed in materials and filtrate during the sealing step. In fact, previous work showed the emission of hydroxyl radicals (305-313 nm) and atomic oxygen (775-778 nm) in the spectra of nitrogen and hydrogen gas with a HVACP treatment at 80 kV [49, 100]. The addition of oxygen by opening the double bond is attributed to an epoxidation. The mechanism of formation of epoxides may involve the direct addition of oxygen formed by electron impact dissociation. Another proposed pathway of oxygen addition involve the formation of a peracid, by a reaction between hydrogen peroxide and a carboxylic group [21, 123], as shown in figure A.2.

Changes in FTIR signal intensities confirm that reactions occurred upon HVACP treatment (Fig. 4.3). The major peaks in FTIR spectra for a fatty acid are due to the presence of carbon-carbon double bonds (stretching of aliphatic alkenes, 3010 cm^{-1}), carbon-carbon simple bonds (stretching of aliphatic alkanes 2930 and 2830 cm^{-1}), and carbonyl group (C=O stretching, 1746 cm^{-1}) [124]. FTIR spectra measured for treated linolenic acid show a reduced signal at 3010 cm^{-1} for all gases, and an increased signal at 2930 and 2830 cm^{-1} for linolenic acid treated with argon and hydrogen. Other peaks observed are: 1239 cm^{-1} as ester antisymmetric stretch, 1378 cm^{-1} as methyl symmetric deformation, 1463 cm^{-1} methyl antisymmetric deformation. As well as the epoxy group that corresponds to 862 cm^{-1}, which it is enhanced for nitrogen and argon treated samples (Fig. 4.3).

FTIR spectra of nitrogen treated linolenic acid show peaks indicative of an amine (amine I band:1630 cm^{-1}; amine II band: 1552 cm^{-1}) as shown in Fig. 4.3 [66]. This finding suggests that nitrogen has been incorporated into linolenic acid upon the treatment, and it is in agreement with a literature report wherein formation of fatty amines (-CH2-CH(NH2)-CH2) was observed when treating soybean oil with nitrogen plasma to produce lubricants. Therefore, nitrogen gas treatment may result in addition of N into the fatty acid in spite of MS results that did not indicate the presence of nitrogen compounds. However, a fraction of the treated sample did not dissolve completely in hexane, acetonitrile, chloroform, or methanol, which prevented

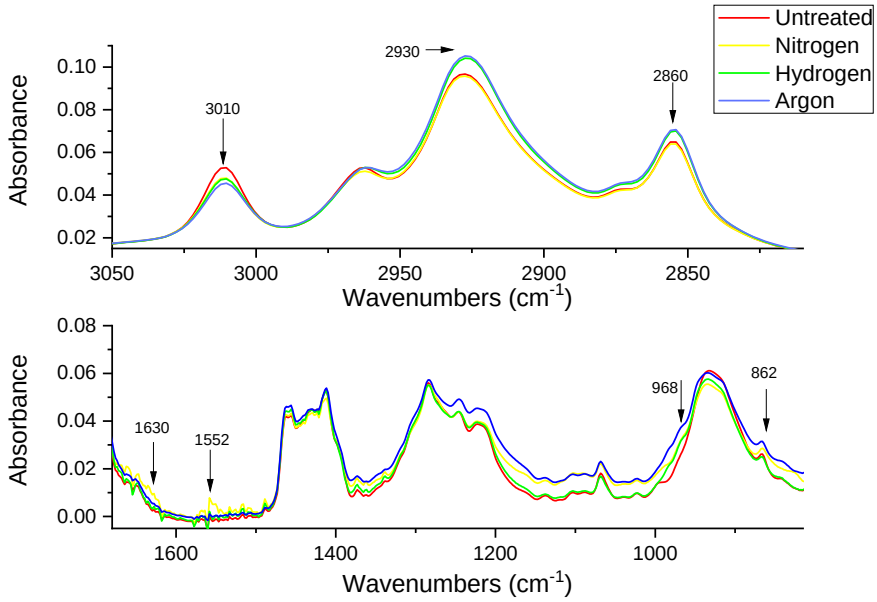


Fig. 4.3. FTIR spectra of untreated linolenic acid and linolenic acid exposed to HVACP when using nitrogen, hydrogen, and argon gas.

the MS analysis of this fraction. The nitrogen compounds may have remained in the insoluble fraction.

It is interesting that similar changes in the structure of linolenic acid were detected when using either nitrogen or argon gas, suggesting that hydrogen atoms may come from another source than the gas in the plasma chamber. It is proposed that the hydrogen atoms may originate from: 1) inter-molecular rearrangement or 2) from water dissociated in the plasma chamber.

Some support for the first hypothesis is provided by Fig. 4.2 that shows MS data for linolenic acid. The mass spectra measured for the treated samples have a small peak corresponding to an ion of m/z 275.20, which was not detected in the untreated sample. This ion corresponds to a compound with four double bonds (C18:4), i.e., linolenic acid that has lost two hydrogen atoms to generate an extra double bond.

In order to explore the effects of water on the plasma hydrogenation reactions, trilinolenin was treated in the presence of dry and humid argon (for details of these

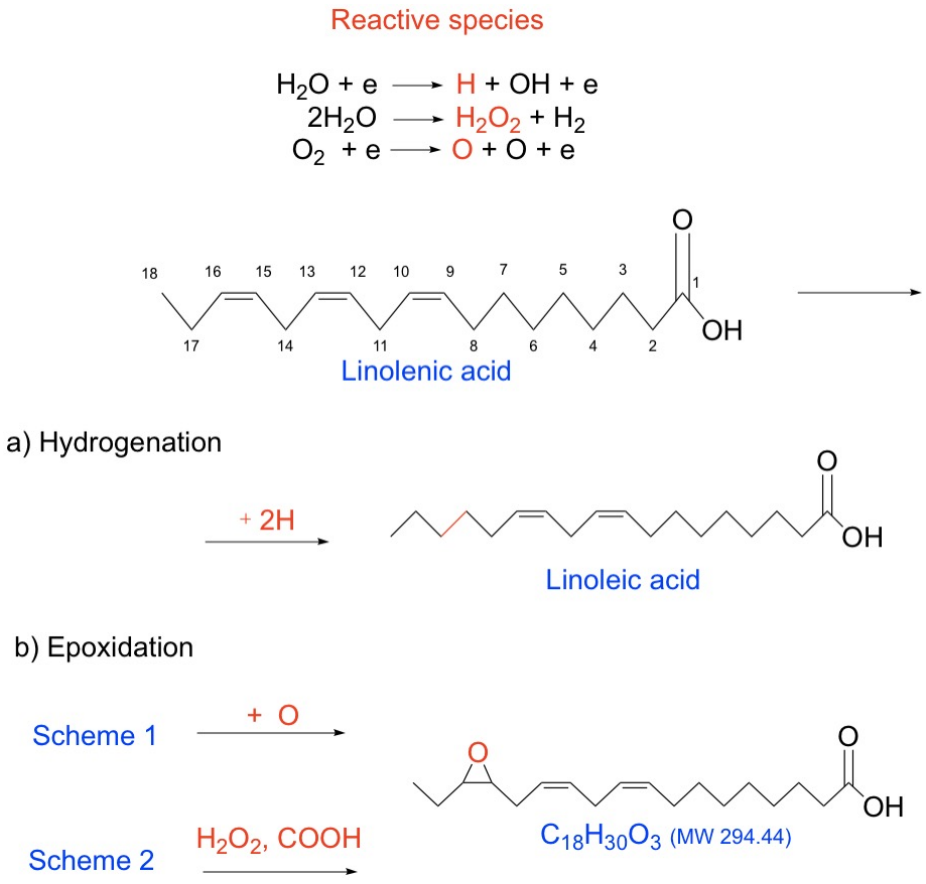


Fig. 4.4. Possible pathway for the formation of linoleic [M+2H], as well as the formation of epoxides [M+O], from HVACP treatment of linolenic acid [M].

experiments, see the experimental section). Results are shown in Fig. 4.5, where untreated and treated trilinolenin samples were ionized in the mass spectrometer using (+)ESI doped with an ammonium salt [125]. Samples treated with humid argon contained increased amounts of the hydrogenated products, as indicated by the ions $[M+2H+NH_4]^+$ and $[M+4H+NH_4]^+$, with m/z 898.8 and m/z 900.8, respectively. As can be seen in appendix A.1, the ions corresponding to m/z 898.8 are also detected in the untreated sample. The ions corresponding to m/z 900.8 are not detected for the untreated sample, and the high humidity treated sample shows a small increment (4.7%). In spite of the increment in the peak relative abundance, this result does

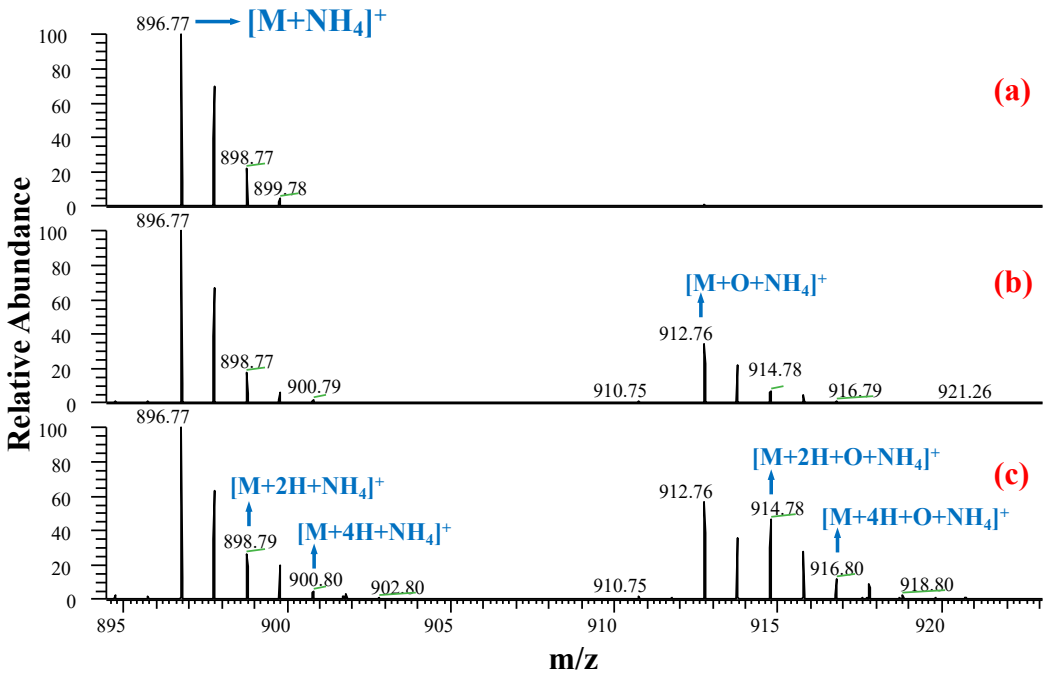


Fig. 4.5. Positive mode ESI mass spectra (obtained using ammonium salt dopant) of (a) untreated trilinolein and trilinolein exposed for 1 h to HVACP at 80 kV under (b) dry and (c) humid argon atmosphere.

not provide a strong evidence to confirm that a high humidity modified atmosphere promote the hydrogenation reaction.

Water dissociation generates H and HO radicals as primary products. The presence of water in the plasma chamber increases the current, by a higher flow of electrons and collisions that may increase rate of reactions such as dissociation and electron/ion recombination. Mass spectra measured for linolenic acid treated with nitrogen and argon showed a higher relative abundance of ions $[M+O+2H-H]^-$ and $[M+O+4H-H]^-$, compared to the untreated linolenic acid. Suggesting that both hydrogenation and epoxidation reactions occurred upon HVACP. Indeed, trilinolein treated with argon gas with low and high humidity showed the same reactions. The mass spectra

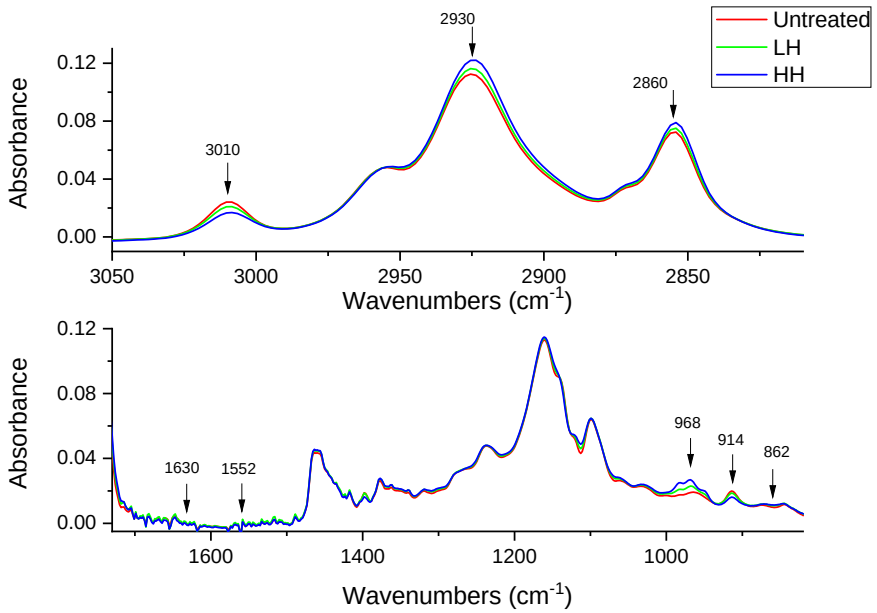


Fig. 4.6. FTIR spectra of trilinolein exposed for 1 h to HVACP at 80 kV under low (LH) and high (HH) humidity conditions.

measured for treated trilinolein samples showed $[M+O+NH_4]^+$, $[M+O+2H+NH_4]^+$ and $[M+O+4H+NH_4]^+$ ions with m/z values of 912.8, 914.8 and 916.8, respectively. These ions have a higher abundance for samples treated under high moisture, again suggesting that water dissociation products hydrogenate and epoxidize unsaturated fatty acids.

The structural modifications of trilinolein upon HVACP treatment were further supported by FTIR spectroscopy (Fig. 4.6). The reduction of double bonds (decrease in signal at 3010 cm^{-1}), and the increase in the number of single bonds (increase in signal at 2930 cm^{-1} and 2830 cm^{-1}), were observed. Peaks corresponding to nitrogen compounds were not detected in the spectra of argon gas treated samples (no signals at 1630 cm^{-1}-1, or 1552 cm^{-1}). An increase in the signal intensity at 968 cm^{-1} was observed in figure 4.6 for argon gas treated samples, and it corresponds to the *trans-* isomer that may be formed by an incomplete hydrogenation that leads to the restoration of a double bond into the isomer at the lower energy level [126]. This signal

was not obvious the FTIR spectra measured for linolenic acid because of overlap with signals due to the carboxylic acid end of the fatty acid.

4.5 Conclusions

HVACP treatment of soybean oil by using nitrogen gas (but less so for hydrogen and argon gases) showed changes in the fatty acid composition, with a reduction in the amount of polyunsaturated fatty acids. Pure standards, linolenic acid and trilinolein, were treated similarly and the products analyzed with high resolution mass spectrometry. Hydrogenation and epoxidation of unsaturated double bonds were observed, where nitrogen gas treatment was recognized as the medium with higher amount of hydrogenated products. The generation of hydrogen atoms was identified mainly by water dissociation, hence increasing the relative humidity from 22% to 63% allowed to determine a significant increment in two reactions: epoxidation and hydrogenation with an argon modified atmosphere. In addition, an intermolecular rearrangement was also proposed as a mechanism to obtain a hydrogenated product. However, this reaction may involve transferring hydrogen atoms from one molecule (forming a double bond) to another (that lose the double bond). Epoxidation may involve a reaction with reactive species from gas impurities, including oxygen and also water dissociation.

5. CHARACTERIZATION OF SOYBEAN OIL TREATED WITH HVACP AND HYDROGEN GAS

5.1 Abstract

Since the removal of partially hydrogenated oil from GRAS status, many studies have been focused in finding a replacement that can provide the texture and oxidation stability of partially hydrogenated oils (PHO). Until now, tropical oils such as palm and coconut oil have been the main replacement for PHO. As a consequence, locally produced soybean oil has decreased its consumption as a food ingredient. The objective of this study is to analyze the effect of cold plasma in the chemical structure of soybean oil, using hydrogen gas, as a non-thermal processing aid to partially hydrogenate soybean. As well as to identify parallel reactions that may occur during HVACP hydrogenation of soybean oil.

HVACP was produced by a dielectric barrier discharge system, and it was used to treat soybean oil (15g) with treatment times up to 6h, by triplicate. Plasma reactive species interact with the sample producing three fractions: liquid, gel, and solid. Fatty acid composition, FTIR, 1H-NMR, 2D-NMR, thermal properties, and peroxide value, was used to characterize their chemical structure.

Results of fatty acid composition showed a lower content of polyunsaturated fatty acids, an increased content of saturated fatty acids, and a low content of isomers. A hexane non-soluble fraction was identified as a cross-linked polymer, corresponding to a 30-40% of the 6h treated samples. It was proposed that plasma species collide with double bonds of unsaturated fatty acids, producing two main reactions: polymerization and hydrogenation.

This technology has the capability of modifying the chemical structure of soybean oil, by a plasma-liquid reaction, creating a stable oil with less double bonds and a

cross-linked polymer. Cold plasma can be used as a processing technology to produce oleogels from soybean oil, without adding a gelator.

5.2 Introduction

As a food ingredient, soybean oil is used mainly as cooking oil or salad dressing. The high content of unsaturated fatty acids makes this oil susceptible to oxidation and limits its range of applications. Modifications to the fatty acid composition have been realized to increase its spectrum of uses. The partial hydrogenation process reduces the polyunsaturated fatty acid content, to obtain a semi solid fat that is less susceptible to oxidation and has extended shelf life. However, this process isomerizes the monounsaturated fatty acid, generating 25-40% of trans fatty acids [93,122]. This component is linked to cardiovascular diseases, and nowadays partially hydrogenated oils (PHO) are not allowed to use as food ingredient [10].

Cold plasma has been studied as a processing aid to functionalize organic compounds [127, 128]. Collisions of electrons with molecular or atomic gases, generate a series of reactions that lead to the formation of ions, radicals, and other excited plasma species that are highly reactive. This technology has been used to add chemical groups (deposition) to organic structures, and it has been studied as a method to modify edible films, polymers or graphene [44, 97, 101, 129].

Plasma treatment can fix nitrogen, oxygen, or hydrogen on graphene to improve its properties [118]. Nitrogen-doped graphene has been synthesized using ammonia or nitrogen gas [130, 131]. Hydrogen plasma species can saturate a monolayer of carbon atoms tightly packed, by exposure to a low pressure cold plasma [82, 132]. Plasma hydrogenation of graphene converts a highly conductive material into an insulator, by the deposition of hydrogen atoms on its structure. These are examples of the versatility of this technology to form new products by adding specific atoms or molecules. The reaction products can be controlled by gas composition, input energy, frequency, or treatment time.

Hydrogenation of soybean oil using cold plasma is a novel approach to reduce the formation of trans fatty acids [121] and produce a semi solid fat that may replace partially hydrogenated vegetable oil. The goal of this study is to identify parallel reactions that occur during HVACP hydrogenation of soybean oil. Two main reactions are analyzed, polymerization and oxidation. Fatty acid composition is measured to determine changes in the unsaturation degree. Analysis such as FTIR, ^{1}H-NMR, and 2D-NMR, provide information related to structural modifications. Thermal properties and peroxide value are also investigated.

5.3 Materials and Methods

Setup and experimental design

HVACP system employs a transformer that converts an energy input of 120VAC /60Hz, into an energy output of 0-130 kV. This device has a dielectric barrier discharge configuration, as described in Chapter 1. A plasma state is formed between two aluminum electrodes. Electrodes were positioned at top and bottom of the plasma chamber, allowing the sample to receive a direct exposure to the electric field (Fig. 5.1a-b). Dielectric layers (4mm thickness, polypropylene) were added to maintain a uniform glow, free from arcing. The box (polypropylene, 175×275×44mm) used as a plasma chamber was flushed with hydrogen gas for 5min at 1 l/min to allow a uniform composition. Then sealed with a high barrier film (Cryovac, Sealed Air Corporation, NJ). The experiments were carried out inside a hood, to extract and neutralize the reactive species generated through the treatment. A nitrogen gas blanket was adapted to cover entirely the electrodes and diminish the risk of flammability. All experiments were conducted at 80kV.

Soybean oil was procured from a local store, and samples (15g) were treated in a glass petri dish for 0, 2, 4, and 6 hours, by triplicate. Treated samples were collected from 3 points of the plasma chamber: 1) Liquid, inside the petri dish; 2) Gel, outside the petri dish; and, 3) Solid, from the top of the box (Fig. 5.1c).

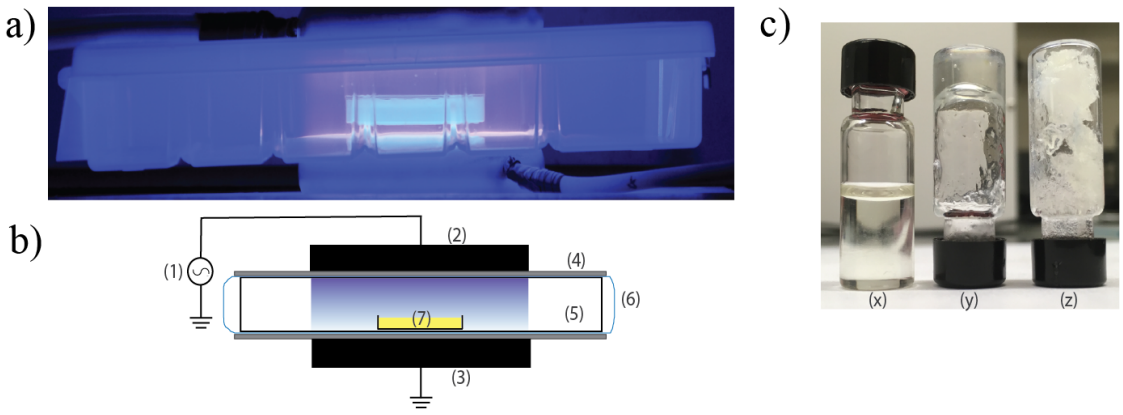


Fig. 5.1. High voltage atmospheric cold plasma system for soybean oil treatment. Picture (a). Schematic diagram (b): transformer (1), high voltage electrode (2), ground electrode (3), dielectric barriers (4), plasma chamber (5), packing film(cryovac) (6), sample of soybean oil (7). Treated samples (c): liquid (x), gel (y), and solid (z).

Samples were collected after treatment and maintained at -18°C for further analysis. Partially hydrogenated oil was obtained using nickel as a catalyst, under 170-190°C, and hydrogen pressure of 20-25 psi.

Fatty acid composition

Same method as in section 4.3.3

FTIR

Same method as in section 4.3.5

Nuclear Magnetic Resonance

Same method as in section 3.3.7

Thermal Analysis

Thermal stability was determined using a TGA 55 (TA instruments, New Castle, DE). Samples (12±2mg) were heated from room temperature up to 600°C, at a rate of 10°C/min under nitrogen gas. Dielectric scanning calorimetry (DSC) experiments were performed using a Discovery Series (TA instruments, New Castle, DE), calibrated with indium and sapphire. Samples 10±2mg were weighed in hermetic pans, and an empty pan was used as reference. Melting point (Tm) was measured according to AOCS method. Enthalpy (ΔHm) were also reported in association with the melting point.

Peroxide Value (PV)

Peroxide value (peroxide milliequivalent/kg of oil) was tested according to the International Dairy Federation standard method 74A. A sample of oil (20 mg) was dissolved with 3:2 (v/v) dichloromethane/methanol solution. Peroxides react with iron(II) chloride and ammonium thiocyanate, followed by absorbance measurements at 560 nm, as explained by Chew and collaborators [133].

Fat extraction

Separation of insoluble material from treated samples of soybean oil was applied using about 220ml of hexane in a Soxhlet apparatus. The sample (100-300mg) was introduced in cellulose thimbles, and the extraction was continued for five hours. The insoluble material was dried at 70°C.

Statistical Analysis

Data analysis were performed using SAS (version 9.3) for Windows (SAS Institute Inc, 2008). Analysis of variances was conducted using the GLM procedure. A Tukey test was used to determine significance differences at 95% level of confidence ($p<0.05$)

5.4 Results and discussion

Soybean oil samples were exposed to the direct electric field between electrodes, yet the intense treatment drove out a portion of the sample outside the glass petri dish. Therefore, three fractions were analyzed from each treated sample: liquid, gel, and solid (Fig. 5.1c). Liquid sample (94.5%) was collected from the petri dish and had less contact with reactive plasma species. Gel sample (2.5%) was collected from the bottom of the box, as it escaped from the petri dish due to the intense treatment. Solid sample (3%) was collected from the top of the box, and it was the sample with the higher exposure to plasma treatment.

Fatty acid composition

Results from fatty acid composition are shown in Fig. 5.2. An increment in saturated, and reduction of unsaturated fatty acids were observed in all treated samples. Solid samples had the higher exposure to plasma species and showed higher changes in their composition. A significant increment of stearic acid (C18:0) was observed in solid samples, in a range of 12.2-13.7%, and total saturated fatty acids increased from 16.6% to 36.5-38.8%. Linolenic acid decreased from 8.3% to 3-2.3%, and linoleic acid from 53% to 33.1-30%. In summary, the fatty acid composition for the 6h solid sample showed a saturated content of 38.8%, the monounsaturated fatty acids (MUFA) 24.3%, and the polyunsaturated fatty acid (PUFA) with a 36.5%. This fatty acid composition corresponds to an iodine value of 86.6.

Column SLB-IL60 allows to separate oleic acid (C18:1), with double bond in 9c or 11c position in the hydrocarbon chain, being 11c a small fraction of MUFA. The former increased significantly over treatment time, from 20.5% to 23.7%. In contrast with C18:1-11c that did not have significant differences. A new peak was observed corresponding to elaidic acid (C18:1-9t) that reached 1.3% as the highest value for treated samples.

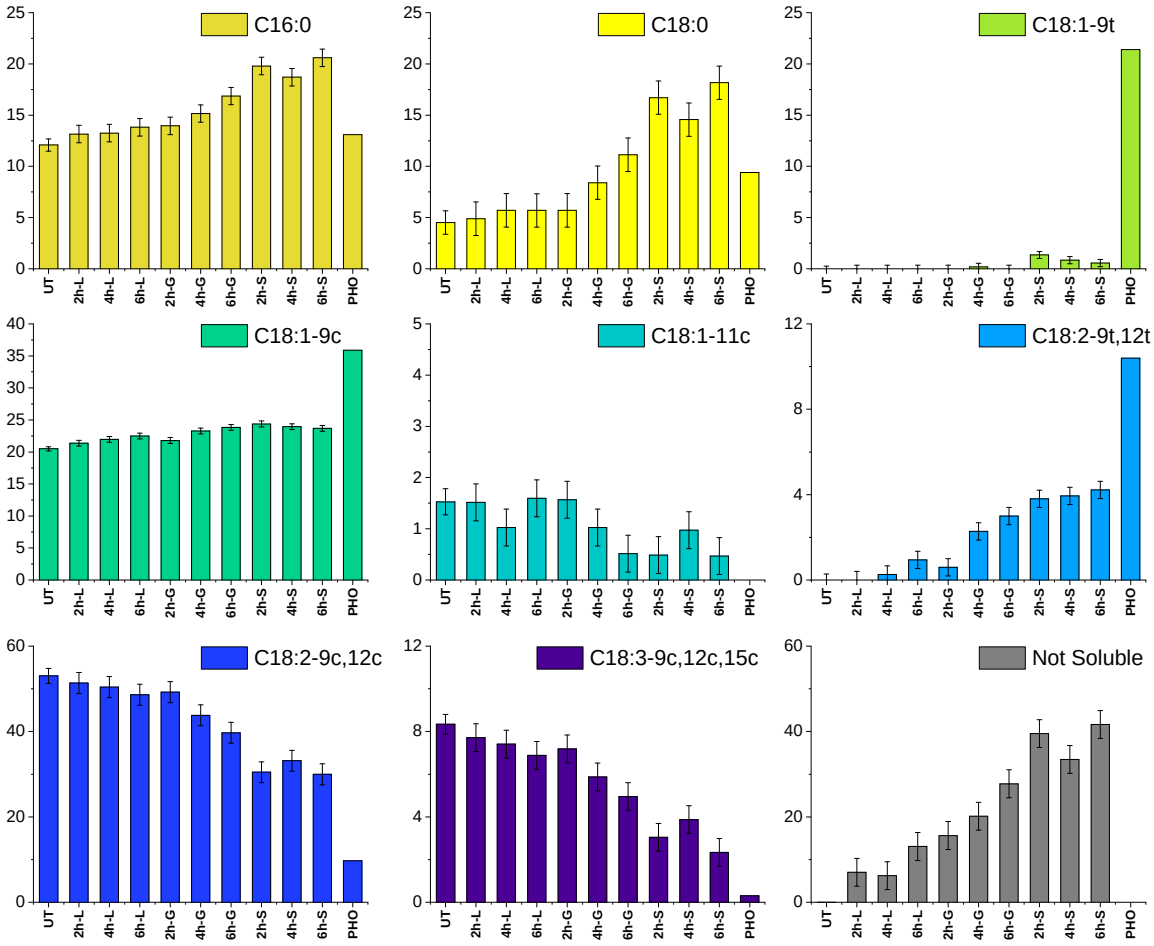


Fig. 5.2. Fatty acid composition of soybean oil treated with HVACP at 80 kV. Results show untreated (UT), liquid (L), gel (G), and solid (S) fractions, treated for 2h, 4h, and 6h.

Traditional partial hydrogenation of soybean oil form 25-40% of trans fatty acid, mostly as elaidic acid C18:1-9t [93,122]. Trans isomers are formed through an incomplete hydrogenation, as a product of the absorption-desorption of unsaturated fatty acids from the catalyst surface, and it is isomerized to its lower energy positional structure. The fatty acid composition of a traditional PHO with an iodine value of 84 is shown in Fig. 5.2, with a total trans fatty acid content of 31.8%. HVACP treatment can reduce IV to the level of a traditional PHO, but with a different fatty

acid composition and remarkable lower content of trans fatty acids, in the range of 4.8-5.2%.

Trans fatty acid content is much lower for HVACP-treated samples, mostly formed by linoelaidic acid (C18:2-9t,12t), in contrast with PHO that mainly have elaidic acid (C18:1-9t). The mechanism of formation of trans isomers in HVACP is not well understood, because there is not a catalyst surface where the double bonds are opened, neither absorption-desorption. Isomerization may occur due to attachment/detachment of hydrogen atoms, when the reaction activation energy is not reached, and double bonds change to a *trans-* position.

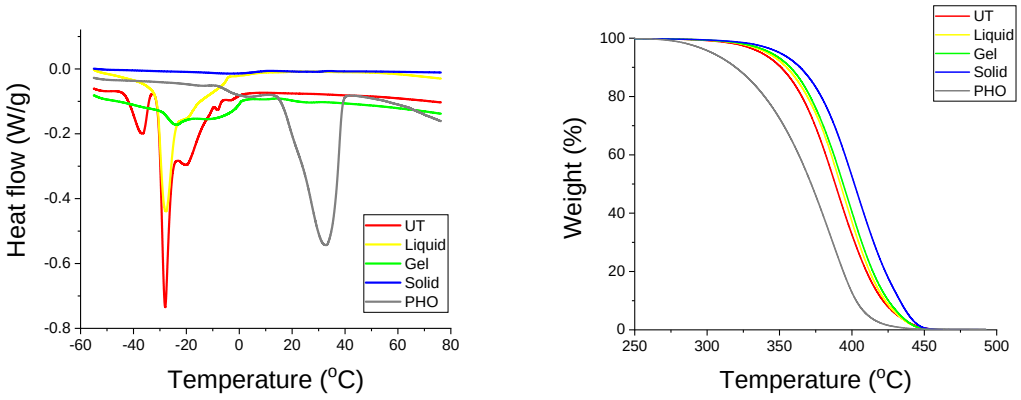
The insoluble fraction was quantified by the amount of sample used for fatty acid composition analysis, and this fraction may be associated with a parallel reaction of HVACP treatment such as epoxidation or polymerization. During derivatization of the triglycerides into fatty acid methyl esters, a fraction of the treated sample did not dissolve completely using hexane. The sample was filtrated (0.2μm) before injecting into the GC, and losses were quantified. The amount of non-soluble fraction increased consistently with longer treatment times, reaching up to 41.6% for a 6h solid sample. From this sample, the remaining hexane-soluble oil (58.4%) contained 13.1±0.2% of C16:0, 10.4±0.7% of C18:0, 14.4±0.2% of C18:1, 21.2±2.6% of C18:2, and 1.5±0.5% of C18:3 (Appendix A.4). These values were calculated based on AOAC method 996.06, using the known amount of internal standard, and the initial weight of the sample. The amount of stearic acid (C18:0) increased by 5.9% compared with the untreated oil, linoleic and linolenic acid reduced by 35.7% and 6.9% respectively. It is suggested that the formation of insoluble material is related to the reduction of unsaturated fatty acids, as the most reactive components in the sample. A hydrogenation reaction may have occurred as the saturated fatty acid increased in the 6h solid sample. Further analysis were conducted to identify the structure of the non-soluble fraction, using thermal properties, FTIR, and NMR.

Thermal properties

The effect of hydrogenation is mainly reflected in the thermal properties, including melting point and thermal degradation. It is known that an increased content of saturated bonds increase the melting point. Untreated soybean oil has a melting point of -28±0.4°C, while PHO is solid at room temperature with a melting point of 32.3±0.3°C. HVACP treated samples showed a melting point of -27.6±0.3°C, -23.5±0.4°C and -4.9±0.3 °C for liquid, gel and solid samples treated for 6h, respectively. HVACP treated samples increased the melting point but not to the level of a PHO, because of the high content of PUFA (36.6%) compared to a PHO (20.4%).

Additionally, a reduction of melting enthalpy was also observed in treated samples, from 59.1±3.3 J/g to 9.7±3 J/g for the untreated and treated 6h-solid samples, respectively. A reduction of the endothermic peak is linked with less amount of crystals or a reduction of crystals size. It seems that the triglyceride structure was modified as it could not crystallizes completely, so it may correspond to a complex structure that can have a relationship with the insoluble fraction. This complex structure may involve cross-links junctions that disturb the crystals formation by preventing molecular chain folding. In addition, the solid sample does not show any other peak until 80°C.

Thermal degradation involves scission, side groups elimination, and de-polymerization [134]. Samples were exposed to a temperature up to 600°C, where a 100% of sample degradation was achieved (Fig. 5.3b). The onset temperature (T_0, temperature corresponding to a 10% degraded sample) of untreated soybean oil was 357±1.5°C, and this temperature increased with treated samples, reaching 369±1.2°C for 6h-solid sample. Therefore, a 12-14°C shift was observed in treated samples through the whole thermal degradation process, and this increment was statistically different from untreated soybean oil. In contrast, PHO showed an onset temperature of 344.48°C, hence it was characterized by a thermal degradation shifted to a lower temperature, which means that triglycerides with simple bonds requires less energy



(a) DSC. (b) TGA

Fig. 5.3. Thermal properties of soybean oil untreated and treated for 6h with hydrogen gas.

to be degraded, in contrast with untreated soybean oil that have more double bonds and requires more energy to degrade the molecule. Moreover, HVACP treated samples showed a thermal degradation shifted to a higher temperature compared with the untreated soybean oil, indicating the formation of complex bonds that require additional energy to degrade [135].

FTIR

Untreated and treated samples (6h) were analyzed with FTIR as shown in Fig. 5.4. FTIR chromatograms do not show the same intensity for all functional groups, therefore they are used to identify bond changes and not for quantification. Likewise, it is important to mention that FTIR results represent the whole sample, without previous dilution with solvents as it is required with GC or NMR analysis. FTIR may allow to identify bonds formed in the insoluble fraction of treated samples.

FTIR results suggest a HVACP hydrogenation reaction. Fig. 5.4a shows the hydrogen stretching region including the range of 2800-3050cm^{-1}. Liquid, gel, and solid samples showed an increment in the number of simple bonds (2930 cm^{-1}, 2860 cm^{-1} asymmetric stretch bands), and a reduction of cis-double bonds (3010 cm^{-1}, stretch

band). Further information about bonds modifications are included in the fingerprint region (Fig. 5.4b), in the range of 800-1300 cm^{-1}. Peak at 914 cm^{-1} corresponds to cis-olefinic group, that shows a reduction from untreated to treated samples [124]. However, an increment of trans- double bonds bending vibration was also observed in the 968 cm^{-1} band, with a higher peak for PHO. These results are in accordance with the fatty acid composition. An additional modification in bonding was observed in peak 1121 cm^{-1}, corresponds to stretching vibrations of C-O.

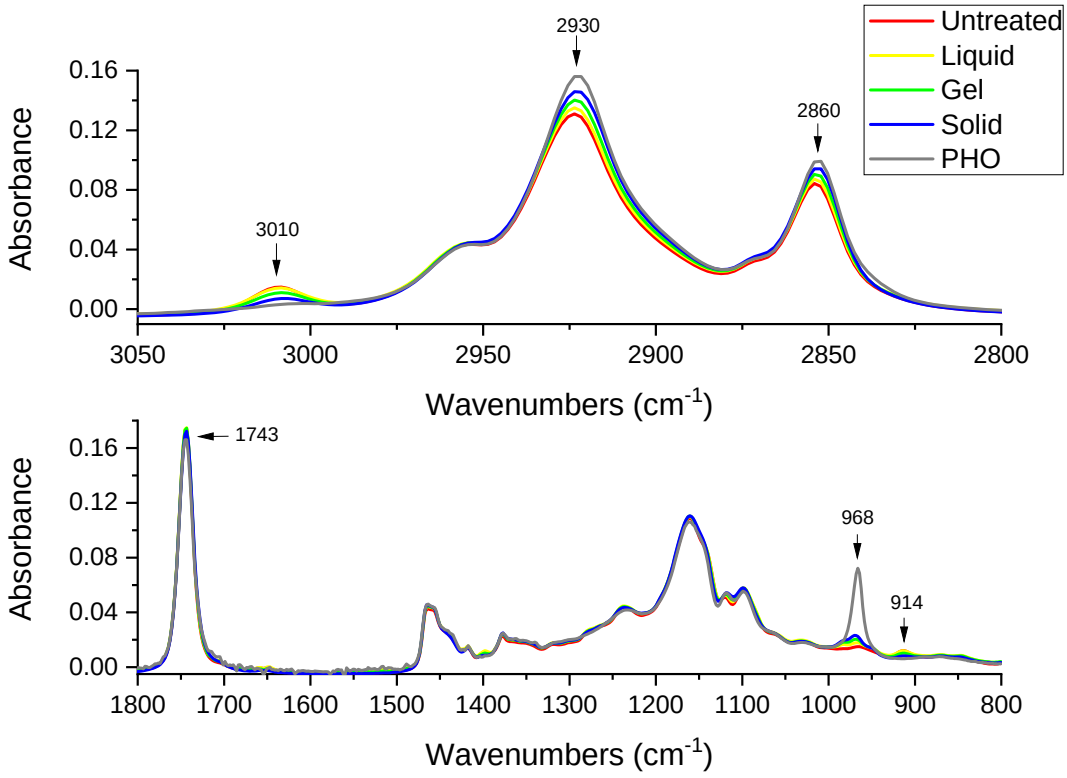


Fig. 5.4. FTIR spectra of soybean oil untreated and treated for 6h with hydrogen gas

The solid sample obtained by HVACP treatment of soybean oil with hydrogen gas, as well as nitrogen and argon gas from chapter 4 were further analyzed with FTIR. The soluble liquid oil from the solid sample was extracted with hexane, then

the insoluble fraction was isolated and analyzed as shown in Fig. 5.5. This material is difficult to dissolve in solvents, because it may form a crosslinked polymer with a high molecular weight which plays an important role in solubility. A strongly crosslinked polymer forms a network that inhibit the interaction of the solvent molecules with the polymer chains, preventing the molecules for being transported into solution.

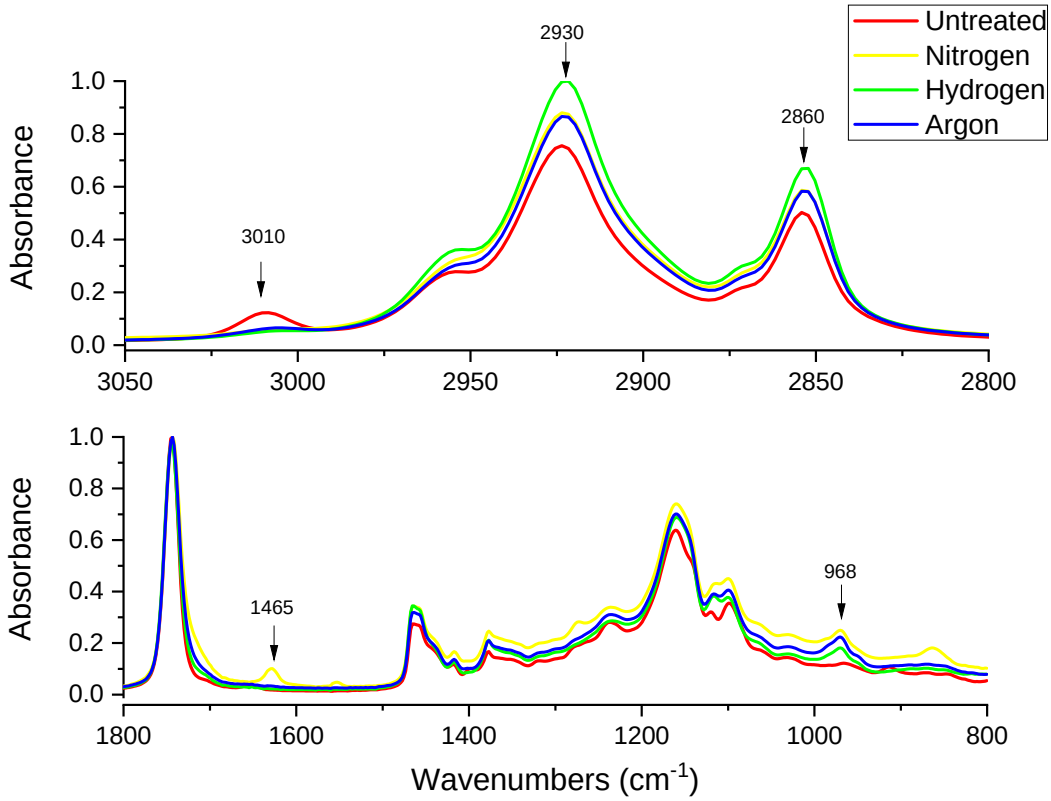


Fig. 5.5. FTIR spectra of insoluble fraction of soybean oil treated for 6h with hydrogen gas, and 4h with nitrogen and argon gas.

The insoluble fraction was characterized by a reduced content of double bonds (peak at 3010cm^{-1}) and a higher content of simple bonds, in accordance with values observed in Fig. 5.4. From the FTIR figure there is no additional peaks that provide information about structure modifications rather than changes in simple and double bonds. It is proposed that the mechanism of formation of this polymer may occur by

the "Diels Alder" reaction, in which a conjugated double bond rich in pi-electrons, react with a dienophile forming a crosslink between two hydrocarbon chains. This reaction has been reported in literature as "cationic polymerization of soybean oil" [136]. Soybean oil with a catalyst (superacid: fluoroboric acid HBF_4) reacts at room temperature forming cationic active centers and conjugated double bonds. Then, it is heated to 80-90°C for 6 hours where the reaction occurs and the viscosity increases. The electric field of HVACP treatment and the formation of reactive species may allow the conditions to promote the polymerization reaction of soybean oil by the "Diels Alder" reaction. The mechanism of the reaction includes the carbocation by addition of protons to double bonds, transfer reaction of carbocations with the hydrogen atoms from mono- or bis- allyl positions (Fig. A.3). Then, the reaction of allyl cations with double bonds of conjugated double bonds. As a result, the triglyceride fatty acid chains are linked by cyclic structures. A rearrangement of the double bonds from cis- to trans-, as a consequence of the reaction is also reported. This effect can be observed in results from FTIR and fatty acid composition, by an increment of the trans isomer of 18:1 and 18:2.

Changes in rheological behavior imply a modification of the chemical structure. The literature reports a Newtonian behavior for vegetables oils, ranging from 0.04-0.06 Pa.s for sunflower, soybean, corn, or olive oil [137]. Vegetable oils with a high content of *cis*- double bonds have a lower viscosity, because the fatty acid molecules do not stack together and are more fluid. Partially hydrogenated oils maintained a Newtonian behavior with an increased viscosity that can be duplicated with this reaction [138]. Samples treated for 12 hours with 5% hydrogen and 95% nitrogen gas in chapter 3, change the rheological behavior of the untreated soybean oil and reaching 9.5 Pa.s at a shear rate of $10s^{-1}$. In this case the viscosity increases 190 times. Such increment has been reported for polymerized soybean oil by superacid catalysis [136,139]. Supporting the hypothesis that HVACP promote a polymerization reaction.

NMR

Peak assignments of the triglyceride structure of soybean oil in the ^{1}H-NMR spectra are shown in Fig. 5a/5b, corresponding to: 1) Methyl terminal group, 0.8-0.9; 2) Methyl terminal group for linolenic acid, 0.9-1.0; 3) Methylene protons -CH2-CH2-CH2-, 1.2-1.4; 4) Methylene protons C(=O)-CH2-CH2-CH2- 1.5-1.7; 5) Methylene protons -CH2-CH2-CH=CH-, 1.9-2.1; 6) Methylene protons C(=O)-CH2-CH2-CH2- 2.2-2.3; 7) Allylic group =CH-CH2-CH=, 2.7-2.9; 8) Glycerol protons n-1 and n-3, doublet: 4.0-4.3; 9) Glycerol protons n-2, 5.2-5.3; 10) Olefinic protons CH2-CH=CH-CH2, 5.3-5.4. Values obtained from peak integration were used to compare differences between untreated and treated samples (Table 1). Soybean oil treated for 6h showed a reduction of double bonds (peak 10) from 7.4% to 4.7%, and an increasing percentage of simple bonds (peak 3) from 53.5% to 59.5%. Moreover, if double bonds are hydrogenated, peaks 5 and 7 related to simple bonds close to double bonds will reduce as well, as it occurs with treated samples. The reduction of peaks 5 and 7 have a correlation with the increased amount of peak 3, because hydrogenated double bonds on carbon 9 or 12 will increase the signal of peak 3, that is the moiety of a simple bond not close to a double bond. The glycerol moiety did not show major modifications, peaks 8 and 9. Suggesting that treated samples maintain a triglyceride structure.

Oxidation products include hydroperoxides (chemical shift 8.3-8.9 ppm), aldehydes (chemical shift 9.5-9.8), alcohols (chemical shift 3.43-3.62), epoxides (chemical shift 2.63,2.88-2.90, 3.1 ppm), ketones (chemical shift 6.08, 6.82 ppm), or conjugated double bonds (chemical shift 5.7-6.4ppm) [140–142]. From these compounds, treated samples showed an increased peak only for conjugated double bonds, as can be seen in the expanded spectra from Fig. 5.6b, corresponding to 5.7-6.3 ppm. Moreover, spectra from 2D-NMR (HSQC) of treated sample was used to identify a link between the conjugated double bond with any other moiety in the triglyceride structure. From this analysis, only two points were identified on treated samples: (6.2;126.3) and

Table 5.1.
^{1}H-NMR peak integration (%) for untreated and 6h treated samples of soybean oil with HVACP at 80 kV.

Peak	**Untreated**	**Liquid**	**Gel**	**Solid**	**PHO**
1	7.0	7.0	7.1	7.4	8.4
2	0.4	0.3	0.3	0.4	0.1
3	53.5	55.2	56.5	59.5	60.2
4	6.4	6.5	6.6	6.7	5.4
5	10.1	9.7	9.1	7.9	10.1
6	6.4	6.5	6.5	6.4	5.9
7	3.7	3.0	2.5	1.5	0.0
8	4.1	4.2	4.3	4.3	4.0
9	1.0	1.0	1.1	1.3	1.3
10	7.4	6.7	5.9	4.7	3.7

(5.9;128.7), as can be seen in figure 5b. These peaks are related to the double bonds region of the hydrocarbon chain, in the ^{13}C-NMR spectra. According to Martinez and collaborators, these peaks may correspond to conjugated double bonds associated with hydroperoxides (-CH(OOH)-CH=CH-) [103], that were identified in corn oil samples submitted to thermal treatments. Suggesting that double bonds can migrate to a conjugated arrangement, forming a ring structure that can result in dimerization of triglycerides [104]. It is important to note that samples analyzed with ^{1}NMR was previously dissolved with chloroform, and the signal identified as the link of polymerization is low because the polymer may not completely dissolve with chloroform.

HVACP treatment not only has been used to modify chemical structures, but has been studied mainly as a processing technique to reduce the microbial load of food [33]. The mechanisms of microbial inactivation may include the production of UV light, oxidation of membrane lipids, or protein oxidation [143, 144]. Therefore, this technology is known as an oxidizer tool. In this study, the goal is to use hydrogen

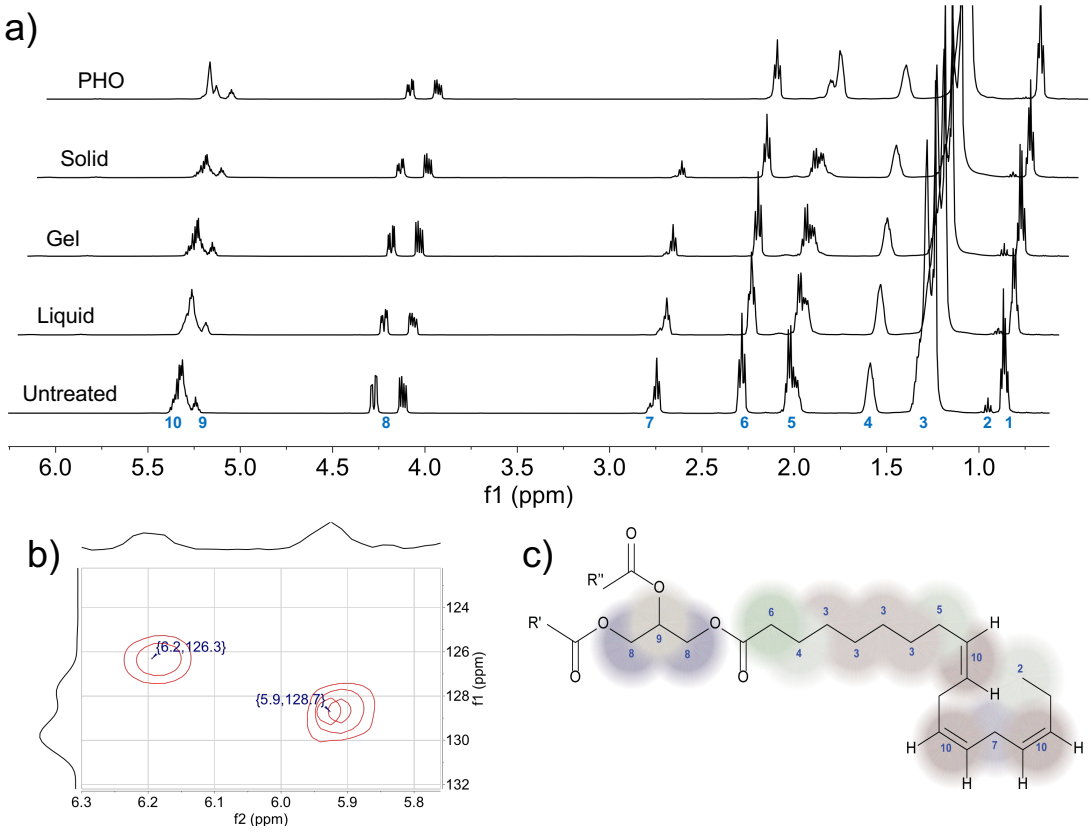


Fig. 5.6. ^{1}H-NMR spectra of soybean oil untreated and treated for 6h with hydrogen gas (a) ^{1}H-NMR spectra (b) Expanded spectra 5.7-6.4ppm (c) 2D-NMR HSQC of treated sample 6h-solid.

gas as a source of hydrogen atoms, to hydrogenate double bonds of unsaturated fatty acids. Consequently, the presence of oxygen was avoided because any trace could form oxygen reactive species that may initiate oxidation reactions, as a matter of fact hydrogen gas was flushed for 5 minutes to reduce oxygen content below 0.01%. However, treated samples of soybean oil (1.9-5.0 meq/kg) showed a higher peroxide value (PV) than untreated (0.19 meq/kg) (Fig. 5.7). These values were below 10 meq/kg, which is the limit of peroxide content requirement for fats and oils [145]. PV is a critical parameter that should be monitored, then further precautions can be

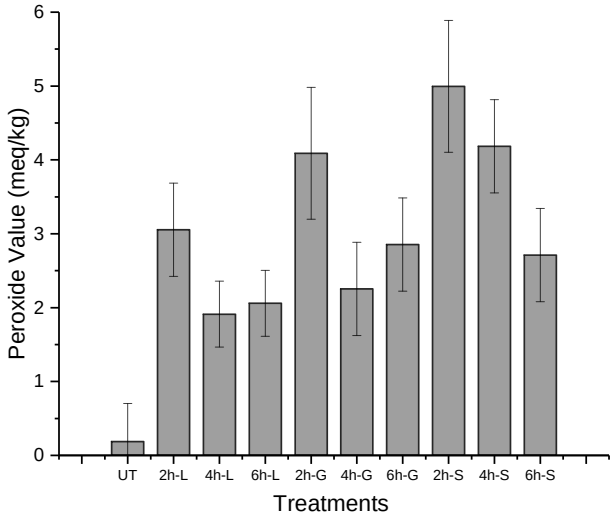


Fig. 5.7. Peroxide value of soybean oil untreated and treated for 2, 4, and 6h with hydrogen gas at 80kV.

adopted to remove oxygen from the plasma chamber, or it would be required the use of antioxidants as an additive. Interestingly, results showed that longer treatment times reduce PV. Samples treated for 6h-solid have a PV of 2.71±0.6, being lower than 5.0±0.9 meq/kg for the 2h-solid treatment. Samples treated for 6h have less amount of double bonds available to react with oxygen, which means that samples treated for longer treatment times are less susceptible to lipid oxidation.

5.5 Conclusions

This study investigated the modifications of soybean oil using HVACP hydrogen plasma. Soybean oil was treated for 2, 4, and 6h. Samples were characterized by fatty acid composition, FTIR, 1H-NMR, 2D-NMR, oxidation and thermal properties. HVACP treatment of soybean oil using hydrogen gas can transform the liquid oil into a semi-solid product. This processing technology change the chemical structure by the interaction with plasma reactive species. Treated samples showed an increased

content of saturated fatty acids, and a decreased content of unsaturated fatty acids. Samples treated for 6h reduced their iodine value from 133 to 86.6. These changes include a reduction of PUFA of 24.9%, an increment of 22.9% of saturated fatty acids, and the formation of an insoluble fraction that reached 41.6%. It is suggested that HVACP provides the conditions to catalyze hydrogenation of double bonds. However, a hexane insoluble fraction was identified and linked to polymerization reaction. The insoluble fraction changes the oil texture, suggesting that it is a cross-linked polymer that entraps the liquid oil. This polymer has a higher resistance to thermal degradation.

Reactions can be accelerated by increasing the oil surface exposed highly reactive species. Hydrogenation is produced at specific conditions of treatment, that may be influenced by voltage, current, and the availability of particular hydrogen plasma species. It would be interesting to identify the conditions required to hydrogenate the sample and minimize the polymerization reaction.

6. SUMMARY

In 2015, the FDA released a final determination regarding the use of partially hydrogenated oils. It was stated that partially hydrogenated oils (PHO) were not classified as safe food ingredients, because they contain 25-40% of *trans* fatty acids, which have a significant risk factor of increasing cardiovascular diseases. PHO were produced industrially from liquid vegetable oils through catalytic hydrogenation. The food industry has been trying to find similar products without the detrimental effect of *trans* fatty acids. At the moment, tropical and high-oleic soybean oils are the best options to replace PHO. However, there is resistance in the public towards the use of these alternatives due to their environmental impact, and their genetically-modified status.

The **objective** of this study is to develop a technology for partially-hydrogenating vegetable oil without the formation of *trans* fatty acids, and to identify the reactions that occur between the liquid vegetable oil and the reactive species that form in the process. Considering recent adaptations to the regulations about the use and consumption of partially hydrogenated oils, a novel technology called High Voltage Atmospheric pressure Cold Plasma (HVACP) is proposed, as an alternative to obtain PHO without *trans* fatty acids. Cold plasma technology operates at low temperatures and introduces hydrogen atoms within an electric field which may hydrogenate double bonds of unsaturated fatty acids, with a reaction that causes a change of the oil from liquid into a solid fat.

Based upon the hypothesis that cold plasma generates hydrogen atoms within the surrounding electric field created in the enclosure, an experimental implementation is reported in **Chapter 2**, in which soybean oil was treated with a modified atmosphere of 5% hydrogen - 95% nitrogen. In this configuration, hydrogen gas is used as the source of hydrogen atoms, and nitrogen gas as an inert gas to reduce the flammability risk. The treatment conditions of this experiment obtain a reduction in a iodine value

from 130.9±1.3 to 91.9±1.6, for a 12h treatment time. Overall, the fatty acid profile showed an increased amount of saturated fatty acids and a reduction of unsaturated fatty acids. In these experiments, *trans* fatty acids were not detected. The sample changed its rheological behavior, from a Newtonian to a non-Newtonian fluid, with viscosity at a shear rate of $10s^{-1}$ that increased significantly from 0.05 Pa.s to 9 Pa.s. These findings demonstrate the potential of HVACP to modify the fatty acid composition of soybean oil without *trans* fatty acids formation.

In **Chapter 3**, further experiments are reported where the hydrogenation rate is increased by means of increasing hydrogen concentration. If a 5% of hydrogen gas reduced iodine value to the level of a commercial product, it was hypothesized that increasing hydrogen gas concentration would generate a higher amount of reactant (hydrogen atoms) that could accelerate the reaction. However, the use of a hydrogen gas concentration over 5% entails flammability risk, particularly if electrical energy is involved in the treatment. Therefore, several safety measures were implemented, including a nitrogen gas blanket used to isolate the electrodes from air, and the use of an oxygen-content meter with an alarm. Results from this experiment were unexpected: a higher concentration of hydrogen gas did not increase the hydrogenation rate. Surprisingly, pure nitrogen gas changes the oil chemistry through a reaction that suggests hydrogenation, with an increment of saturated fatty acids and a reduction of unsaturated fatty acids, with a higher rate than that observed when using hydrogen. Samples treated with hydrogen and nitrogen at different concentrations, reduced iodine value at a similar degree, from 133 to 122-125 in a 1.5h HVACP treatment.

If nitrogen and hydrogen gas species react with double bonds of unsaturated fatty acids, it would be expected to obtain a nitrated fatty acid. Thus, nitrogen-content analysis were conducted and samples treated with high concentrations of nitrogen gas showed a concentration of 218.3-242.9 ppm of nitrogen. This suggests that the nitrated compounds were not soluble in hexane and therefore were not identified through GC.

Next, in **Chapter 4**, experiments were conducted to explore the hydrogenation and nitration reactions, with the objective of identifying reaction products and propose a reaction mechanism. For this purpose, pure standards ($> 99\%$) of polyunsaturated fatty acid (linolenic acid, C18:3) and triglyceride (trilinolenin) were treated with nitrogen, hydrogen, and argon gas, to identify the reaction products through high resolution mass spectrometry. Results show that hydrogenation occurs with all three gases used by the formation of linoleic, oleic and stearic acid. In fact, hydrogenated products were identified in the modified gas atmosphere in decreasing order: from nitrogen gas being the highest rate, then argon, and finally hydrogen gas. An epoxide was identified in treated samples with MS, as the main ion with an oxygen adduct. Neither nitrated fatty acids nor nitrated triglyceride were found in any of the treated samples. A hydrogenation reaction mechanism is proposed, where hydrogen atoms that hydrogenate double bonds may be generated by (a) hydrogen-gas-modified atmosphere; (b) intermolecular rearrangement; or, (c) water dissociation from gas impurities.

Finally in **Chapter 5**, a study was conducted to determine if parallel reactions may occur during HVACP treatment of soybean oil. The hypothesis tested was that the presence of unsaturated double bonds may decrease because reactions like polymerization or epoxidation could occur, when HVACP treatment opens the double bond and two triglyceride molecules join together or absorb an oxygen (epoxide). Previous results showed that a fraction of the treated soybean oil did not dissolve completely neither did hexane, chloroform, or ethanol (appendix A.1). Here, the insoluble fraction was quantified after treating soybean oil for up to 6h with hydrogen gas, and samples were characterized with fatty acid composition, thermal properties, FTIR, and NMR. Results show that the insoluble fraction reaches up to 40% for a 6h treatment with an iodine value in the range of 86.6-96.5. It is suggested that this fraction is a polymer because thermal degradation implies a complex structure, and results from FTIR and NMR suggest a link formed through the double bonds.

In **summary**, HVACP treatment of 6h with hydrogen gas achieved a product with a saturated content of 38.8%, MUFA of 24.3%, PUFA of 36.5%, and trans of 5.2%; hence, it has an iodine value of 86.6, peroxide value of 2.7 meq/g, and melting point of -4.9°C. HVACP treatment of 12h with a modified atmosphere of 5% hydrogen and 95% nitrogen gas achieved a fatty acid composition with a saturated content of 32%, MUFA of 26%, PUFA of 42%, and no trans fatty acids. In comparison with a commercial partially hydrogenated oil that has a saturated content of 22.5%, MUFA of 57.3%, PUFA of 20.4%, and trans of 31.8%; hence, it has an iodine value of 84.9, and melting point of 32.3°C.

Overall, this study provides insights in cold plasma treatment of vegetable oils. Here, unsaturated fatty acids exposed to an electric field and the reactive species resulting from a modified atmosphere, change their double bonds with reactions identified as hydrogenation, epoxidation, and polymerization. These are proposed as plausible outcomes, based upon the evidence provided in this thesis.

The results of this project may open paths towards additional new applications. Investigation is suggested for further exploration of this technology as a means to obtain bio-based gelators, plasticizers, lubricants or greases. The main characteristic of "cold plasma" is that it can be used as an environment-friendly processing technology, which can be categorized within the "green chemistry" area as it may replace the use of chemicals with potentially negative impact on the environment.

Future work can be focused in the electric field characterization to provide information about the hydrogen species. The hydrogenation reaction requires the dissociation of molecular hydrogen into atoms, and these atoms react with the double bonds as has been described in literature for the catalytic hydrogenation. Therefore, further studies are strongly advised to identify the atomic hydrogen species that have affinity to react with the double bonds.

This study is an initial examination of the use of high voltage atmospheric cold plasma treatment of vegetable oils in various gases including: hydrogen, nitrogen, argon. Soybean oil and unsaturated fatty acids were exposed to an electric field

between 60 kV to 100 kV for up to 12 hours. These conditions resulted in a range of chemical reactions, without a catalysts at room temperature (less than 50°C). Identified chemical reactions include hydrogenation, epoxidation, and polymerization.

The results of this study demonstrates that high voltage atmospheric cold plasma can produce useful industrial chemicals without a catalyst, at room temperatures, and atmospheric pressure. Further exploration and optimization of the HVACP technology as a means to obtain commercial scale bio-based gelators, plasticizers, lubricants and greases is recommended. The main characteristic of "cold plasma" is that it can be used as an environment-friendly processing technology, which can be categorized within the "green chemistry" area as it may replace the use of chemicals with potentially negative impact on the environment.

Future work should examine the electric field characteristics of high voltage atmospheric cold plasma including the electron energy density and the associated reactive gas chemistry. Although not quantified in this study, HVACP can create many atomic elements from common gases including atomic hydrogen, atomic nitrogen, atomic oxygen and others that can be used to produce many novel products including partially hydrogenated soybean oil without any trans-fat, ammonia, and complex amine chemistry. HVACP is a novel technology that could be applied to current, high energy chemical and industrial manufacturing processes to produce bio-based green products.

Future directions

The challenges associated with implementing HVACP treatment at an industrial scale can be related with:

(1) Long treatment times. Samples of soybean oil at a laboratory scale were 5-10 g, and treatment times between 2-12 h. Even if the energy consumption was low (80-150 W) similar to the consumption of a lightning bulb, the treatment time was long for a small sample. An additional experiment was conducted with a 200 ml sample

of soybean oil, treated for 20 h which resulted in a reduction of iodine value to 106 (results in appendix A.2). Therefore, treatment time increases with a larger sample, but this is not a directly proportional relation. Increasing the surface contact of the sample with reactive species, may accelerate the reaction.

(2) Improved equipment design. As a method to accelerate the reaction rate it is proposed to increase the sample surface exposure, with a thin layer, spraying, or bubbling gas. Limitations to scale up the technology could include materials (non-metallic), safety, and yield. These aspects are important at the moment of designing an equipment for larger scale treatments.

(3) Raw material specifications. In chapter 4, oleic and linoleic acid pure standards were preliminary tested, and the obtained results did not sow significant changes with a 1h treatment time. Therefore, experiments were conducted using a highly unsaturated fatty acid such as linolenic acid. Similarly, triglyceride with three linolenic acids was the standard that resulted most useful in the identification of significant changes with a 1h treatment. Consequently, this reaction is related to the physical availability to reach double bonds within the hydrocarbon chain, and the reaction rate may decrease when a sample has fewer double bonds available. The reaction rate depends on the fatty acid composition of the raw material.

(4) Purification. Results from chapter 5 show the formation of a polymer as the PUFA are involved in this reaction. Therefore, two fractions are obtained that can be separated with a solvent (hexane). This additional purification step would be required to obtain a vegetable oil that can be used as a food ingredient. This product will have a modified fatty acid composition rich in saturated fatty acids with no *trans*- isomers. Further studies could be conducted for analyzing the benefits of an additional purification process.

In the future, the capability of HVACP to change a double bond through the addition of water and oxygen in the presence of an inert gas can be used with other molecules or ingredients. Non-food applications of modified vegetable oils include the production of bio-lubricants, greases, or plasticizers. This topic was studied briefly,

by treating oleic acid with air as a catalytic reaction to obtain a product with similar characteristics as estolides [23, 146]. The electric field may open the double bonds of oleic acid, form an epoxide that may polymerize with other molecules nearby. Results of preliminary experiments of this particular case can be found in Appendix A.3.

REFERENCES

REFERENCES

[1] F. D. Gunstone, *Production and Trade of Vegetable Oils*, 2011. [Online]. Available: http://dx.doi.org/10.1002/9781444339925.ch1

[2] R. O'Brien, *Fats and oils*. CRC Press, 2008.

[3] C. A. Martin, M. C. Milinsk, J. V. Visentainer, M. Matsushita, and N. E. De-Souza, "Trans fatty acid-forming processes in foods: A review," *Anais da Academia Brasileira de Ciencias*, vol. 79, no. 2, pp. 343–350, 2007.

[4] J. W. E. Coenen, "Hydrogenation of Edible oils," *Journal of the American Oil Chemists Society*, vol. 53, no. 6, pp. 382–389, 1976.

[5] A. J. Dijkstra, "Revisiting the formation of trans isomers during partial hydrogenation of triacylglycerol oils," *European Journal of Lipid Science and Technology*, vol. 108, no. 3, pp. 249–264, 2006.

[6] H. B. W. Patterson, *Hydrogenation Methods*, 2nd ed., G. R. List and J. W. King, Eds. AOCS press, 2010. [Online]. Available: http://www.sciencedirect.com/science/article/pii/B9781893997936500142

[7] G. R. List, W. E. Neff, R. L. Holliday, J. W. King, and R. Holser, "Hydrogenation of soybean oil triglycerides: Effect of pressure on selectivity," *Journal of the American Oil Chemists' Society*, vol. 77, no. 3, pp. 311–314, 2000.

[8] G. R. List, W. C. Byrdwell, K. R. Steidley, R. O. Adlof, and W. E. Neff, "Triacylglycerol structure and composition of hydrogenated soybean oil margarine and shortening basestocks," *Journal of Agricultural and Food Chemistry*, vol. 53, no. 12, pp. 4692–4695, 2005.

[9] R. H. Eckel, S. Borra, A. H. Lichtenstein, and S. Y. Yin-Piazza, "Understanding the complexity of trans fatty acid reduction in the American diet." *Circulation*, vol. 115, no. 16, pp. 2231–2246, 2007.

[10] FDA, "Final Determination Regarding Partially Hydrogenated Oils," *The Daily Journal of the United States Goverment*, vol. 80, no. 116, pp. 34 650–34 670, 2015.

[11] F. C. Wang, A. J. Gravelle, A. I. Blake, and A. G. Marangoni, "Novel trans fat replacement strategies," *Current Opinion in Food Science*, vol. 7, pp. 27–34, 2016. [Online]. Available: http://dx.doi.org/10.1016/j.cofs.2015.08.006

[12] T. P. Labuza and L. R. Dugan, "Kinetics of lipid oxidation in foods," pp. 355–405, 1971.

[13] E. N. Frankel, *Lipid Oxidation*. Elsevier, 2014.

[14] E. Choe and D. B. Min, "Mechanisms and factors for edible oil oxidation," pp. 169–186, 2006.

[15] E. N. Frankel, "Volatile lipid oxidation products," *Progress in Lipid Research*, vol. 22, no. 1, pp. 1–33, 1983.

[16] A. G. Marangoni, "Organogels: An Alternative Edible Oil-Structuring Method," *Journal of the American Oil Chemists' Society*, vol. 89, no. 5, pp. 749–780, 5 2012.

[17] A. R. Patel and K. Dewettinck, "Edible oil structuring: an overview and recent updates," *Food Function*, vol. 7, no. 1, pp. 20–29, 2016. [Online]. Available: http://xlink.rsc.org/?DOI=C5FO01006C

[18] A. R. Patel, N. Cludts, M. D. B. Sintang, A. Lesaffer, and K. Dewettinck, "Edible oleogels based on water soluble food polymers: preparation, characterization and potential application." *Food & function*, vol. 5, no. 11, pp. 2833–41, 2014.

[19] M. Davidovich-Pinhas, S. Barbut, and A. G. Marangoni, "The role of surfactants on ethylcellulose oleogel structure and mechanical properties," *Carbohydrate Polymers*, vol. 127, pp. 355–362, 2015. [Online]. Available: http://dx.doi.org/10.1016/j.carbpol.2015.03.085

[20] A. J. Gravelle, S. Barbut, and A. G. Marangoni, "Ethylcellulose oleogels: Manufacturing considerations and effects of oil oxidation," *Food Research International*, vol. 48, no. 2, pp. 578–583, 2012.

[21] M. Desroches, M. Escouvois, R. Auvergne, S. Caillol, and B. Boutevin, "From vegetable oils to polyurethanes: Synthetic routes to polyols and main industrial products," *Polymer Reviews*, vol. 52, no. 1, pp. 38–79, 2012.

[22] K. M. Doll, S. C. Cermak, J. A. Kenar, and T. A. Isbell, "Synthesis and Characterization of Estolide Esters Containing Epoxy and Cyclic Carbonate Groups," *JAOCS, Journal of the American Oil Chemists' Society*, vol. 93, no. 8, pp. 1149–1155, 2016.

[23] J. McNutt and Q. He, "Development of biolubricants from vegetable oils via chemical modification," *Journal of Industrial and Engineering Chemistry*, vol. 36, pp. 1–12, 2015. [Online]. Available: http://dx.doi.org/10.1016/j.jiec.2016.02.008

[24] T. A. Isbell and R. Kleiman, "Characterization of estolides produced from the acid-catalyzed condensation of oleic acid," *Journal of the American Oil Chemists' Society*, vol. 71, no. 4, pp. 379–383, 1994.

[25] K. Kiefer, "Derivatization of Corn Oil for Analysis by GC," *Supelco Reporter*, vol. 16, no. 3, p. 6, 1997.

[26] A. Barison, C. W. P. da Silva, F. R. Campos, F. Simonelli, C. A. Lenz, and A. G. Ferreira, "A simple methodology for the determination of fatty acid composition in edible oils through 1H NMR spectroscopy." *Magnetic resonance in chemistry : MRC*, vol. 48, no. 8, pp. 642–50, 2010.

[27] G. Knothe and J. A. Kenar, "Determination of the fatty acid profile by 1H-NMR spectroscopy," *European Journal of Lipid Science and Technology*, vol. 106, no. 2, pp. 88–96, 2004.

[28] A. Martínez-Yusta and M. D. Guillén, "Deep-frying food in extra virgin olive oil: A study by 1H nuclear magnetic resonance of the influence of food nature on the evolving composition of the frying medium," *Food Chemistry*, vol. 150, pp. 429–437, 2014.

[29] F. Shahidi and U. N. Wanasundara, "Methods for Measuring Oxidative Rancidity in Fats and Oils," *Food Lipids: Chemistry, Nutrition, and Biotechnology*, no. 1, pp. 387–407, 2002.

[30] P. Bruggeman and C. Leys, "Non-thermal plasmas in and in contact with liquids," *Journal of Physics D: Applied Physics*, vol. 42, no. 5, 2009.

[31] A. Fridman, A. Chirokov, and A. Gutsol, "Non-thermal atmospheric pressure discharges," *Journal of Physics D: Applied Physics*, vol. 38, no. 2, 2005.

[32] M. Capitelli, C. Ferreira, B. Gordiets, and A. Osipov, *Plasma kinetics in atmospheric gases*. Springer Berlin Heidelberg, 2000, vol. 162.

[33] N. Misra, X. Yepez, L. Xu, and K. Keener, "In-package cold plasma technologies," *Journal of Food Engineering*, vol. 244, pp. 21–31, 3 2019. [Online]. Available: https://www.sciencedirect.com/science/article/pii/S0260877418304072

[34] N. N. Misra, B. K. Tiwari, K. S. M. S. Raghavarao, and P. J. Cullen, "Nonthermal Plasma Inactivation of Food-Borne Pathogens," pp. 159–170, 2011.

[35] H. Lu, S. Patil, K. M. Keener, P. J. Cullen, and P. Bourke, "Bacterial inactivation by high-voltage atmospheric cold plasma: Influence of process parameters and effects on cell leakage and DNA," *Journal of Applied Microbiology*, vol. 116, no. 4, pp. 784–794, 2014.

[36] D. X. Liu, P. Bruggeman, F. Iza, M. Z. Rong, and M. G. Kong, "Global model of low-temperature atmospheric-pressure He + H2O plasmas," *Plasma Sources Science and Technology*, vol. 19, no. 2, 2010.

[37] J. Meichsner, M. Schmidt, R. Schneider, and H. Wagner, *Nonthermal plasma chemistry and physics*. CRC Press, 2013.

[38] D. X. Liu, M. Z. Rong, X. H. Wang, F. Iza, M. G. Kong, and P. Bruggeman, "Main species and physicochemical processes in cold atmospheric-pressure He + O2Plasmas," *Plasma Processes and Polymers*, vol. 7, no. 9-10, pp. 846–865, 2010.

[39] S. Blanksby and G. Ellison, "Bond dissociation energies of organic molecules," *Acc. Chem. Res.*, vol. 36, no. 4, pp. 255–263, 2003.

[40] S. Jung, J. Lee, Y. Lim, W. Choe, H. I. Yong, and C. Jo, "Direct infusion of nitrite into meat batter by atmospheric pressure plasma treatment," *Innovative Food Science and Emerging Technologies*, vol. 39, pp. 113–118, 2017. [Online]. Available: http://dx.doi.org/10.1016/j.ifset.2016.11.010

[41] S. Patil, T. Moiseev, N. N. Misra, P. J. Cullen, J. P. Mosnier, K. M. Keener, and P. Bourke, "Influence of high voltage atmospheric cold plasma process parameters and role of relative humidity on inactivation of Bacillus atrophaeus spores inside a sealed package," *Journal of Hospital Infection*, vol. 88, no. 3, pp. 162–169, 2014.

[42] F. Judée, S. Simon, C. Bailly, and T. Dufour, "Plasma-activation of tap water using DBD for agronomy applications: Identification and quantification of long lifetime chemical species and production/consumption mechanisms," *Water Research*, vol. 133, pp. 47–59, 2018.

[43] Z. Wang, "Sabatier-Senderens Reduction," *Comprehensive Organic Name Reactions and Reagents*, pp. 2454–2457, 2010. [Online]. Available: http://onlinelibrary.wiley.com/doi/10.1002/9780470638859.conrr554/full

[44] D. Das and B. Sain, "Electrical transport phenomena prevailing in undoped nc-Si/a-SiNx:H thin films prepared by inductively coupled plasma chemical vapor deposition," *Journal of Applied Physics*, vol. 114, no. 7, 2013.

[45] L. Carnel, I. Gordon, K. Van Nieuwenhuysen, D. Van Gestel, G. Beaucarne, and J. Poortmans, "Defect passivation in chemical vapour deposited fine-grained polycrystalline silicon by plasma hydrogenation," *Thin Solid Films*, vol. 487, no. 1-2, pp. 147–151, 2005.

[46] B. Swatowska and T. Stapinski, "Amorphous hydrogenated silicon-nitride films for applications in solar cells," *Vacuum*, vol. 82, no. 10, pp. 942–946, 2008.

[47] N. Cherkasov, A. Ibhadon, P. F. C. E. and, and u. 2015, "A review of the existing and alternative methods for greener nitrogen fixation," *Elsevier*. [Online]. Available: https://www.sciencedirect.com/science/article/pii/S0255270115000409

[48] K. Sugiyama, K. Akazawa, M. Oshima, H. Miura, T. Matsuda, and O. Nomura, "Ammonia synthesis by means of plasma over MgO catalyst," *Plasma Chemistry and Plasma Processing*, vol. 6, no. 2, pp. 179–193, 6 1986. [Online]. Available: http://link.springer.com/10.1007/BF00571275

[49] J. Winter, K. Wende, K. Masur, S. Iseni, M. Dünnbier, M. U. Hammer, H. Tresp, K. D. Weltmann, and S. Reuter, "Feed gas humidity: A vital parameter affecting a cold atmospheric-pressure plasma jet and plasma-treated human skin cells," *Journal of Physics D: Applied Physics*, vol. 46, no. 29, 2013.

[50] A. Lacombe, B. A. Niemira, J. B. Gurtler, X. Fan, J. Sites, G. Boyd, and H. Chen, "Atmospheric cold plasma inactivation of aerobic microorganisms on blueberries and effects on quality attributes," *Food Microbiology*, vol. 46, pp. 479–484, 2015. [Online]. Available: http://dx.doi.org/10.1016/j.fm.2014.09.010

[51] B. G. Dasan, I. H. Boyaci, and M. Mutlu, "Nonthermal plasma treatment of Aspergillus spp. spores on hazelnuts in an atmospheric pressure fluidized bed plasma system: Impact of process parameters and surveillance of the residual viability of spores," *Journal of Food Engineering*, vol. 196, pp. 139–149, 2017. [Online]. Available: http://dx.doi.org/10.1016/j.jfoodeng.2016.09.028

[52] L. Bárdos and H. Baránková, "Cold atmospheric plasma: Sources, processes, and applications," pp. 6705–6713, 2010.

[53] M. Y. Won, S. J. Lee, and S. C. Min, "Mandarin preservation by microwave-powered cold plasma treatment," *Innovative Food Science and Emerging Technologies*, vol. 39, pp. 25–32, 2017. [Online]. Available: http://dx.doi.org/10.1016/j.ifset.2016.10.021

[54] C. Hertwig, K. Reineke, J. Ehlbeck, B. Erdodu, C. Rauh, and O. Schlüter, "Impact of remote plasma treatment on natural microbial load and quality parameters of selected herbs and spices," *Journal of Food Engineering*, vol. 167, pp. 12–17, 2015.

[55] M. Gavahian, Y. H. Chu, A. Mousavi Khaneghah, F. J. Barba, and N. N. Misra, "A critical analysis of the cold plasma induced lipid oxidation in foods," *Trends in Food Science and Technology*, vol. 77, no. April, pp. 32–41, 2018. [Online]. Available: https://doi.org/10.1016/j.tifs.2018.04.009

[56] J. A. Slezak, B. D. Zion, and S. J. Sibener, "Enhanced oxidation rate of Ni(111) by atomic oxygen," *Surface Science*, vol. 442, no. 1, 1999.

[57] I. Albertos, A. B. Martín-Diana, P. J. Cullen, B. K. Tiwari, S. K. Ojha, P. Bourke, C. Álvarez, and D. Rico, "Effects of dielectric barrier discharge (DBD) generated plasma on microbial reduction and quality parameters of fresh mackerel (Scomber scombrus) fillets," *Innovative Food Science and Emerging Technologies*, vol. 44, no. August 2016, pp. 117–122, 2017. [Online]. Available: http://dx.doi.org/10.1016/j.ifset.2017.07.006

[58] I. Albertos, A. Martin-Diana, P. Cullen, B. Tiwari, S. Ojha, P. Bourke, and D. Rico, "Shelf-life extension of herring (Clupea harengus) using in-package atmospheric plasma technology," *Innovative Food Science & Emerging Technologies*, no. September, 2017. [Online]. Available: http://linkinghub.elsevier.com/retrieve/pii/S146685641730718X

[59] C. Sarangapani, D. Ryan Keogh, J. Dunne, P. Bourke, and P. J. Cullen, "Characterisation of cold plasma treated beef and dairy lipids using spectroscopic and chromatographic methods," *Food Chemistry*, vol. 235, pp. 324–333, 2017. [Online]. Available: http://dx.doi.org/10.1016/j.foodchem.2017.05.016

[60] J. D. McClurkin-Moore, K. E. Ileleji, K. M. Keener, H. Shi, B. Cooper, R. L. Stroshine, K. E. Ileleji, K. M. Keener, S. S. Siddique, G. E. S. J. Hardy, K. L. Bayliss, H. Shi, K. E. Ileleji, R. L. Stroshine, K. M. Keener, J. L. Jensen, P. Basaran, N. Basaran-Akgul, and L. Oksuz, "Cold plasma: a potential new method to manage postharvest diseases caused by fungal plant pathogens. S. S. Siddique," *Food and Bioprocess Technology*, vol. 10, no. 30, pp. 1431–1440, 2017.

[61] M. Korachi, F. Ozen, N. Aslan, L. Vannini, M. E. Guerzoni, D. Gottardi, and F. Y. Ekinci, "Biochemical changes to milk following treatment by a novel, cold atmospheric plasma system," *International Dairy Journal*, vol. 42, pp. 64–69, 2014. [Online]. Available: http://linkinghub.elsevier.com/retrieve/pii/S0958694614002209

[62] J. Vandamme, A. Nikiforov, K. Dujardin, C. Leys, L. De Cooman, and J. Van Durme, "Critical evaluation of non-thermal plasma as an innovative accelerated lipid oxidation technique in fish oil," *Food Research International*, vol. 72, pp. 115–125, 2015.

[63] J. Van Durme and J. Vandamme, "Non-thermal plasma as preparative technique to evaluate olive oil adulteration," *Food Chemistry*, vol. 208, pp. 185–191, 2016.

[64] J. Van Durme, A. Nikiforov, J. Vandamme, C. Leys, and A. De Winne, "Accelerated lipid oxidation using non-thermal plasma technology: Evaluation of volatile compounds," *Food Research International*, vol. 62, pp. 868–876, 2014. [Online]. Available: http://dx.doi.org/10.1016/j.foodres.2014.04.043

[65] J. Vandamme, A. Nikiforov, M. De Roose, C. Leys, L. De Cooman, and J. Van Durme, "Controlled accelerated oxidation of oleic acid using a DBD plasma: Determination of volatile oxidation compounds," *Food Research International*, vol. 79, pp. 54–63, 2016. [Online]. Available: http://dx.doi.org/10.1016/j.foodres.2015.11.028

[66] X. Zhao, J. Yang, D. Tao, and X. Xu, "Synthesis and Tribological Properties of Air Plasma Polymerized Soybean Oil with N-Containing Structures," *Journal of the American Oil Chemists' Society*, vol. 91, no. 5, pp. 827–837, 5 2014.

[67] ——, "Tribological study of nitrogen plasma polymerized soybean oil with nitrogen heterocyclic structures," *Industrial Crops and Products*, vol. 51, pp. 236–243, 2013.

[68] D. Patient, "The Chemistry of Pastry Products," *Nutrition and Food Science*, vol. 94, no. 4, pp. 33–35, 1994.

[69] P. Sabatier, *Catalysis in organic chemistry*. D. Van Nostrand, 1922.

[70] I. Horiuti and M. Polanyi, "Exchange reactions of hydrogen on metallic catalysts," *Trans Faraday Soc.*, vol. 30, p. 1164, 1934.

[71] R. D. O. Brien, *Oils and fats: Formulating and Processing for Applications*, 2004.

[72] A. E. W. Beers, "Low trans hydrogenation of edible oils," *Technology*, vol. 19, no. 3, pp. 56–58, 2007.

[73] I. Karabulut, M. Kayahan, and S. Yaprak, "Determination of changes in some physical and chemical properties of soybean oil during hydrogenation," *Food Chemistry*, vol. 81, no. 3, pp. 453–456, 2003.

[74] ASA, "Soybean Oil: U.S. Vegetable oils consumption," 2018. [Online]. Available: http://soystats.com/soybean-oil-u-s-vegetable-oils-consumption/

[75] M. T. Tarrago-Trani, K. M. Phillips, L. E. Lemar, and J. M. Holden, "New and Existing Oils and Fats Used in Products with Reduced Trans-Fatty Acid Content," *Journal of the American Dietetic Association*, vol. 106, no. 6, pp. 867–880, 2006.

[76] E. Anderson, "Process for electrical treatment of liquids," 1926.

[77] A. Hemptinne, "Manufacture of lubricating oils," p. 3, 1910.

[78] W. J. Sweeney, "Voltolization of hydrogenated products," pp. 6–8, 1941.

[79] K. M. Keener, J. Jensen, V. Valdramidis, E. Byrne, J. Connolly, J. P. Mosnier, and P. J. Cullen, *Plasma for Bio-Decontamination, Medicine and Food Security*, ser. NATO Science for Peace and Security Series A: Chemistry and Biology, Z. Machala, K. Hensel, and Y. Akishev, Eds. Springer Netherlands, 2012.

[80] R. R. Allen, "Principles and catalysts for hydrogenation of fats and oils," *Journal of the American Oil Chemists' Society*, vol. 55, no. 11, pp. 792–795, 1978.

[81] A. Grill, *Cold plasma in materials fabrication: from fundamental to applications.* IEEE Press, 1994.

[82] D. C. Elias, R. R. Nair, T. M. G. Mohiuddin, S. V. Morozov, P. Blake, M. P. Halsall, A. C. Ferrari, D. W. Boukhvalov, M. I. Katsnelson, A. K. Geim, and K. S. Novoselov, "Control of graphene's properties by reversible hydrogenation: evidence for graphane." *Science (New York, N.Y.)*, vol. 323, no. 5914, pp. 610–3, 2009. [Online]. Available: http://www.ncbi.nlm.nih.gov/pubmed/19179524

[83] M. Keidar and I. I. Beilis, *Plasma Engineering*, 2013.

[84] I. W. Wu, T. Y. Huang, W. B. Jackson, A. G. Lewis, and A. Chiang, "Passivation Kinetics of Two Types of Defects in Polysilicon TFT by Plasma Hydrogenation," *IEEE Electron Device Letters*, vol. 12, no. 4, pp. 181–183, 1991.

[85] A. Kramida, Y. Ralchenko, J. Reader, and NIST Atomic Spectra Database Team, "NIST Atomic Spectra Database (version 5.3)," 2015.

[86] F. Rigas and S. Sklavounos, "Evaluation of hazards associated with hydrogen storage facilities," *International Journal of Hydrogen Energy*, vol. 30, no. 13-14, pp. 1501–1510, 2005.

[87] D. Firestone, "Official Methods and Recommended Practices of the AOCS," in *Iodine value of fats and oils cyclohexane method*, 6th ed., 2009, vol. Cd 1b-87 r.

[88] H. Tawara, Y. Itikawa, H. Nishimura, and M. Yoshino, "Cross Sections and Related Data for Electron Collisions with Hydrogen Molecules and Molecular Ions," *Journal of Physical and Chemical Reference Data*, vol. 19, no. 3, pp. 617–636, 1990.

[89] A. Bogaerts and R. Gijbels, "Effects of adding hydrogen to an argon glow discharge: overview of relevant processes and some qualitative explanations," *Journal of Analytical Atomic Spectrometry*, vol. 15, no. 4, pp. 441–449, 2000.

[90] J. Connolly, V. P. Valdramidis, E. Byrne, K. a. Karatzas, P. J. Cullen, K. M. Keener, and J. P. Mosnier, "Characterization and antimicrobial efficacy against E. coli of a helium/air plasma at atmospheric pressure created in a plastic package," *Journal of Physics D: Applied Physics*, vol. 46, p. 35401, 2013.

[91] P. Andre, J. Aubreton, M. F. Elchinger, P. Fauchais, and A. Lefort, "A new modified pseudoequilibrium calculation to determine the composition of hydrogen and nitrogen plasmas at atmospheric pressure," *Plasma Chemistry and Plasma Processing*, vol. 21, no. 1, pp. 83–105, 2001.

[92] A. Beers, R. Ariaansz, and D. Okonek, "Trans Isomer Control in Hydrogenation of Edible Oils," *Trans Fatty Acids*, vol. 1911, pp. 147–180, 2008.

[93] J. W. Veldsink, M. J. Bouma, N. H. Schöön, and A. a. C. M. Beenackers, "Heterogeneous Hydrogenation of Vegetable Oils: A Literature Review," *Catalysis Reviews*, vol. 39, no. 3, pp. 253–318, 1997.

[94] ERS, "USDA Economic Research Service," 2016. [Online]. Available: http://usda.mannlib.cornell.edu/MannUsda/viewDocumentInfo.do?documentID=1290

[95] L. Xu, A. L. Garner, B. Tao, and K. M. Keener, "Microbial Inactivation and Quality Changes in Orange Juice Treated by High Voltage Atmospheric Cold Plasma," *Food and Bioprocess Technology*, vol. 10, no. 10, pp. 1778–1791, 2017.

[96] D. Ziuzina, S. Patil, P. Cullen, K. Keener, and P. Bourke, "Atmospheric cold plasma inactivation of ¡i¿Escherichia coli¡/i¿ in liquid media inside a sealed package," *Journal of Applied Microbiology*, vol. 114, no. 3, pp. 778–787, 3 2013. [Online]. Available: http://doi.wiley.com/10.1111/jam.12087

[97] S. Pankaj, C. Bueno-Ferrer, N. Misra, L. ONeill, B. Tiwari, P. Bourke, and P. Cullen, "Characterization of dielectric barrier discharge atmospheric air cold plasma treated gelatin films," *Food Packaging and Shelf Life*, vol. 6, pp. 61–67, 2015.

[98] L. Scally, J. Lalor, P. J. Cullen, and V. Milosavljević, "Impact of atmospheric pressure nonequilibrium plasma discharge on polymer surface metrology," *Journal of Vacuum Science & Technology A: Vacuum, Surfaces, and Films*, vol. 35, no. 3, p. 03E105, 2017.

[99] AOAC, *Official Methods of Analysis of AOAC International*, 2005.

[100] X. V. Yepez and K. M. Keener, "High-voltage Atmospheric Cold Plasma (HVACP) hydrogenation of soybean oil without trans-fatty acids," *Innovative Food Science & Emerging Technologies*, vol. 38, pp. 169–174, 2016.

[101] S. Pankaj, C. Bueno-Ferrer, N. Misra, V. Milosavljević, C. O'Donnell, P. Bourke, K. Keener, and P. Cullen, "Applications of cold plasma technology in food packaging," *Trends in Food Science & Technology*, vol. 35, no. 1, pp. 5–17, 2014. [Online]. Available: http://linkinghub.elsevier.com/retrieve/pii/S092422441300229X

[102] J. Keeler, *Understanding NMR spectroscopy*, 2nd ed. Wiley, 2010.

[103] A. Martínez-Yusta, E. Goicoechea, and M. D. Guillén, "A Review of Thermo-Oxidative Degradation of Food Lipids Studied by 1H NMR Spectroscopy: Influence of Degradative Conditions and Food Lipid Nature," *Comprehensive Reviews in Food Science and Food Safety*, vol. 13, no. 5, pp. 838–859, 2014.

[104] A. Biswas, A. Adhvaryu, D. G. Stevenson, B. K. Sharma, J. L. Willet, and S. Z. Erhan, "Microwave irradiation effects on the structure, viscosity, thermal properties and lubricity of soybean oil," *Industrial Crops and Products*, vol. 25, no. 1, pp. 1–7, 2007.

[105] E. Hatzakis, A. Agiomyrgianaki, S. Kostidis, and P. Dais, "High-resolution NMR spectroscopy: An alternative fast tool for qualitative and quantitative analysis of diacylglycerol (DAG) oil," *JAOCS, Journal of the American Oil Chemists' Society*, vol. 88, no. 11, pp. 1695–1708, 2011.

[106] C. O. Laux, T. G. Spence, C. H. Kruger, and R. N. Zare, "Optical diagnostics of atmospheric pressure air plasmas," *Plasma Sources Sci. Technol.*, vol. 12, pp. 125–138, 2003.

[107] C. Parigger, J. W. L. Lewis, and D. Plemmons, "Electron number density and temperature measurement in a laser-induced hydrogen plasma," *Journal of Quantitative Spectroscopy and Radiative Transfer*, vol. 53, no. 3, pp. 249–255, 1995.

[108] K. J. Clay, S. P. Speakman, G. Amaratunga, and S. R. P. Silva, "Characterization of a-C:H:N deposition from CH4/N2 rf plasmas using optical emission spectroscopy," *Journal of Applied Physics*, vol. 79, no. 9, p. 7227, 1996.

[109] D. M. El-Zeer, A. Samir, F. Elakshar, and A. A. Garamoon, "Decaying of Nitrogen Second Positive System by Addition of H2 Gas in Air DB Discharge," *Journal of Modern Physics*, vol. 04, no. 02, pp. 160–167, 2 2013. [Online]. Available: http://www.scirp.org/journal/doi.aspx?DOI=10.4236/jmp.2013.42022

[110] N. N. Misra, A. Martynenko, F. Chemat, L. Paniwnyk, F. J. Barba, and A. R. Jambrak, "Thermodynamics, transport phenomena, and electrochemistry of external field-assisted nonthermal food technologies," pp. 1–32, 2017.

[111] J. Van Durme, J. Dewulf, C. Leys, and H. Van Langenhove, "Combining nonthermal plasma with heterogeneous catalysis in waste gas treatment: A review," *Applied Catalysis B: Environmental*, vol. 78, no. 3-4, pp. 324–333, 2008.

[112] J. Hong, M. Aramesh, O. Shimoni, D. H. Seo, S. Yick, A. Greig, C. Charles, S. Prawer, and A. B. Murphy, "Plasma Catalytic Synthesis of Ammonia Using Functionalized-Carbon Coatings in an Atmospheric-Pressure Non-equilibrium Discharge," *Plasma Chemistry and Plasma Processing*, vol. 36, no. 4, pp. 917–940, 2016.

[113] Y. Kubota, K. Koga, M. Ohno, and T. Hara, "Synthesis of Ammonia through Direct Chemical Reactions between an Atmospheric Nitrogen Plasma Jet and a Liquid," *Plasma and Fusion Research*, vol. 5, pp. 042–042, 2010. [Online]. Available: http://joi.jlc.jst.go.jp/JST.JSTAGE/pfr/5.042?from=CrossRef

[114] J. J. Max and C. Chapados, "Aqueous ammonia and ammonium chloride hydrates: Principal infrared spectra," *Journal of Molecular Structure*, vol. 1046, pp. 124–135, 2013. [Online]. Available: http://dx.doi.org/10.1016/j.molstruc.2013.04.045

[115] T. Mizushima, K. Matsumoto, J. I. Sugoh, H. Ohkita, and N. Kakuta, "Tubular membrane-like catalyst for reactor with dielectric-barrier- discharge plasma and its performance in ammonia synthesis," *Applied Catalysis A: General*, vol. 265, no. 1, pp. 53–59, 2004.

[116] L. Bromberg, "Plasma catalytic reforming of methane," *International Journal of Hydrogen Energy*, vol. 24, no. 12, pp. 1131–1137, 1999.

[117] E. C. Neyts, K. Ostrikov, M. K. Sunkara, and A. Bogaerts, "Plasma Catalysis: Synergistic Effects at the Nanoscale," *Chemical Reviews*, vol. 115, no. 24, pp. 13 408–13 446, 2015.

[118] P. Attri, B. Arora, and E. H. Choi, "Retracted Article: Utility of plasma: a new road from physics to chemistry," *RSC Adv.*, vol. 3, no. 31, pp. 12 540–12 567, 2013. [Online]. Available: http://xlink.rsc.org/?DOI=C3RA41277F

[119] P. A. Klockow and K. M. Keener, "Safety and quality assessment of packaged spinach treated with a novel ozone-generation system," *LWT - Food Science and Technology*, vol. 42, no. 6, pp. 1047–1053, 2009. [Online]. Available: http://dx.doi.org/10.1016/j.lwt.2009.02.011

[120] N. N. Misra and C. Jo, "Applications of cold plasma technology for microbiological safety in meat industry," *Trends in Food Science and Technology*, vol. 64, pp. 74–86, 2017. [Online]. Available: http://dx.doi.org/10.1016/j.tifs.2017.04.005

[121] X. Yepez and K. Keener, "High-voltage Atmospheric Cold Plasma (HVACP) hydrogenation of soybean oil without trans-fatty acids," *Innovative Food Science and Emerging Technologies*, vol. 38, 2016.

[122] D. Jovanovic, "Nickel hydrogenation catalyst for tallow hydrogenation and for the selective hydrogenation of sunflower seed oil and soybean oil," *Catalysis Today*, vol. 43, no. 1-2, pp. 21–28, 1998. [Online]. Available: http://www.sciencedirect.com/science/article/pii/S0920586198001333

[123] D.-x. Liu, F. Iza, X.-h. Wang, D. Liu, B. Sun, F. Iza, D. Xu, and X. Wang, "atmospheric pressure plasma jets propagating into humid air Main species and chemical pathways in cold atmospheric-pressure Ar + H 2 O plasmas," 2017.

[124] M. D. Guillén and N. Cabo, "Characterization of edible oils and lard by fourier transform infrared spectroscopy. Relationships between composition and frequency of concrete bands in the fingerprint region," *Journal of the American Oil Chemists' Society*, vol. 74, no. 10, pp. 1281–1286, 1997.

[125] A. M. McAnoy, C. C. Wu, and R. C. Murphy, "Direct qualitative analysis of triacylglycerols by electrospray mass spectrometry using a linear ion trap," *Journal of the American Society for Mass Spectrometry*, vol. 16, no. 9, pp. 1498–1509, 2005.

[126] D. Lin-Vien, N. B. Colthup, W. B. Fateley, and J. G. Graselli, "Characteristic Raman Vibrational Frequencies of Organic Groups," in *The Handbook of Infrared and Raman Characteristic Frequencies of Organic Molecules*, 1991, no. 1103, pp. 1–2.

[127] R. Thirumdas, A. Trimukhe, R. R. Deshmukh, and U. S. Annapure, "Functional and rheological properties of cold plasma treated rice starch," *Carbohydrate Polymers*, vol. 157, pp. 1723–1731, 2017. [Online]. Available: http://dx.doi.org/10.1016/j.carbpol.2016.11.050

[128] S. K. Pankaj, Z. Wan, J. E. De León, C. Mosher, W. Colonna, and K. M. Keener, "High-voltage atmospheric cold plasma treatment of different types of starch films," *Starch - Stärke*, vol. 1700009, p. 1700009, 2017. [Online]. Available: http://doi.wiley.com/10.1002/star.201700009

[129] K. Ostrikov, E. C. Neyts, and M. Meyyappan, "Plasma nanoscience: From nano-solids in plasmas to nano-plasmas in solids," *Advances in Physics*, vol. 62, no. 2, pp. 113–224, 2013.

[130] H. Wang, T. Maiyalagan, and X. Wang, "Review on recent progress in nitrogen-doped graphene: Synthesis, characterization, and its potential applications," *ACS Catalysis*, vol. 2, no. 5, pp. 781–794, 2012.

[131] Y. Shao, S. Zhang, M. H. Engelhard, G. Li, G. Shao, Y. Wang, J. Liu, I. A. Aksay, and Y. Lin, "Nitrogen-doped graphene and its electrochemical applications," *Journal of Materials Chemistry*, vol. 20, no. 35, p. 7491, 2010. [Online]. Available: http://xlink.rsc.org/?DOI=c0jm00782j

[132] J. S. Burgess, B. R. Matis, J. T. Robinson, F. A. Bulat, F. Keith Perkins, B. H. Houston, and J. W. Baldwin, "Tuning the electronic properties of graphene by hydrogenation in a plasma enhanced chemical vapor deposition reactor," *Carbon*, vol. 49, no. 13, pp. 4420–4426, 2011. [Online]. Available: http://dx.doi.org/10.1016/j.carbon.2011.06.034

[133] S. C. Chew and K. L. Nyam, "Oxidative Stability of Microencapsulated Kenaf Seed Oil Using Co-extrusion Technology," *JAOCS, Journal of the American Oil Chemists' Society*, vol. 93, no. 4, pp. 607–615, 2016.

[134] R. Crompton, "Carbon hydrogen polymers," *Thermal stability of polymers*, pp. 1–44, 2012.

[135] G. Levchik, K. Si, S. Levchik, G. C. P. d. . . . , , and u. 1999, "The correlation between cross-linking and thermal stability: Cross-linked polystyrenes and polymethacrylates," *Elsevier*. [Online]. Available: https://www.sciencedirect.com/science/article/pii/S0141391099000282

[136] M. Ionescu and Z. Petrovic, "Cationic polymerization of biological oils with superacid catalysts," 2009.

[137] J. Kim, N. Kim, S. H. Lee, S.-H. Yoo, and S. Lee, "Correlation of fatty acid composition of vegetable oils with rheological behaviour and oil uptake."

[138] H. Topallar, Y. Bayrak, M. I. J. o. t. A. O. Chemists', and u. 1995, "Effect of hydrogenation on density and viscosity of sunflowerseed oil," *Springer*. [Online]. Available: https://link.springer.com/article/10.1007/BF02577846

[139] A. Ionascut-Nedelcescu, C. Carlone, U. Kogelschatz, D. V. Gravelle, and M. I. Boulos, "Calculation of the gas temperature in a throughflow atmospheric pressure dielectric barrier discharge torch by spectral line shape analysis," *Journal of Applied Physics*, vol. 103, no. 6, 2008.

[140] B. Muik, B. Lendl, A. Molina-Díaz, and M. J. Ayora-Cañada, "Direct monitoring of lipid oxidation in edible oils by Fourier transform Raman spectroscopy," *Chemistry and Physics of Lipids*, vol. 134, no. 2, pp. 173–182, 2005.

[141] F. R. Voort, A. A. Ismail, J. Sedman, and G. Emo, "Monitoring the oxidation of edible oils by Fourier transform infrared spectroscopy," *J. Am. Oil Chem. Soc.*, vol. 71, no. 3, pp. 243–253, 1994.

[142] N. Vlachos, Y. Skopelitis, M. Psaroudaki, V. Konstantinidou, A. Chatzilazarou, and E. Tegou, "Applications of Fourier transform-infrared spectroscopy to edible oils," *Analytica Chimica Acta*, vol. 573-574, pp. 459–465, 2006.

[143] X. Liao, D. Liu, Q. Xiang, J. Ahn, S. Chen, X. Ye, and T. Ding, "Inactivation mechanisms of non-thermal plasma on microbes: A review," *Food Control*, vol. 75, pp. 83–91, 2017. [Online]. Available: http://dx.doi.org/10.1016/j.foodcont.2016.12.021

[144] A. I. Muhammad, Q. Xiang, X. Liao, D. Liu, and T. Ding, "Understanding the Impact of Nonthermal Plasma on Food Constituents and MicrostructureA Review," *Food and Bioprocess Technology*, vol. 11, no. 3, pp. 463–486, 2018.

[145] R. B. Pegg, "Measurement Of Primary Lipid Oxidation Products," *Handbook of Food Analytical Chemistry*, vol. 1-2, pp. 515–529, 2005.

[146] S. C. Cermak and T. A. Isbell, "Synthesis of estolides from oleic acid and saturated fatty acids," vol. 78, no. 6, pp. 557–565, 2001.

[147] C. Sarangapani, G. O'Toole, P. J. Cullen, and P. Bourke, "Atmospheric cold plasma dissipation efficiency of agrochemicals on blueberries," *Innovative Food Science and Emerging Technologies*, vol. 44, pp. 235–241, 2017. [Online]. Available: http://dx.doi.org/10.1016/j.ifset.2017.02.012

[148] A. Adhvaryu and S. Z. Erhan, "Epoxidized soybean oil as a potential source of high-temperature lubricants," *Industrial Crops and Products*, vol. 15, no. 3, pp. 247–254, 2002.

APPENDIX

A. APPENDIX

A.1 Relative abundance of MS-ESI from linolenic acid and trilinolein.

a. LINOLENIC ACID UNTREATED AND TREATED WITH NITROGEN, HYDROGEN, AND ARGON GAS.

UNTREATED			NITROGEN GAS			HYDROGEN GAS			ARGON GAS		
m/z	Intensity	Relative (%)	m/z	Intensity	Relative (%)	m/z	Intensity	Relative (%)	m/z	Intensity	Relative (%)
275.2	336434.2	0.6	275.2	156916.4	3.34	275.2	1202691	1.16	275.2	128328.8	3.35
277.2	83116.5	0.15	276.21	28601.6	0.61	277.21	409250.5	0.39	276.2	20280.8	0.53
277.2	94394.2	0.17	277.21	22282.3	0.47	277.22	1.04E+08	100	277.21	15834.7	0.41
277.2	170045.8	0.3	277.22	4700777	100	277.23	288296	0.28	277.22	3827488	100
277.22	55982872	100	278.22	907995.6	19.32	277.23	222044.8	0.21	278.22	737719.1	19.27
277.23	147251.8	0.26	279.22	70232.3	1.49	278.22	20889880	20.15	279.22	47473.4	1.24
277.23	128000.6	0.23	279.23	1320715	28.1	279.22	1668645	1.61	279.23	551565.1	14.41
278.22	10747665	19.2	280.24	245772	5.23	279.23	10908883	10.52	280.24	88303.4	2.31
279.22	776684.4	1.39	281.25	723193.4	15.38	280.24	2065694	1.99	281.25	93288.3	2.44
279.23	495123.5	0.88	282.25	134081.8	2.85	281.25	921053.1	0.89	282.25	15467	0.4
280.24	86600.1	0.15	283.26	306066	6.51	283.26	220401.9	0.21	283.26	24911.6	0.65
290.21	86600.8	0.15	284.27	54561.2	1.16	291.2	1792460	1.73	289.18	30748	0.8
291.2	1482553	2.65	289.18	23753.2	0.51	292.2	323675.4	0.31	291.2	91364.9	2.39
292.2	268938.1	0.48	291.2	65790.9	1.4	293.21	2438419	2.35	292.2	14937.9	0.39
293.21	4208063	7.52	293.21	353291.5	7.52	294.22	431583.8	0.42	293.21	412955.7	10.79
294.21	718099.1	1.28	294.22	62084.8	1.32	295.23	2346539	2.26	294.21	65719.8	1.72
295.23	2550804	4.56	295.23	353861.3	7.53	296.23	421397.2	0.41	295.23	317890.4	8.31
296.23	458448.9	0.82	296.23	66262.6	1.41	297.15	241688	0.23	296.23	48537.2	1.27
301.23	128466	0.23	297.24	101080.9	2.15	297.24	275367.2	0.27	297.24	38674.5	1.01
302.21	965295.1	1.72	301.23	30036.5	0.64	302.21	249553.2	0.24	301.23	15011.3	0.39

b. TRILINOLEIN UNTREATED AND TREATED ARGON GAS.

UNTREATED			LOW HUMIDITY			HIGH HUMIDITY		
m/z	Intensity	Relative (%)	m/z	Intensity	Relative (%)	m/z	Intensity	Relative (%)
896.77	139349.7	100	894.75	1675.2	1.07	894.75	475.5	1.21
897.77	95591.3	68.6	896.73	1811.4	1.15	895.73	565.8	1.44
898.3	837.1	0.6	896.77	157163.4	100	896.77	39255.9	100
898.77	30270.7	21.72	897.74	1430.6	0.91	897.77	23321	59.41
899.78	6046.1	4.34	897.77	101036.8	64.29	898.79	10645.8	27.12
912.76	1067.1	0.77	898.77	27399.4	17.43	899.79	7781.7	19.82
			899.79	8042.4	5.12	900.8	1849.8	4.71
			900.04	708.2	0.45	901.72	426.1	1.09
			900.21	615.1	0.39	901.8	1368.5	3.49
			900.79	1561.6	0.99	910.74	754.8	1.92
			901.25	637.5	0.41	912.76	22017.3	56.09
			908.67	789.6	0.5	913.77	13478	34.33
			912.76	45572.5	29	914.78	17738.7	45.19
			913.76	27857.2	17.72	915.78	11147.6	28.4
			914.78	8775.1	5.58	916.76	650.4	1.66
			915.78	7093.2	4.51	916.78	3174.4	8.09
			916.78	2126.3	1.35	916.8	4258.8	10.85
			916.8	1351.7	0.86	917.8	3466.3	8.83
			917.8	1296.9	0.83	918.8	760	1.94
			922.35	693.3	0.44	919.81	652.4	1.66

Fig. A.1. Relative abundance of MS-ESI from linolenic acid and trilinolein.

A.2 Reaction mechanism

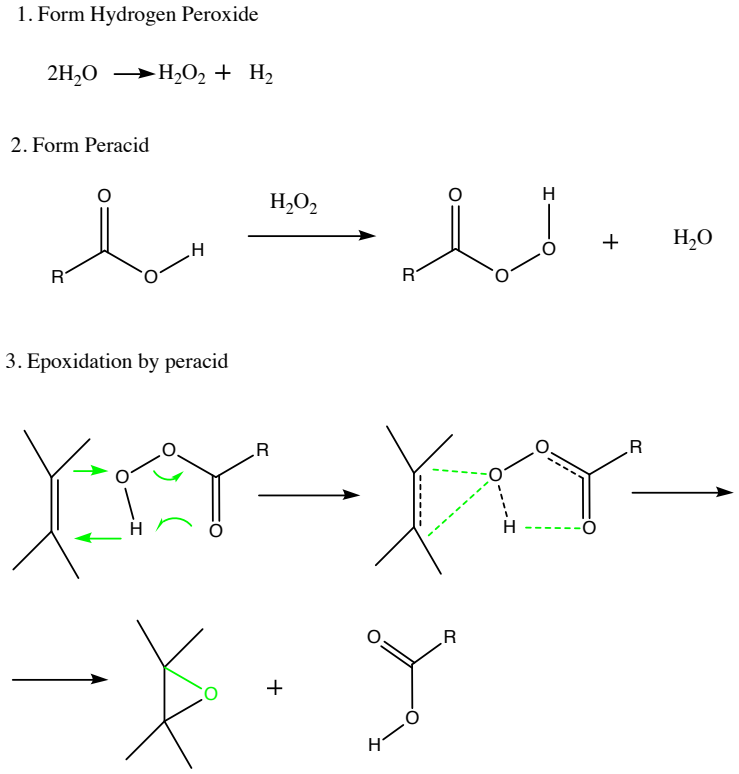


Fig. A.2. Mechanism of formation of epoxides with a carboxylic group and hydrogen peroxide.

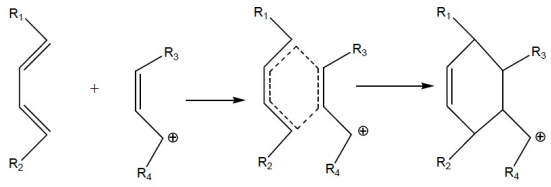


Fig. A.3. Diels Alder reaction mechanism.

A.3 Fatty acid composition

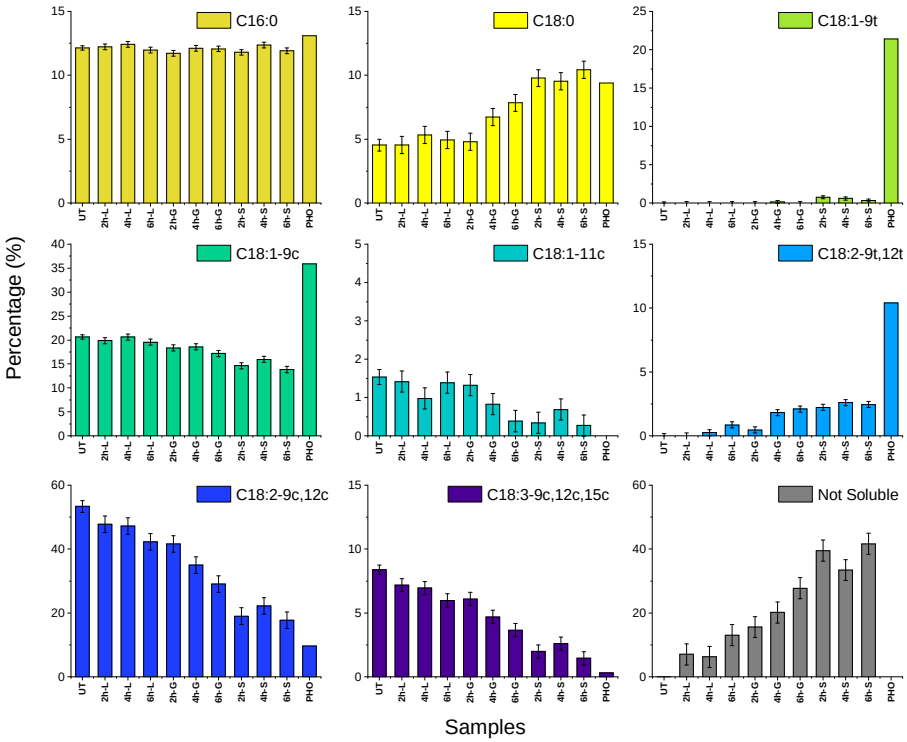


Fig. A.4. Fatty acid composition

A.4 Infrared absorption frequencies for fatty acids and triglycerides.

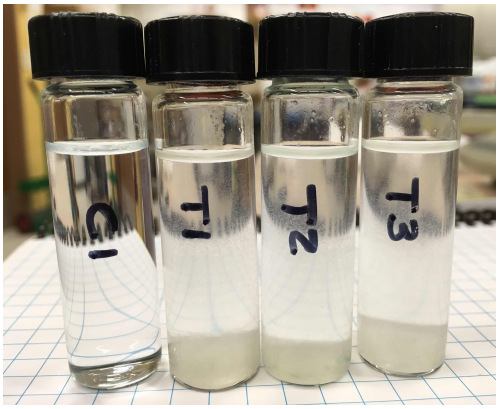
Table A.1.: Infrared absorption frequencies for functional groups of fatty acids and triglycerides .

Frequency	**Functional group**	**Structure**	**Intensity**	**Ref.**
3470	Peroxide	C-O	m	[147]
3468	Glyceril ester carbonyl	C-O	m	[124]
3010	Alkene stretching (C=C)	C-H	s	[66, 124, 142]
2960	Alkane methyl end	C-CH_3	s	[66, 124, 142]
2930	Alkane antisymmetric stretching	C-H	s	[124, 142]
2900	Aldehyde	-CHO	w	[59]
2860	Alkane symmetric stretching	C-H	s	[124, 142]
2677, 2730	Carbonyl			
1801	Carbonated estolide			
1746	Carbonyl	C=O		
1725	Aldehyde			
1715	Ketone			
1700, 1711	Free fatty acid			
1654	Aliphatic alkene			
	Cis- isomer			
1630	Amine primary			
1552	Amine secondary			
1460	Methylene scissoring	C-H	m	[124]
1239	C-O			[66, 124, 142]
1195	Ozonide			[59]
1160	C-O stretching	C-O	m	[66, 124, 142]
1100	Ozonide	C-O	m	[59, 66, 124]
1050	Polymeric	C-O-C		[?]

continued on next page

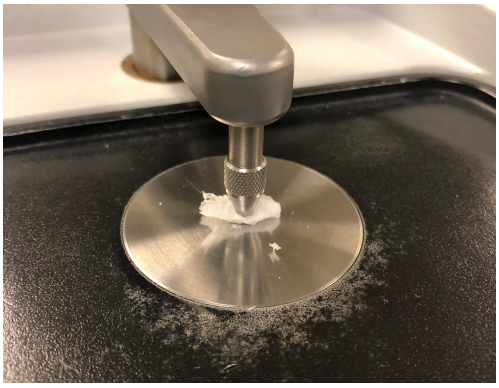
Table A.1 – *Continued from previous page*

Frequency	**Functional group**	**Structure**	**Intensity**	**Ref.**
972	*Trans-* conjugated	C-H	m	[66]
968	*Trans-* isomer bending	C-H	m	[124]
914	Cis isomer bending	C-H	m	[124]
895	Epoxide ring			[24]
862	Epoxy group			[148]

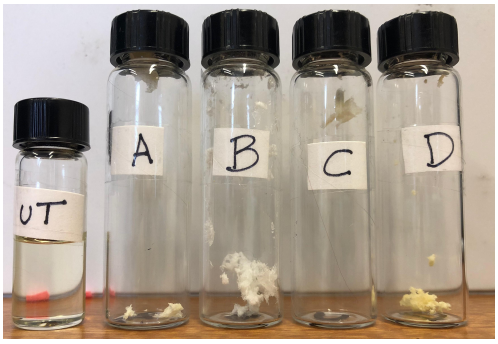


(a) Solvents

(b) Isolated with Soxhlet



(c) FTIR measurement



(d) Samples

Fig. A.5. Pictures of insoluble fraction of treated sample of soybean oil

A.5 Insoluble fraction of HVACP treated samples of soybean oil.

(a) Picture of untreated soybean oil (C1) diluted in hexane, and treated soybean oil dilute in hexane (T1), tetrahydrofuran (T2), and chloroform (T3). Solution of 50mg of sample with 4ml of solvent.

(b) Insoluble fraction isolated with Soxhlet, using hexane as solvent.

(d) Isolated insoluble fraction from hydrogen (A,B), argon (C), and nitrogen (D) gas treatment. Untreated soybean oil (UT).

A.6 Soybean oil treated with hydrogen gas for extended time.

Table A.2.
Technical data sheet of untreated and treated soybean oil (200 ml) for 20h with HVACP at 80 kV on direct field exposure, using hydrogen gas

Parameter	**Untreated**	**Treated**
C16:0	$12.81 \pm 0.16_a$	$12.75 \pm 0.16_a$
C18:0	$6.27 \pm 0.09_a$	$7.06 \pm 0.09_b$
C18:1-9t	0_a	$1.07 \pm 0.03_b$
C18:1-9c	$19.59 \pm 0.02_a$	$19.47 \pm 0.02_a$
C18:1-11c	$1.61 \pm 0.05_a$	$1.51 \pm 0.05_a$
C18:2-9t,12t	0_a	0_a
C18:2-9c,12c	$49.24 \pm 0.06_a$	$40.28 \pm 0.06_b$
C18:3-9c,12c,15c	$10.26 \pm 0.51_a$	$6.71 \pm 0.51_b$
Insoluble	$0.23 \pm 1.04_a$	$11.17 \pm 1.04_b$
Saturated	19.08	19.81
MUFA	21.20	22.04
PUFA	59.50	46.99
Total trans	0	1.07
Iodine Value (calculated)	130.4	106.3
Peroxide Value (meq/kg)	$0.73 \pm 0.02_a$	$0.84 \pm 0.02_a$
Free fatty acids (%)	0.03	0.04

A.7 HVACP treatment of oleic acid

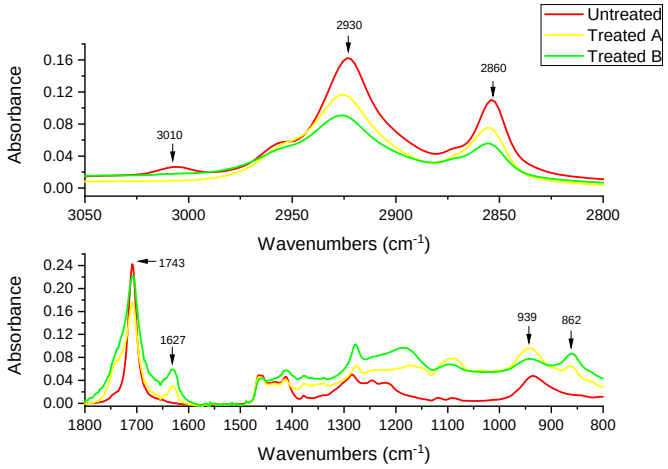


Fig. A.6. FTIR spectra of oleic acid treated with air for 2h at 80 kV. Sample from outside the petri dish (treated A), and sample inside the petri dish (treated B).

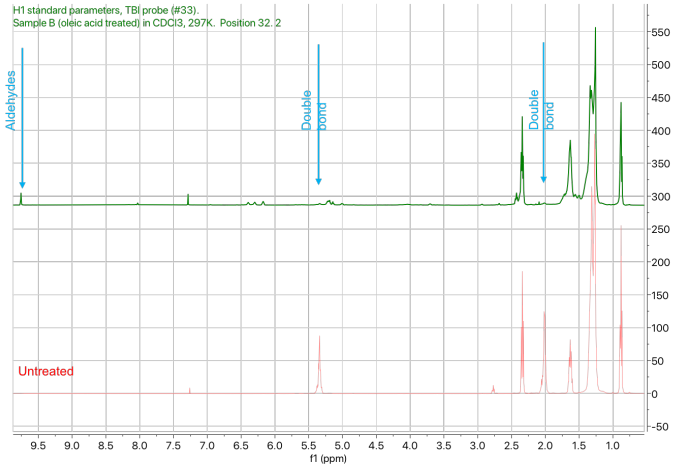


Fig. A.7. NMR spectra of oleic acid untreated (top) and treated (bottom) with air for 2h at 80 kV.

VITA

XIMENA V. YEPEZ

EDUCATION

- Ph.D. Food Science
  Purdue University, West Lafayette, IN, USA
  Jan. 2015 – Dec. 2018
- M.S. Food Science
  Purdue University, West Lafayette, IN, USA
  Aug. 2012 – Dec. 2014
- B.S., Food Engineering
  Escuela Superior Politécnica del Litoral, Guayaquil-Ecuador.
  Sept. 1997 - Feb. 2003

PEER REVIEWED PUBLICATIONS

- Misra N.N., Yepez X., Xu L., Keener K.M. 2019. In-package cold plasma technologies. J. of Food engineering, 24, 21-31.
- Yepez, X. V., & Keener, K. M. 2016. High-voltage Atmospheric Cold Plasma (HVACP) hydrogenation of soybean oil without trans-fatty acids. *Innovative Food Science & Emerging Technologies*, *38*, 169-174.
- Undergraduate Thesis: Yepez, X., Martinez, E. 2005. "Analysis and Improvement of a Chocolate-covered Honey Nougat Manufacturing Process". *Degree Thesis Articles.*
- Yepez X., Misra N.N., Keener K.M. Non-thermal Plasma. In Demirci, Feng, and Krishnamurthy (Eds). *Food Safety Engineering*. Springer. (Submitted)

RESEARCH PROJECTS

- Main project: Developing of Cold Plasma hydrogenation of soybean oil without trans-fatty acids. Main analytical techniques: GC/FID, GC/MS. Data analysis from Mass Spectrometry, and NMR.
- Collaboration: "Development of a Sanitizing Treatment to Improve Safety and Quality of Indiana Cantaloupe", "High voltage atmospheric cold plasma (HVACP) treatment of Listeria monocytogenes from ready-to-eat deli-style, chicken breast meat inside a package", "Evaluation of In-Package High Voltage Atmospheric Cold Plasma Treatment for Mold Spore Reduction in Wheat and Corn Whole Grain and Flour", "Extrusion of whole and decorticated pearl millet flour".

TEACHING EXPERIENCE

- Food Sanitation
  Universidad Espíritu Santo, Guayaquil, Ecuador. Aug - Dec 2009
- Food Analysis Laboratory
  Teaching assistantship. Jan. - May 2015; Jan. - May 2018

AWARDS

- (2012) SENESCYT (National Secretariat of Science, Technology and Innovation of Ecuador). Master of Science Grant under the Universities of Excellence Program, for a two-year period.
- (2015) SENESCYT (National Secretariat of Science, Technology and Innovation of Ecuador). Ph.D. Grant under the Universities of Excellence Program, for a three-and-a-half-year period.

LIST OF CONFERENCE AND POSTER PRESENTATIONS

1. Ximena V. Yepez, Kevin M. Keener. Poster presentation: 'High Voltage Atmospheric Cold Plasma (HVACP) Hydrogenation of Soybean Oil Without Trans-Fatty Acids'. IFT Annual meeting. Chicago, IL. July 2016. Finalist of 'Non-thermal Processing Division Graduate Student Research Paper Poster Competition'.
2. Ximena V. Yepez, Hanna S. Gracz, Kevin M. Keener. E-Poster presentation: 'Modifying Soybean Oil Chemistry Using High Voltage Atmospheric Cold Plasma (HVACP) Treatment with Hydrogen and Nitrogen Gas'. IFT Annual meeting. Chicago, IL. July 2016.
3. Ximena V. Yepez, Hanna S. Gracz, Kevin M. Keener. Oral presentation 'Modifying Soybean Oil Chemistry Using High Voltage Atmospheric Cold Plasma (HVACP) Treatment with Hydrogen and Nitrogen Gas'. 2017 AOCS Annual meeting and industry showcases. Orlando, FL. April 30 - May 4, 2017.
4. Ximena V. Yepez, Hanna S. Gracz, Kevin M. Keener. Oral presentation 'Modifying Soybean Oil Chemistry Using High Voltage Atmospheric Cold Plasma (HVACP) Treatment with Hydrogen and Nitrogen Gas'. *17th AOCS Latin American Congress and Exhibition on Fats, Oils, and Lipids. Cancun, Mexico.* September 11-14, 2017.
5. Ximena V Yepez, Lei Xu, Bernard Tao, Hanna S. Gracz, Kevin M. Keener. Poster presentation: "Characterization of soybean oil treated with cold plasma and hydrogen gas". IFT Annual meeting. Chicago, IL. July 2018.

MEMBERSHIPS

IFT (Institute of Food Technology), since 2013
AOCS (American Oil Chemist Society), since 2014

INDUSTRY EXPERIENCE

- (2007-2012) Independent Consultant, Guayaquil, Ecuador

Consultant for food industries, in regulatory affairs and developing products, to obtain mandatory Food Registry Certificates. Clients: Congelados Ecuatorianos Ltd (Frozen tropical fruits), Modercorp Ltd. (Frozen Shrimp), Ecuacocoa Ltd. (Cocoa and chocolate products), Casa Luker del Ecuador Ltd. (Cocoa, fruit concentrates), Productos Cris (Peanuts), L. Henriques & Cia. Ltd (Cookies). Projects for food product development: mango wine, Andean grains cereal bar, banana plantains.

- (2006-2007) CONGELADOS ECUATORIANOS, Guayaquil, Ecuador. Quality Assurance Manager

Responsible for the quality of the production line. The company's main business was to manufacture tropical frozen fruit for export (papaya, pineapple, banana, and mangoes chunks). During this period, I lead the process to obtain the Organic Production Certification, and worked in GMP, SSOP, and HACCP fields within the organization.

COURSES

Certification "Preventive controls for human food", October 2018. Purdue University/FSPCA.
Firestarter, April-May 2018. Burton D Morgan Center for Entrepreneurship, Purdue University.
Certification "Better Process Control", May 8-11, 2017. Purdue University/Grocery Manufacturers Association/U.S. Food and Drug Administration.
"Avances en la Tecnología de Extrusión de Alimentos", ESPOL
"Implementación de HACCP y Seguridad alimentaria" Universidad de Nebraska
"Transferencia de calor en sistemas radiantes e Intercambiadores de Placas" ESPOL

LANGUAGES

Spanish, English.